ARSENIC IRRIGATED VEGETABLES: RISK ASSESSMENT FOR SOUTH ASIAN HORTICULTURE

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ABSTRACT

Arsenic (As) contaminated water is often used in South Asia to irrigate vegetables. These vegetables accumulate As in their edible tissues and once ingested, increase As burden in humans. Despite the apparent risk, the As uptake potential of vegetable species when irrigated with As-contaminated water is not well defined. Most research on As-irrigated vegetables are monitoring surveys that only describe the As concentration levels in various vegetable species from affected areas. Because of the great variability in As concentration of irrigation water, soil type, vegetable species and cultivars, agronomic practices and climatic factors, As uptake potential of an individual vegetable species cannot be described from the monitoring data. Identifying vegetable species and soil conditions that result in high As concentrations in the edible tissues of vegetables is prerequisite for risk assessment and proposing As mitigation strategies.

The objectives of this study were to (i) determine the As uptake response of common vegetable species when irrigated with As-contaminated water, (ii) calculate the risk to humans upon ingestion of As-contaminated vegetable species, (iii) elucidate factors that may increase As concentrations in vegetable species, and (iv) propose management strategies for South Asian countries where As-contaminated water is used for vegetable cultivation.

In the first glasshouse experiment (Chapter 4), four common vegetables, carrot (*Daucus carota*), radish (*Raphanus sativus*), spinach (*Spinacia oleracea*), and tomato (*Solanum esculentum*) were irrigated with a range of As^V enriched water (50 to 1000 µg L^{-1}) using two irrigation techniques. These irrigation techniques were (i) non-flooded, where soil moisture was maintained to 70% field capacity (Fc) of soil, and (ii) flooded, where the water was maintained at 110% Fc initially followed by drainage and onset of aerobic conditions until the next irrigation event. Only the 1000 µg As L^{-1} treatment showed a significant increase of As concentration in the vegetables compared to all other treatments. There was a higher concentration of As in the vegetables grown under flood irrigation relative to non-flood irrigation. The trend of As uptake among vegetable species was spinach > tomato > radish > carrot. Only in spinach leaves, the As concentration was above the Chinese food safety standard for inorganic As (0.05 µg g^{-1} fresh weight) by a factor of 1.6 to 6.4 times, when irrigated with 100, 200, and 1000 µg As L^{-1} under flood irrigation and with 1000 µg As L^{-1}

under non-flood irrigation. The USEPA carcinogenic and non-carcinogenic risk parameters for the scenario where vegetables are consumed 500 grams per day were calculated. The USEPA Hazard Quotient (HQ) value for spinach leaves ranged from 0.32 to 1.26 for adults and 0.38 to 1.51 for adolescents while the Cancer Risk (CR) value ranged from 1.4 x 10^{-4} to 5.7 x 10^{-4} for adults and 1.7 x 10^{-4} to 6.8 x 10^{-4} for adolescents for treatment water concentrations 100 µg As L^{-1} or greater. An HQ value greater than 1 represents an unacceptable non-carcinogenic risk and a CR value greater than 10^{-4} represents an unacceptable carcinogenic risk.

A laboratory batch experiment (Chapter 5) was conducted using four soils to determine their As adsorption behavior and the soil properties that control As retention in these soils. Soils used in this study were (i) Rangitikei silt loam (the soil which was used in glasshouse experiment 1), (ii) Rangitikei silt loam soil amended with calcium hydroxide to raise the pH to 7.5, to model the soil pH level of South Asian countries, and (iii) two New Zealand soils, Korokoro silt loam and Tokomaru silt loam. Both arsenate (As^{V}) and arsenite (As^{III}) were investigated in the experiment because these As species are mainly present in irrigation water. The results showed that the As^{V} was adsorbed to a greater degree than As^{III} as defined by high adsorption maxima, bonding energy and As partition coefficient values of Langmuir and Freundlich isotherms. Adsorption of both As^{V} and As^{III} was mainly controlled by amorphous Al, total C and Olsen P content of selected soils.

A glasshouse experiment (Chapter 6) was conducted to explore those factors which can promote As concentration in plants. The following factors which are likely to affect horticulture in South Asia were included: two As species (As^{V} and As^{III}), four As concentration levels of irrigation water (50 to 1000 µg L^{-1}), two soil pH levels (6.1 and 7.5), and two soil amendments (biochar and cattle manure). The control treatment for this experiment was no As in irrigation water and no soil amendment. Spinach was selected for this work due to its high uptake potential described in the earlier glasshouse experiment (Chapter 4). The findings of this experiment showed that the As concentration in spinach leaves was dependent on As concentrations in water and soil amendments and was independent of soil pH and As species under flood irrigation. Spinach plants grown in biochar and cattle manure amended soils had significantly higher As concentration in their leaves when compared with spinach plants grown with no amendment. In both biochar and cattle manure amended soils, the As concentration in spinach leaves exceeded the Chinese food safety standard (0.05 $\mu g\ g^{-1}$ fresh weight) by a factor of 1.6 to 8.3 times, where the concentration of As in irrigation water was 200 $\mu g\ L^{-1}$ or greater. The CR values for spinach grown in cattle manure amended soil was greater than the critical value of 1×10^{-4} for the scenarios where vegetable consumption is 205 grams and/or 500 grams per day. This increase was found where the As concentration in irrigation water was 200 $\mu g\ L^{-1}$ or greater. The HQ value was above the critical value of 1 for the scenario where the vegetable consumption is 500 grams per day. This increase was observed for spinach grown in cattle manure amended soil with an As concentration in irrigation water 500 $\mu g\ L^{-1}$ or greater. Arsenic daily intake (mg kg^{-1} body weight) associated with the ingestion of spinach leaves corresponds to proposed ATSDR (Agency for Toxic Substances and Disease Registry) and drinking water daily intake values that may lead to development of cancer (bladder, lung and skin), skin lesions, and intellectual impairment in children. The As intake through ingestion of spinach correlates to an As concentration in drinking water that is 10 $\mu g\ L^{-1}$ or greater.

Overall, the results of glasshouse studies indicate that the As concentrations greater than 50 $\mu g\ L^{-1}$ should be avoided for spinach cultivation where flood irrigation is practiced. Addition of cattle manure can further intensify the risk by increasing the As concentration in plant tissues, therefore its usage in South Asian horticulture is questionable. I propose that the As concentration in vegetables should not be overlooked as they can alone be a major source of As poisoning in humans.

DEDICATION

This work is dedicated to my parents for their hard work

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Arsenic (As) is a chemical element that is naturally present throughout the biosphere and at high concentrations, it is toxic to all forms of life. Although As contamination is a global concern, some parts of the world are more severely affected. In Asia, serious As toxicity symptoms in humans have been linked to the consumption of As-contaminated drinking water and food (Samal *et al.*, 2011; Tondel *et al.*, 1999; Williams *et al.*, 2006). The situation is worst in South and Southeast Asian countries, where around 110 million people are under the threat of As poisoning (Brammer & Ravenscroft, 2009). Arsenic exposure can cause several carcinogenic (tumours of the skin, lung, urinary bladder and other organs) and non-carcinogenic (gastrointestinal disturbances, skin lesions, peripheral vascular diseases, reproductive toxicity and neurological disorders) effects in humans (Schuhmacher-Wolz *et al.*, 2009). Arsenic contamination also has economic consequences through increased healthcare costs, loss of agricultural productivity, a deterioration of natural resources (water and soil) leading to increased cost for their rehabilitation, and trade barriers that may prevent the export of food products (Mahimairaja *et al.*, 2005).

There has been an increased concern in recent years over the presence of As in food crops. Rice and vegetables contribute a substantial share to overall As ingestion (Alam *et al.*, 2003; Saha & Ali, 2010; Samal *et al.*, 2011). Vegetables accumulate this element through growing in contaminated soils and/or in soils that are irrigated with As-contaminated water (Baig & Kazi, 2012; Cao & Ma, 2004; Moyano *et al.*, 2009; Rahman *et al.*, 2004). Under conditions of high As exposure, vegetables can continue to grow and accumulate As to a concentration that can exceed food safety standards (Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b; Rahman & Naidu, 2009).

Despite the apparent risk, there is a lack of information on As uptake by vegetables irrigated with As-contaminated water. Most reports on As in vegetables are monitoring surveys where various vegetable species, soil and water samples are collected from affected areas, analysed and the As concentration is reported (Arain *et al.*, 2009; Husaini *et al.*, 2011a; Husaini *et al.*, 2011b; Saha & Ali, 2010). A few monitoring studies have calculated daily As intake through consumption of water and food crops including vegetables (Arain *et al.*, 2009; Roychowdhury *et al.*, 2003). Because of the great variability in As concentration of irrigation water, soil type, vegetable species and cultivars, agricultural practices and climatic factors, a general model for As uptake potential of an individual vegetable species cannot be described. Identifying vegetable species that accumulate high As concentrations in their edible tissues when irrigated with As-contaminated water, is therefore essential to safeguard human health.

1.2 THEME OF THE CURRENT RESEARCH

Some studies have been conducted using hydroponic or soil media with various levels of As to determine uptake potential of vegetables and associated risk (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999a; Miteva, 2002; Rahman & Naidu, 2009; Smith *et al.*, 2009; Tlustos *et al.*, 2002). A central premise of this thesis is that As-irrigated soil studies are different to hydroponic studies and to those where As-contaminated soil is used as a medium for plant growth. This is because:

(i) arsenic in irrigation water is initially available to plants before it is adsorbed onto soil particles,
(ii) arsenic can be directly absorbed by plant leaves during the event of irrigation, and
(iii) an excessive use of irrigation water will develop anaerobic conditions in soil which will result in increased solubility and release of As from As-binding minerals (Marin *et al.*, 1993; Masscheleyn *et al.*, 1991).

Only two studies have investigated the effect of As-contaminated water (as a treatment) for vegetable cultivation in soil (Marconi *et al.*, 2010; Mayorga *et al.*, 2013). Both studies showed that an increase in the As concentration of irrigation water corresponded to an increase in the As concentration of carrot and radish tubers and caused several changes to the radish tubers' internal structure (black spots over the hypocotyl, and changes in the thickness and structure of the outermost cell layer). However, there are two major limitations to these studies. Firstly, there was no risk assessment in these studies for the scenario where the crops were grown. Secondly, these studies were limited to low levels of As in irrigation water (maximum 104 µg L^{-1} for the radish study and 150 µg L^{-1} for the carrot study) in comparison to the high As concentration in water used to irrigate food crops around the world.

The As concentration in irrigation water used around the world for crop cultivation ranges from < 0.001 to 1.014 mg L^{-1} for groundwater (Bhattacharya *et al.*, 2010; Dahal *et al.*, 2008; Saha & Ali, 2010) and from 5.9 to nearly 100 mg L^{-1} for industrial effluent (Husaini *et al.*, 2011a; Tiwari *et al.*, 2011). These industrial effluents, generally untreated, have been used for cultivation of food crops for many years. Therefore, the potential for As uptake by food crops in some scenarios may be many orders of magnitude higher.

The research described in this thesis has been designed to directly address the identified lack of information on As uptake by vegetables as a function of the As concentration in irrigation water and the risk to humans that would be associated with the ingestion of potentially contaminated vegetables.

1.3 RESEARCH HYPOTHESIS

The hypothesis being tested in this work is that vegetables irrigated with water containing As at concentrations found in South Asia, will accumulate the As in their edible tissues to a concentration that will be risky for humans.

1.4 BRIEF DESCRIPTION OF THE OBJECTIVES OF THIS RESEARCH

The research aims were to (i) determine the As uptake behaviour of common vegetables (carrot, radish, spinach and tomato) when irrigated with As-laden water, (ii) calculate risk to humans that is associated with the ingestion of edible parts of these vegetables, (iii) elucidate the factors which promote the As concentration in vegetables tissues, and (iv) propose management strategies for South Asian countries where vegetables are irrigated with As-enriched water.

1.5 THESIS STRUCTURE

The research constituting this thesis is described in three chapters, which include two glasshouse experiments and one laboratory batch study. In these experiments, a Rangitikei silt loam soil was used as a growth substrate. This soil was chosen because similar light textured soils (silt loams, sandy loams, loams) are most often used for vegetables cultivation in South Asian countries, particularly in Pakistan (Jaffri *et al.*, 1976; Khoso, 1994), the author’s country of origin.

The overall structure of the thesis is briefly described in following paragraphs.

Chapter 2 provides a detailed overview of the available information regarding As sources and its dynamics in soil, water and plants. Special consideration is given to studies where vegetables are irrigated with As-contaminated water and/or grown in As-contaminated hydroponic solutions and soils.

Chapter 3 describes the methodology developed during the study to analyse As concentration in plants and soil. The general physical and chemical methods of soil analysis used are also reported in this chapter.

Chapter 4 presents the results of a glasshouse experiment where the As uptake response of four common vegetables as a function of As concentrations in irrigation water and two irrigation techniques was investigated. Carcinogenic and non-carcinogenic risks to humans were calculated for the vegetable species (spinach) which accumulated the most As in its edible parts.

Chapter 5 presents the results of a laboratory batch experiment where four New Zealand soils were assessed for their abilities to adsorb arsenate (As^{V}) and arsenite (As^{III}). The As adsorption behaviour of these soils was modelled by Freundlich and Langmuir isotherms. Soil properties which control As adsorption in selected soils were also explored.

Chapter 6 presents the results of a glasshouse experiment where the response of spinach (the crop that contained the most As in its edible parts in glasshouse experiment 1, Chapter 4) to a range of factors was investigated. These factors were: As concentrations and species, two soil pH levels, and two soil amendments (biochar and cattle manure). A risk assessment using USEPA (United States Environmental Protection Agency), ATSDR (Agency for Toxic Substances and Disease Registry) and drinking water daily intake models was calculated for the factors which promote As concentration in spinach leaves.

Chapter 7 reviews the defined objectives of this study in relation to research conducted in current thesis. The guidelines for vegetables cultivation with As-contaminated water in South Asia and future research needs are also discussed in this chapter.

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CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

This review compiles information regarding As sources and dynamics in soil, water and plants that is relevant to the current research. The focus is on studies where arsenic interacts with vegetables. The purpose of this review is to identify knowledge gaps and future research requirement in this area.

2.2 SOURCES OF ARSENIC

Arsenic enters the environment (soil, water and air) through both natural and anthropogenic activities (Figure 2.1). Natural sources include rocks and minerals, and atmospheric deposition (Mahimairaja *et al.*, 2005; E. Smith *et al.*, 1998). Sedimentary rocks contain higher As concentrations than igneous rocks; the mean As concentration in sedimentary rocks ranges from 1.7 to 400 mg kg^{-1} and in igneous rocks ranges from 1.5 to 3.0 mg kg^{-1} (E. Smith *et al.*, 1998). Parent materials and soils formed from sedimentary rocks may be expected to contain elevated As concentrations. Arsenic is a major constituent of more than 245 minerals, mostly the sulphide-containing ores of Copper (Cu), Nickel (Ni), Lead (Pb), Cobalt (Co), Zinc (Zn), and Gold (Au) (Mahimairaja *et al.*, 2005). Volcanic activity is the next important natural source of As. The total As emission through volcanism is estimated to be 7.0 Giga grams (7.0 x 10^9 grams) per year (Walsh *et al.*, 1979).

Significant anthropogenic sources of As in the environment include industrial, agricultural, mining and smelting activities (Figure 2.1). Examples of industrial usage include wood preservatives, photoelectric devices, glassware, Pb-acid batteries, cosmetics, fireworks, electronics, paints, Cu-based alloys, and leather tanning (Kabata-Pendias, 2011; Mahimairaja *et al.*, 2005; Matschullat, 2000; E. Smith *et al.*, 1998). Examples of agricultural usage are pesticides and herbicides, seed treatment, fertilizers and As-contaminated irrigation water (Bhattacharya *et al.*, 2007; Mahimairaja *et al.*, 2005).

In addition to these sources, As can be redistributed in the environment by various biological agents. Plants, animals and microbes redistribute As through bioaccumulation (e.g. biosorption), biotransformation (e.g. biomethylation), and transfer (e.g. volatilization) reactions (Mahimairaja *et al.*, 2005). Arsenic accumulated in these biological sources may contaminate soil and water bodies and enter into the food chain.

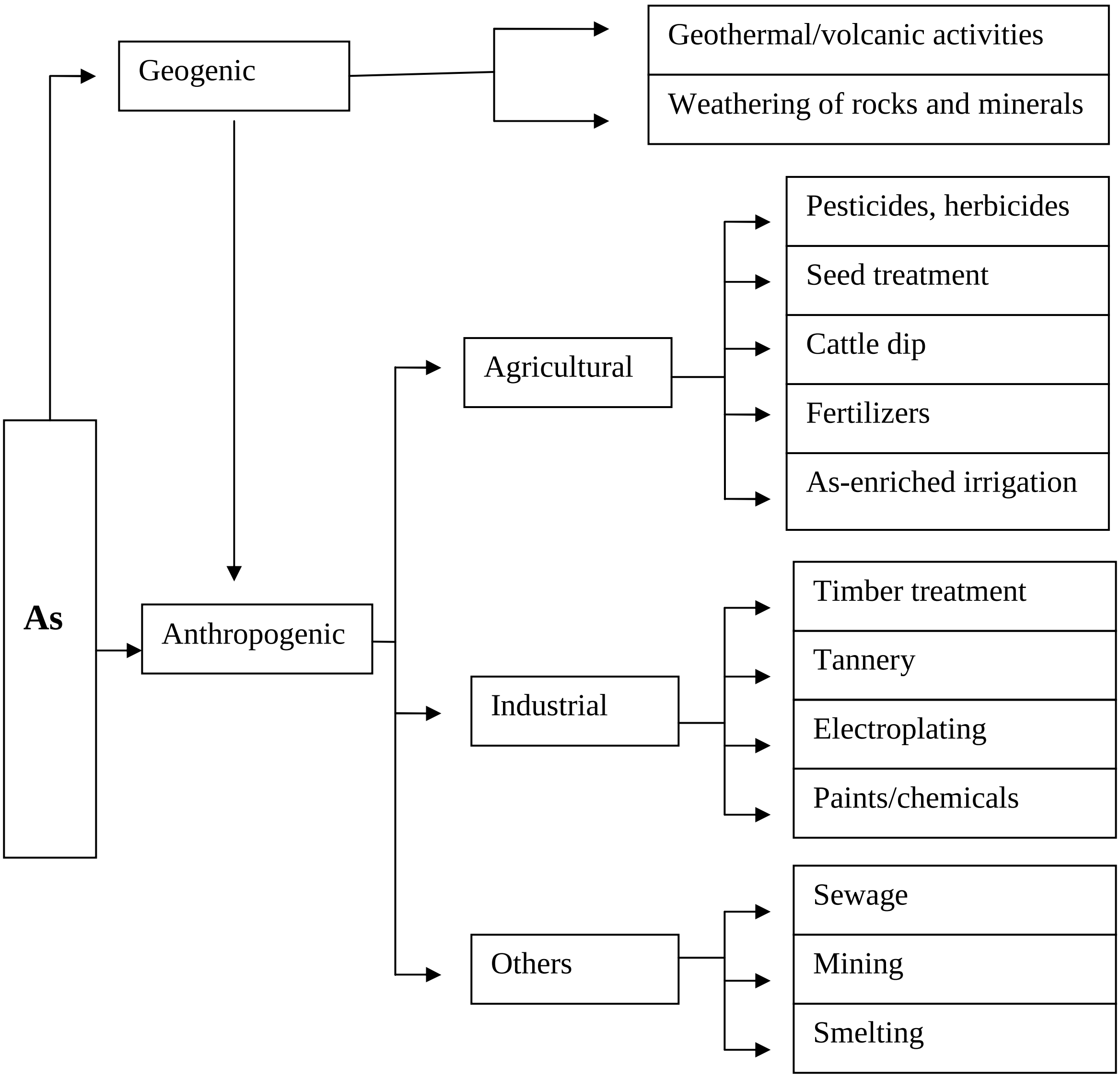

Figure 2.1 Major sources of arsenic in soil and aquatic ecosystems; adopted and modified from Mahimairaja *et al.* (2005)

2.3 ARSENIC IN SOIL

2.3.1 Arsenic concentration in soil

The As concentration in uncontaminated soils is generally less than 10 mg kg^{-1} (Mahimairaja *et al.*, 2005). However, the As concentration in contaminated soils is highly variable and can reach 250,000 mg kg^{-1} depending on the nature and source of contamination (Mahimairaja *et al.*, 2005; E. Smith *et al.*, 1998).

Since the main focus of this review is to describe the effect of As in irrigation water on the soil-plant ecosystem, Table 2.1 presents the As concentration in agricultural soils as a function of As in irrigation water. The selected studies indicate that continuous irrigation with As-enriched water can increase the As level in agricultural soils above the soil limit of 20 mg kg^{-1} (Bhattacharya *et al.*, 2010b; NZWWA, 2003; Pizarro *et al.*, 2003). In Bangladesh, Chile, India, Pakistan and Spain, the As concentration in agricultural soils is two-to-three folds higher than the acceptable critical level. Continuous use of As-contaminated water will lead to further deterioration in soil quality and will make these soils unsuitable for crop cultivation.

2.3.2 Arsenic speciation in soil

Arsenic exists in various oxidation states, and as both organic and inorganic compounds in soils. However, the predominant form of As is one of two inorganic species, arsenate (As^{V}) and arsenite (As^{III}) (Mahimairaja *et al.*, 2005; Masscheleyn *et al.*, 1991; E. Smith *et al.*, 1998). These inorganic species are not stable in soil and remain subjected to various chemical and biological reactions (oxidation-reduction, and methylation) (E. Smith *et al.*, 1998). As a result of these reactions, the valence state of As may change in soil over time (Mahimairaja *et al.*, 2005).

The most prevalent methylated As species in soils are MMA (monomethylarsonic acid) and DMA (Dimethylarsinic acid). These organic As species may change to different methyl forms (for example mono-di-tri methylarsine) and inorganic As species (demethylation process) through the action of soil microorganisms (Mahimairaja *et al.*, 2005; E. Smith *et al.*, 1998). Little is known about the behaviour of organic species in soil and therefore requires further research.

Table 2.1 Arsenic concentration in agricultural soils as a function of As in irrigation water

Country	**Total As concentration in soil (mg kg^{-1})** [a]	**References**
Bangladesh	1.8 to 7.7	Saha and Ali (2010)
Bangladesh	< 0.1 to 42.6	Naidu *et al.* (2009)
Bangladesh	11.8 to 71.5	Panaullah *et al.* (2009)
Bangladesh	14.5 ± 0.1	Rahman *et al.* (2007)
Bangladesh	7.3 to 27.3	Das *et al.* (2004)
Bangladesh	3.1 to 42.5	Meharg and Rahman (2003)
Bangladesh	1.3 to 56.7	Alam and Sattar (2000)
Chile	17.2	Pizarro *et al.* (2003)
Chile	50 to 70	Munoz *et al.* (2002)
India	9.6 ± 0.5	Tiwari *et al.* (2011)
India	3.3 to 9.7	Bhattacharya *et al.* (2010a)
India	10.6 to 38.2	Norra *et al.* (2005)
Nepal	6.1 to 16.7	Dahal *et al.* (2008)
Nigeria	0.3 to 2.4	Agbenin *et al.* (2009)
Pakistan	4.3 to 57.3	Baig and Kazi (2012)
Pakistan	13.5 ± 0.8	Husaini *et al.* (2011a)
Pakistan	19.8 to 21.2	Husaini *et al.* (2011b)
Pakistan	8.7 to 46.2	Arain *et al.* (2009)
Spain	8.0 to 36.0	Moyano *et al.* (2009)

[a] values are range or mean, as described by the selected references; This table can be reviewed in conjunction with Table 2.3 where the As concentration in irrigation water used for cultivation of food crops in most of the described studies is presented.

2.3.3 Biogeochemistry of arsenic in soil

The biogeochemistry of As in soil is controlled by various processes and factors. Two major soil reactions, namely adsorption and redox reactions are discussed in the following paragraphs.

2.3.3.1 Adsorption reaction

Arsenic adsorption or retention in soils determines its persistence, reaction, mobility, transformation and toxicity (Mahimairaja *et al.*, 2005). Adsorption of As in soils and mineral components involves two different reaction mechanisms, namely non-specific and specific adsorption. Non-specific adsorption refers to coulombic attraction between positive charges of adsorbents and negatively charged As anions (Manful *et al.*, 1989; Sadiq, 1997). In contrast, specific adsorption refers to the incorporation of As species as a ligand in the coordinated shell of mineral compounds (Manful *et al.*, 1989; Sadiq, 1997).

2.3.3.2 Factors affecting adsorption reaction

Adsorption of As in soil is controlled by various soil properties and factors including type and amount of oxides/hydroxides and clay minerals, organic matter content, pH, and As competing ions (Frost & Griffin, 1977; Grafe *et al.*, 2001; Manning & Goldberg, 1996b; Pierce & Moore, 1980; Wauchope, 1975).

Effect of mineral constituents

Among the mineral constituents, oxides/hydroxides of Fe, Al and Mn are actively involved in the adsorption of As (Deschamps *et al.*, 2003; Elkhatib *et al.*, 1984; Livesey & Huang, 1981; Smith *et al.*, 1999). Highly oxidic soils can retain an amount of As that is many times higher than that can be held in soils containing low amounts of oxide minerals (Smith *et al.*, 1999). The surface charge of these mineral compounds is pH dependent (Figure 2.2). Under acidic conditions, these mineral compounds have positive surface charge, hence significantly retain As (Sadiq, 1997). However, the positive surface charge varies with the type of metal oxide/hydroxide. The surfaces of Mn, Al and Fe oxides/hydroxides play a limited role in As retention above soil pH 3, 5, and 8 respectively (Sadiq, 1997). In alkaline conditions (mainly between pH 7 and 9), carbonate minerals have positive charge and hence play an important role in As retention (Mahimairaja *et al.*, 2005; Sadiq, 1997).

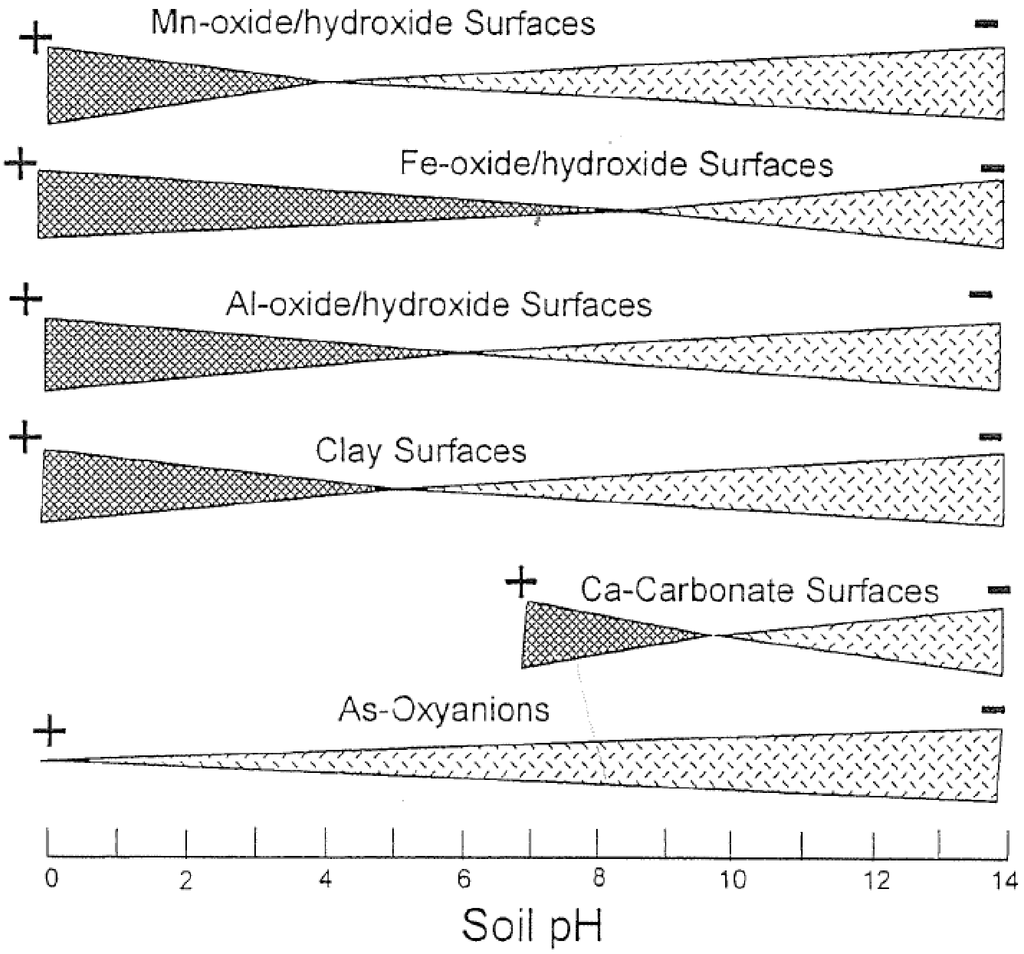

Figure 2.2 Generalized charge distributions on soil colloids, adopted from Sadiq (1997)

Arsenic retention on these mineral compounds also depends on the inorganic As species present (As^V or As^{III}). Arsenic compounds of each of these species are pH dependent and a change in soil pH causes protonation or deprotonation of their neutral or charged compounds (Equation 2.1). At neutral pH, As^V exists as negatively charged $H_2AsO_4^-$ and $HAsO_4^{2-}$, and the negative potential increases as the pH of the medium increases (E. Smith *et al.*, 1998; Zhang & Selim, 2008). In contrast, As^{III} exists as neutral species (H_3AsO_3) below pH 9.2.

Arsenic acid (As^V)

$$H_3AsO_4 + H_2O \Longleftrightarrow H_2AsO_4^- + H_3O^+ \qquad pK_a\ 2.20$$

$$H_2AsO_4^- + H_2O \Longleftrightarrow HAsO_4^{2-} + H_3O^+ \qquad pK_a\ 6.97$$

$$HAsO_4^{2-} + H_2O \Longleftrightarrow AsO_4^{3-} + H_3O^+ \qquad pK_a\ 11.53$$

Arsenous acid (As^{III})

$$H_3AsO_3 + H_2O \Longleftrightarrow H_2AsO_3^- + H_3O^+ \qquad pK_a\ 9.22$$

$$H_2AsO_3^- + H_2O \Longleftrightarrow HAsO_3^{2-} + H_3O^+ \qquad pK_a\ 12.13$$

$$HAsO_3^{2-} + H_2O \Longleftrightarrow AsO_3^{3-} + H_3O^+ \qquad pK_a\ 13.40$$

Equation 2.1 pH dependency of As^V and As^{III} compounds in aqueous solutions; pK_a stands for acid dissociation constant; adopted from E. Smith *et al.* (1998)

An increase in pH increases As^{III} adsorption in soils but decreases As^V adsorption (Smith *et al.*, 1999). The decrease of As^V may be attributed to two interacting factors, (i) increase in negative surface potential on the plane of adsorbents, and (ii) increase in the amount of As^V anions in soil solution because of deprotonation of As^V compounds (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{-2}$; Equation 2.1) (Manful *et al.*, 1989; Smith *et al.*, 1999; Xu *et al.*, 1991). Arsenate and arsenite show a maximum adsorption at a certain pH level and drops thereafter. This critical pH level is variable and depends on the type of adsorbent. For example, As^V maxima was achieved around pH 5 and for As^{III} at pH 7 on alumina, α-Al_2O_3 (Xu *et al.*, 1988, 1991). In the same studies, the As^V adsorption maxima was observed at pH around 6 on hematite. Pierce and Moore (1980) observed the As^{III} adsorption maximum at a pH of around 7 on amorphous iron hydroxide.

Silicate clay minerals also play an important role in As retention in soils (Frost & Griffin, 1977; Manning & Goldberg, 1996a; Wauchope, 1975). Soils having a higher clay content adsorb more As than soils with a low clay content, such as sandy soils (Mahimairaja *et al.*, 2005; Manning & Goldberg, 1997b). The degree of adsorption also varies among various clay minerals. For example, montmorillonite and illite clay minerals adsorb more As (As^V and As^{III}) than kaolinite, possibly because of higher surface area and the interlayer hydroxyl aluminium polymers of the former clay minerals (Frost & Griffin, 1977; Manning & Goldberg, 1997a). In another study, Lin and Puls (2000) found a higher adsorption of As^V by halloysite and chlorite minerals than kaolinite, illite and montmorillonite. These researchers proposed that high As^V adsorption may be because of As^V precipitation leading to the formation of a hydroxyl-arsenate interlayer in halloysite, and the presence of Fe and hydroxide interlayer constituents within chlorite clay minerals. Soils with higher allophanic clays also retain more As than the soils with low allophanic clays; allophanic clays has high surface charge that increases As adsorption (Bolan *et al.*, 2012).

Silicate minerals also differ in their rate of adsorption of the two inorganic As species (As^V and/or As^{III}). In general, As^V is adsorbed in greater quantities than As^{III} by clay minerals, although adsorption is pH dependent (Lin & Puls, 2000). Adsorption of As^{III} increased at pH 7.5 while there was inverse response of As^V adsorption at this pH level (Lin & Puls, 2000). Reported studies describe that As^V adsorption increases at low pH, peaks near pH 5 and then decreases at higher pH for montmorillonite and kaolinite minerals (Frost & Griffin, 1977; Goldberg & Glaubig, 1988). In contrast, As^{III} adsorption continued to rise from pH 4 to 9 on kaolinite, but reached a peak near pH 7 on montmorillonite (Frost & Griffin, 1977).

Arsenic can also be adsorbed onto oxide/hydroxide and clay minerals by specific adsorption (ligand exchange) which can occur even when the surface is uncharged (Bolan *et al.*, 2012; Pierce & Moore, 1982; Sadiq, 1997). Several studies investigating As^V and As^{III} adsorption on mineral compounds (goethite, amorphous Fe and Al oxides) indicate that As ions form inner sphere and/or outer sphere surface complexes on these minerals (Fendorf *et al.*, 1997; Goldberg & Johnston, 2001; Manning *et al.*, 1998; Sun & Doner, 1996). Adsorption-desorption of As ions on these retention sites is affected by many competing ligands for example, hydroxyl, phosphate, molybdate, carbonate, silicic acid, and organic compounds (Appelo *et al.*, 2002; Bolan *et al.*, 2012; Grafe *et al.*, 2001; Manning & Goldberg, 1996b; Quaghebeur *et al.*, 2005; Smith *et al.*, 2002; Waltham & Eick, 2002).

Effect of ions competing with As

Phosphate ions, being an analogue of the $\mathrm{As^{V}}$ ion, compete with As and significantly suppress the adsorption of As onto adsorption sites in soils and mineral compounds (Livesey & Huang, 1981; Manful *et al.*, 1989; Roy *et al.*, 1986). Phosphate can compete with $\mathrm{As^{V}}$ for both non-specific and specific adsorption sites (Qafoku *et al.*, 1999). However, there are some sites which are uniquely specific for arsenate and phosphate (Liu *et al.*, 2001). A few other anions (molybdate, chloride, nitrate, sulphate) also affect the adsorption of $\mathrm{As^{V}}$, but their effect is not profound (Livesey & Huang, 1981; Manful *et al.*, 1989; Manning & Goldberg, 1996a).

Effect of organic matter

The role of organic matter on As adsorption is complex. Some studies have found that organic molecules compete with As for adsorption sites onto mineral compounds and thereby diminish As retention (Grafe *et al.*, 2001; Redman *et al.*, 2002). However, the extent of adsorption depends on the speciation of As, type of competing organic molecules, and pH of the media. Grafe *et al.* (2001) reported that both peat humic and fulvic acids decreased $\mathrm{As^{V}}$ adsorption on goethite while citric acid had no effect. The maximum reduction of $\mathrm{As^{V}}$ adsorption occurred at a pH between 6 and 9 for humic acid and at pH between 3 and 8 for fulvic acid. Further in this study, $\mathrm{As^{III}}$ adsorption was decreased by these organic acids in the order of citric acid > fulvic acid ≈ peat humic acid at a pH between 3 and 8. In another study, Redman *et al.* (2002) reported that organic compounds diminished the extent of $\mathrm{As^{V}}$ and $\mathrm{As^{III}}$ adsorption on hematite surfaces, however, the effect was more profound on $\mathrm{As^{III}}$ than $\mathrm{As^{V}}$. Xu *et al.* (1988) reported that the adsorption of $\mathrm{As^{V}}$ on alumina was greatly affected by fulvic acid at a pH between 3 and 7.5, and fulvic acid concentration of 25 mg $\mathrm{L^{-1}}$. These researchers proposed that the decrease in $\mathrm{As^{V}}$ adsorption may be because of (i) fulvic acid adsorption onto alumina by coulombic attraction leading to the formation of predominantly negative surface charge, and/or (ii) direct competition of fulvic acid with $\mathrm{As^{V}}$ for adsorption sites. In contrary to this discussion, several other studies have described the role of organic matter as an arsenic adsorbent (Cao & Ma, 2004; Cao *et al.*, 2003; Saada *et al.*, 2003). Humic acid amine groups play an important role in the retention of As onto organic matter (Saada *et al.*, 2003). The role of organic matter in As adsorption and mobility appears to be the subject of some degree of conjecture, and is an area that requires further investigation.

2.3.3.3 Redox reactions

Arsenic is a redox-active element and its fate and speciation depends on oxidation-reduction reactions of natural environments. Oxidation and reduction reactions involve transfer of electrons from one compound to another compound. In soil, As is subjected to both abiotic and biotic redox reactions (Jones *et al.*, 2012; Mahimairaja *et al.*, 2005). Among abiotic redox reactions, oxides of Mn (IV) and Fe (III) are effective oxidants and can convert As^{III} to the less toxic As^{V} species (Adriano, 2001; Oscarson *et al.*, 1981; Oscarson *et al.*, 1983a; Oscarson *et al.*, 1983b).

Various microorganisms (e.g. bacteria, fungi and algae) also play an important role in redox reactions involving the oxidation of As^{III} to As^{V} and/or reduction of As^{V} to As^{III} in contaminated soils (Jones *et al.*, 2012; Mahimairaja *et al.*, 2005). Recently, an As^{III}-oxidizing bacterial strain, MM-1 has been isolated which showed an ability to oxidize 500 µM of As^{III} to As^{V} within 3 hours of incubation (Bahar *et al.*, 2012).

2.4 ARSENIC CONCENTRATION IN WATER

Arsenic is widely distributed in waters because of both natural and human activities, although the chief source of As in water is the natural weathering of rocks and minerals. Arsenic occurrence in natural waters is associated with sedimentary rocks of marine origin, weathered volcanic rocks, fossil fuels, geothermal areas, mineral deposits, mining wastes, agricultural use and irrigation practices (Korte & Fernando, 1991). The As concentration in water is highly variable among various countries and water sources, ranging from 0.003 to 13,900 µg L^{-1} (Mahimairaja *et al.*, 2005).

Arsenic contamination of drinking water is the most common source of As poisoning in humans in many parts of the world. Human health has been affected by As in drinking water in Argentina, Bangladesh, Cambodia, Chile, China, India and Nepal (Guo *et al.*, 2001; Hopenhayn-Rich *et al.*, 1998; Mazumder *et al.*, 1998; Phan *et al.*, 2010; Shrestha *et al.*, 2003; A. H. Smith *et al.*, 1998; Tondel *et al.*, 1999). The acceptable concentration of As in drinking water set by the WHO is 10 µg L^{-1} whilst some countries (Bangladesh, China, Nepal, Taiwan, and Vietnam) follow a national guideline of 50 µg L^{-1} (Berg *et al.*, 2001; Chen *et al.*, 1995; Guo *et al.*, 2001; Shrestha *et al.*, 2003; Smith *et al.*, 2000). Arsenic concentration in drinking water has a wide range in various countries, with a maximum reported value of 3700 µg L^{-1} (Table 2.2).

Table 2.2 Arsenic concentration in drinking water in selected studies around the world

Country	**As concentration in water (μg L^{-1})**	**Water source**	**References**
Bangladesh	50 to 830	Hand tube well	Ali and Tarafdar (2003)
Bangladesh	< 0.7 to 640	Tube well	Frisbie *et al.* (2002)
Bangladesh	< 1 to 535	Tube well	Watanabe *et al.* (2001)
Bangladesh	10 to 2040	Well	Tondel *et al.* (1999)
Cambodia	0.1 to 1841	Tube well	Phan *et al.* (2010)
Chile	10 to 870	ND	A. H. Smith *et al.* (1998)
Chile	170 to 600	River	HopenhaynRich *et al.* (1996)
China	non detectable to 1354	Well	Guo *et al.* (2001)
India	up to 3700	Tube well	Mandal *et al.* (1996)
India	3 to 560	ND	Mahata *et al.* (2003)
India	non detectable to 3400	Tube well	Mazumder *et al.* (1998)
India	0.5 to 136	Tube well	Nag *et al.* (1996)
India	non detectable to 2000	Tube well	Mazumder *et al.* (1988)
Japan	10 to 293	Well	Kondo *et al.* (1999)
Mexico	21 to 1070	Well	Gomez-Arroyo *et al.* (1997)
Nepal	up to 2620	Tube well	Shrestha *et al.* (2003)
New Zealand	3 to 121	River, Waikato	Robinson *et al.* (1995)
Taiwan	< 0.7 to 897	Well	Chen *et al.* (1995)
Vietnam	1 to 3050	Tube well	Berg *et al.* (2001)

ND: Not described by researchers

Arsenic-enriched water (groundwater and wastewater) is used for irrigation in many countries. The cultivation of food crops using As-contaminated water and the subsequent transfer of As to crops which can be ingested by humans and animals intensifies the risk of As poisoning. Table 2.3 summarises the As concentration in irrigation waters worldwide. The concentration of As in irrigation water varies as a function of the source of water; the As concentration in groundwater samples used for irrigation has been reported as high as 1014 µg L^{-1}, while the As concentration in industrial effluent used for irrigation has been reported near to 100 mg L^{-1}. This As-enriched, untreated industrial effluent has been used for many years for food crops cultivation in Pakistan, and as a response high As concentration is reflected in food crop tissues (Husaini *et al.*, 2011a). Arsenic exists mainly in the form of As^V and/or As^{III} in irrigation water (Baig & Kazi, 2012; Norra *et al.*, 2005).

2.5 ARSENIC IN PLANTS

This section describes the interaction of As with plants, with emphasis on As phytotoxicity, plant uptake and factors controlling these processes. Some of the factors which control toxicity and uptake are similar and linked; however, they are discussed separately here to elaborate and to provide relevant examples.

2.5.1 Arsenic toxicity to terrestrial plants

Plants are exposed to As when grown in contaminated soils, treated with As containing agro-chemicals and/or irrigated with As-contaminated water. The effect of As toxicity in plants is variable, ranging from a negligible effect to either a significantly positive or negative impact on their growth and yield (Carbonell-Barrachina *et al.*, 1998; Marin *et al.*, 1992; Sheppard, 1992; Tu & Ma, 2002).

Table 2.3 Arsenic concentration in irrigation water used for cultivation of food crops around the world

Country	As concentration in irrigation water ($\mu g\ L^{-1}$) [a]	References
Bangladesh	< 1.0 to 267	Saha and Ali (2010)
Bangladesh	> 100	Naidu *et al.* (2009)
Bangladesh	100 to 130	Panaullah *et al.* (2009)
Bangladesh	70	Rahman *et al.* (2007)
Bangladesh	70 to 420	Das *et al.* (2004)
Bangladesh	Up to 500	Meharg and Rahman (2003)
Bangladesh	10 to 71	Alam and Sattar (2000)
Chile	70 to 280	Munoz *et al.* (2002)
India	5.9 ± 0.2 mg L^{-1} [b]	Tiwari *et al.* (2011)
India	180 to 530	Bhattacharya *et al.* (2010a)
India	519 to 782	Norra *et al.* (2005)
Italy	19 to 104 [c]	Marconi *et al.* (2010)
Nepal	< 5 to 1014	Dahal *et al.* (2008)
Pakistan	5.4 to 8.2	Baig and Kazi (2012)
Pakistan	near to 100 mg L^{-1} [b]	Husaini *et al.* (2011a)
Pakistan	35 to 157	Arain *et al.* (2009)
Spain	20 to 150 [c]	Mayorga *et al.* (2013)
Spain	38 to 136	Moyano *et al.* (2009)

[a] Source of water is groundwater unless mentioned; [b] source of water is industrial effluent; [c] experiments where As-enriched water was used as treatment

The phytotoxic symptoms exhibited by various crops include delayed germination, depressed tillering, chlorosis, necrosis, wilting, stunted growth, biomass reduction and reduced yield, and death under extreme conditions (Carbonell-Barrachina *et al.*, 1997; Jiang & Singh, 1994; Lambkin & Alloway, 2003; Rahman & Naidu, 2009). However, there is a known competition between As (As^V) and P for uptake in plants, and therefore it is difficult to differentiate between As toxicity and P deficiency symptoms (Lambkin & Alloway, 2003).

Plants adopt various strategies to tolerate As. Proposed strategies include (i) avoidance i.e. limited uptake by roots and/or limited transport to shoots or grains, (ii) internal detoxification (subcellular compartmentalization of As or through binding of As to cell walls), and (iii) biochemical tolerance (specialized metabolic pathways and enzymatic adaptations) (Carbonell-Barrachina *et al.,* 1997; Rahman & Naidu, 2009). Plants may adopt either a singular or integrated approach of these mechanisms to tolerate As toxicity (Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b).

2.5.2 Factors affecting As toxicity to plants

Arsenic toxicity to plants depends on various As-plant-growth medium factors.

Effect of As concentration

An increase in As concentration intensifies the As toxicity to plants. Rahman and Naidu (2009) reported that by increasing the level of As^V and As^{III} in nutrient solution and soil, the fresh weight and yield of amaranth and silverbeet was decreased. Similarly, Carbonell-Barrachina *et al.* (1997) reported reduced dry matter production (roots, stem, leaf and fruit) in tomato and bean plants by increasing the As^{III} concentration in hydroponic condition.

The critical toxicity concentration level for As varies among crop species under hydroponic and soil conditions. For example, a significant decrease in plant dry biomass relative to the control in marsh species (*Spartina alterniflora* and *Spartina patens)* was observed at an As level of 2.0 mg L^{-1}, regardless of the As form present in hydroponic culture (Carbonell-Barrachina *et al.,* 1998; Carbonell *et al.*, 1998). In soil, severe yield reduction was observed in ryegrass and barley at 50 and 250 mg As kg^{-1} application (Jiang & Singh, 1994). Miteva (2002) observed a decrease in growth of vegetative and root tissues in tomato plants at 50 and

100 mg As kg^{-1}. Woolson (1973) reported that As^{V} at a concentration of 500 mg kg^{-1} caused the death of vegetables (green beans, lima beans, spinach, cabbage, tomato, and radish).

An increasing concentration of As in growth medium may also be phytotoxic to As hyper-accumulator plant species (e.g. *Pteris vittata* L.). Addition of 500 mg As kg^{-1} reduced frond and root biomass of *Pteris vittata* by 64% and 70% respectively in comparison to control plants (Tu & Ma, 2002). These researchers observed the death of plants at a soil concentration of 1000 mg inorganic As kg^{-1} (as K_2HAsO_4) and at 50 mg organic As kg^{-1} (as sodium dimethylarsonate). This discussion highlights the toxic effect of As to all plants at some critical level of concentration in the growth medium. Furthermore, this discussion highlights the lack of information on the As toxicity threshold concentration for various crop species in soil and hydroponic conditions.

Effect of As forms

The speciation of As in a growth media profoundly affects the phytotoxicity of As (Carbonell-Barrachina *et al.*, 1998; Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b). Among the inorganic species, As^{III} is more phytotoxic than As^{V}. Rahman and Naidu (2009) found As^{III} five times more toxic to amaranth and silverbeet plants than As^{V}. Similarly, Jiang and Singh (1994) reported that As^{III} generally caused more yield reduction than As^{V} in ryegrass and barley crops. VandenBroeck *et al.* (1997) reported As^{III} to be more phytotoxic to mung bean than As^{V} under hydroponic conditions, with a lethal concentration of As^{III} defined as less than 10 µM. Reduced dry matter production and death of bean plants with As^{III} application has also been reported (Carbonell-Barrachina *et al.*, 1997).

Interesting results have been observed when these inorganic arsenicals were compared with organic arsenicals for various crop species. Marin *et al.* (1992) reported that As^{III} and MMA were phytotoxic; As^{V} had no effect; while DMA increased total dry matter production in rice. In other studies, MMA and/or DMA were found to be more phytotoxic to various plant species (tomato, radish, turnip, and marsh plant species) than As^{III} and As^{V} (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1998; Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b). Sheppard (1992) proposed that organic sources are more toxic than inorganic sources. However, the results in many cases are conflicting, and the key point we can derive from these findings is that the toxicity of a particular As species varies with crop species.

Crop species and cultivars

The literature provides evidence that the phytotoxicity of As varies among plant species and cultivars. Carbonell-Barrachina *et al.* (1998) observed a significantly lower biomass production in *Spartina patens* than *Spartina alterniflora* under similar growth conditions (As concentrations and species). Similarly, bean plants were found more sensitive to As (as As^{III}) toxicity than tomato plants (Carbonell-Barrachina *et al.*, 1997). Soybeans are reported more sensitive to As than cotton (Deuel & Swoboda, 1972). Woolson (1973) calculated the amount of available As (As^{V}) causing a 50% growth reduction in selected vegetables. The crop sensitivity to available As follows the order: green beans > lima beans ≈ spinach > radish > tomato > cabbage. Walsh *et al.* (1977) described the tolerance of several crops and categorized them as: highly tolerant (potatoes, cabbage, tomatoes, carrots, tobacco, rye, Sudan grass and grapes), moderately tolerant (strawberries, corn, beets and squash), and low tolerant (onion, cucumbers and legumes).

The effect of As toxicity is variable among plant cultivars. For example, in a comparison of two rice varieties at the same As concentration, the variety Mercury yielded a significantly lower root dry weight than Lemont (Marin *et al.*, 1992). Similarly, in a comparison of tomato varieties, Marmande yielded a significantly lower total dry biomass (shoot and root) than Muchamiel (Burlo *et al.*, 1999).

Effect of soil type

The toxic effect of As on plants could be expected to vary under various soil types. Toxicity is generally higher in sand than in loam and clayey soils due to a lower content of chemical moieties (e.g. Fe and Al oxides, clay, and organic matter) that can adsorb the contaminant in sandy soils (Jiang & Singh, 1994; Sheppard, 1992). Woolson (1973) reported a higher toxicity of As to vegetable crops (green beans, lima beans, spinach, cabbage, tomato, and radish) in Lakeland loamy sand than other soil types (Hagerstown silty clay loam and Christiana clay loam).

Effect of competing ions

The effect of various competing ions (particularly phosphate) in aggravating As toxicity should not be overlooked. Phosphate has either an antagonistic or synergistic effect on As, and can thereby influence toxicity (Mahimairaja *et al.*, 2005). Phosphate application to soils can increase As toxicity by displacing the adsorbed As ions at adsorption sites and thereby increase its bioavailability (Davenport & Peryea, 1991). However, this increased phytotoxicity of As by P addition depends on various factors including As and P concentration and their ratio in soil solution. Wang *et al.* (2002) observed phytotoxicity symptoms in *Pteris vittata* when P supply was low (20 µM) and As^V supply was high (416 and 2080 µM). Woolson (1973) observed more toxic effects of As on vegetables growth in Lakeland loamy sand than Christiana clay loam soil. These researchers attributed this effect to the available P/As ratio of the Christiana soil which was 2.5 to 4.4 times greater than Lakeland soil. The background P concentration of the Christiana and Lakeland soil was 59.4 and 10.3 mg kg^{-1} respectively.

Indirect toxic effect of As

The toxicity of As to plants is aggravated by its indirect effect on the deficiency of essential macro and micro nutrients in soil. Lambkin and Alloway (2003) found that as the concentration of As increased in soil, plant yield decreased and the barley plants showed symptoms of As toxicity and P deficiency. A reduction or increase in P concentration in radish, turnip and tomato plants by application of arsenicals has been reported (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b). Carbonell *et al.* (1998) reported that both the concentration and form of As (As^{III}, As^V, MMA, and DMA) affected macro and micro nutrients concentration in *Spartina alterniflora*. These researchers attributed the high phytotoxicity of MMA to a reduction in macro (P, K, Ca and Mg) and micro (B, Cu and Fe) nutrients in plant tissues. Addition of MMA to soil caused a reduction of the Zn and Cu concentration in rice plant tissues in another study (Marin *et al.*, 1993). A possible mechanism of this effect was not described. The addition of As to soils may increase the pH of soil solution by release of OH^- from soil surfaces by ligand exchange (Lambkin & Alloway, 2003) which may alter rhizosphere chemistry.

Positive effects of As

In contrast to the above discussion, As has been found to stimulate plant growth at low concentrations. Rahman and Naidu (2009) observed an increase in yield of amaranth and silverbeet at a concentration of 5 mg As^V L^{-1} under hydroponic conditions. A similar yield increase for silverbeet was also observed at a concentration of 25 mg kg^{-1} under soil conditions in the same study. Similarly, total dry biomass of *Spartina alterniflora* and

Spartina patens was increased in a hydroponic experiment when As^{V} was applied at the rate of 0.2 and 0.8 mg L^{-1} and As^{III} at the rate of 0.2 mg L^{-1} (Carbonell-Barrachina *et al.*, 1998). An addition of 50 and 100 mg As kg^{-1} to a sandy soil significantly increased the aboveground biomass (frond) by 107% and 64% respectively relative to the control for *Pteris vittata* (Tu & Ma, 2002). Miteva (2002) observed an increase in the height and weight of stem and roots of tomato plants when grown in soil supplemented with a low As concentrations (15 and 25 mg As^{III} kg^{-1}). The possible reason for this increase in growth and yield has been linked to P nutrition. In plants, arsenate and phosphate are taken up by the same uptake system; however, the uptake carriers have much greater affinity for phosphate than arsenate (Meharg & Macnair, 1990). Carbonell-Barrachina *et al.* (1999a) suggested that although As^{V} is taken up by the same uptake system, As^{V} is unable to carry out P's role in energy transfer, therefore the plants reacts as if there is a P deficiency. As a result, the plants increase their P uptake which ultimately increase plant growth and yield parameters.

2.5.3 Arsenic accumulation in vegetables

Arsenic is accumulated by vegetables when they are grown in As-contaminated soil and/or irrigated with As-contaminated water (Husaini *et al.*, 2011c; Moyano *et al.*, 2009; Rahman *et al.*, 2004; Warren *et al.*, 2003). The scenario of As-contamination of vegetables has led to many hydroponic and soil studies that have been conducted to understand the As uptake potential of various vegetable species and the subsequent risk that might be expected for humans. Many researchers refer to the WHO recommended maximum permissible As concentration (MPC) of 2.0 mg kg^{-1} (fresh weight) or Australian MPC level of 1.0 mg kg^{-1} (fresh weight) to evaluate the risk from contaminated vegetables (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999a; Rahman & Naidu, 2009). When expressed on a dry weight basis, this value will depend on the water content of vegetables. For example, a fresh weight value of 1 mg kg^{-1} will be equivalent to 10 mg kg^{-1} dry weight if the water content of vegetables is 90% or 20 mg kg^{-1} if the water content is 95% (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999a). Many hydroponic and soil studies reveal that the As concentration in the edible parts of most vegetable species is lower than the MPC levels (Table 2.4). However, certain vegetable species can accumulate As in their edible tissues to a concentration that exceeds the critical level. These vegetables are amaranth, radish, silverbeet, spinach, tomato and turnip (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b; Rahman & Naidu, 2009; Woolson, 1973).

Table 2.4 Total As concentrations (µg g^{-1}) in edible parts of various vegetable species under different growth conditions

Plant	**Plant part**	**As in plant (µg g^{-1}) [a]**	**As in growing media**	**Growing medium**	**Purpose of Study (to determine)**	**Country**	**Reference**
Amaranth	Shoot	0.002 to 0.168 (fresh weight)	Soil: 0.34 to 2.37 mg kg^{-1}	Soil (monitoring survey)	risk assessment	Nigeria	Agbenin *et al.* (2009)
Amaranth (Red)	Edible part (stem & leaf)	0.31 to 1.24	Irrigation water: 1.0 to 267 µg L^{-1} Soil: 2.17 to 6.59 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	Bangladesh	Saha and Ali (2010)
Amaranth (Stem)	Edible part (stem & leaf)	0.33 to 1.14	Irrigation water: 1.1 to 166 µg L^{-1} Soil: 2.16 to 5.52 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	Bangladesh	Saha and Ali (2010)
Asparagus	Stem	0.081 to 0.132 (fresh weight)	Irrigation water: 70 to 280 µg L^{-1} Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Beans	Fruit	0.022 ± 0.003	Irrigation water: 70 to 280 µg L^{-1} Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Beans	Fruit	3.3 to 4.4	2 and 5 mg L^{-1} (As^{III})	Hydroponic	As uptake and distribution in tissues	Spain	Carbonell-Barrachina *et al.* (1997)
Beans	Fruit	0.18 to 1.72	23.3 to 408 mg kg^{-1}	Mixture of soil and mine waste (pot experiment)	Heavy metal(loid)s concentration in vegetables	USA	Cobb *et al.* (2000)

[a] concentration is in dry weight unless mentioned

Table 2.4 continued

Plant	**Plant part**	**As in plant (µg g^{-1})** [a]	**As in growing media**	**Growing medium**	**Purpose of Study (to determine)**	**Country**	**Reference**
Beetroot	Root	0.129 to 0.268 (fresh weight)	Irrigation water: 70 to 280 µg L^{-1} Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Cabbage	Leaves	2.73± 0.34	13.55 ± 0.8 mg kg^{-1}	Soil (monitoring survey)	As concentration in crops and soils to assess toxicity level	Pakistan	Husaini *et al.* (2011a)
Cabbage	Edible part	0.20 to 0.95	Irrigation water: < 1.0 to 180 µg L^{-1} Soil: 1.81 to 5.11 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	Bangladesh	Saha and Ali (2010)
Cabbage	Leaves	0.016 ± 0.002 (fresh weight)	Irrigation water: 70 to 280 µg L^{-1} Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Cabbage	Leaves	6.5	Irrigation water: 5.92 ± 0.19 mg L^{-1} Soil: 9.62 ± 0.48 mg kg^{-1}	Soil (monitoring survey)	Metal(loid)s concentration in crops, water and soil	India	Tiwari *et al.* (2011)
Cauliflower	Edible part	0.01 to 0.78	Irrigation water: 1.6 to 267 µg L^{-1} Soil: 2.91 to 7.72 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	Bangladesh	Saha and Ali (2010)
Cauliflower	Inflorescence	0.014 to 0.060 (fresh weight)	Irrigation water: 70 to 280 µg L^{-1} Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)

[a] concentration is in dry weight unless mentioned

Table 2.4 continued

Plant	**Plant part**	**As in plant (μg g^{-1}) ^{a}**	**As in growing media**	**Growing medium**	**Purpose of Study (to determine)**	**Country**	**Reference**
Carrot	Edible root	0.73 ± 0.01	Soil: 13.5 ± 0.8 mg kg^{-1}	Soil (monitoring survey)	As concentration in crops and soils to assess toxicity level	Pakistan	Husaini *et al.* (2011a)
Carrot	Root	0.135 ± 0.014	Irrigation water: 35 to 157 μg L^{-1}; Soil: 8.7 to 46.2 mg kg^{-1}	Soil (monitoring survey)	As concentration in water, crops and soils and risk assessment	Pakistan	Arain *et al.* (2009)
Carrot	Peel Peeled root	0.025 to 0.924 0.034 to 0.135	Soil: 1.97 to 110 mg kg^{-1}	Soil (pot experiment)	As concentration in carrot from polluted soil	USA	Zandstra and De Kryger (2007)
Carrot	Carrots (without peel) Carrot peels Carrots (with peel)	0.03 to 0.283 0.165 to 2.950 0.049 to 0.608	Soil: < 3 to 50 mg kg^{-1}	Soil (pot experiment)	As uptake and risk assessment	USA	Rahman *et al.* (2004)
Carrot	Edible root (without skin)	0.078 to 0.138 (fresh weight)	Irrigation water: 70 to 280 μg L^{-1} Soil: 50 to70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Carrot	Core Peel Whole	< 0.098 to 0.387 < 0.098 to 1.04 1.85 ± 0.09	Soil: 6.5 to 338 (As^{V} and As^{III})	Soil (field experiment)	Uptake, species distribution and risk assessment	Denmark	Helgesen and Larsen (1998)
Celery	Edible part	0.006 to 0.338 (fresh weight)	Soil: 1.29 to 25.28 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	China	Huang *et al.* (2006)

[a] concentration is in dry weight unless mentioned

Table 2.4 continued

Plant	**Plant part**	**As in plant ($\mu g\ g^{-1}$)** [a]	**As in growing media**	**Growing medium**	**Purpose of Study (to determine)**	**Country**	**Reference**
Chard	Leaves	3.13 ± 0.77	2 mg L^{-1} (As^V)	Hydroponic	As uptake and species distribution in tissues	Australia	Smith *et al.* (2009)
Chard	Leaves	0.195 to 0.266	Irrigation water: 70 to 280 $\mu g\ L^{-1}$ Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Chive	Shoots	BDL to 4.120	Soil: 31.4 to 184 mg kg^{-1}	Soil (Pot experiment)	As uptake and risk assessment	Hungary	Sipter *et al.* (2009)
Garlic	Edible root (without skin)	0.030 to 0.368 (fresh weight)	Irrigation water: 70 to 280 $\mu g\ L^{-1}$ Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Garlic	Edible part	0.0035 to 0.1595 (fresh weight)	Soil: 1.29 to 25.28 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	China	Huang *et al.* (2006)
Lettuce	Shoot	0.002 to 0.38 (fresh weight)	Soil: 0.34 to 2.37 mg kg^{-1}	Soil (monitoring survey)	risk assessment	Nigeria	Agbenin *et al.* (2009)
Lettuce	Leaves	3.18 ± 1.03	2 mg L^{-1} (As^V)	Hydroponic	uptake and species distribution in tissues	Australia	Smith *et al.* (2009)
Lettuce	Leaves	< 0.2 to 0.7	Soil: 0.4 to 35.6 mg kg^{-1}	Soil (pot experiment)	uptake and risk assessment	New Zealand	Gaw *et al.* (2008)

[a] concentration is in dry weight unless mentioned; BDL= Below detection limit

Table 2.4 continued

Plant	**Plant part**	**As in plant ($\mu g\ g^{-1}$) [a]**	**As in growing media**	**Growing medium**	**Purpose of Study (to determine)**	**Country**	**Reference**
Lettuce	Leaves	0.002 to 0.105 (fresh weight)	Soil: 1.29 to 25.28 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	China	Huang *et al.* (2006)
Lettuce	Leaves	0.068 to 0.448 (fresh weight)	Irrigation water: 70 to 280 $\mu g\ L^{-1}$ Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Lettuce	Leaves	5.5 to 34.9	23.3 to 408 mg kg^{-1}	Mixture of soil and mine waste (pot experiment)	Heavy metal(loid)s concentration in vegetables	USA	Cobb *et al.* (2000)
Onion	Edible part	0.005 to 0.157 (fresh weight)	Soil: 1.29 to 25.28 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	China	Huang *et al.* (2006)
Potato	Edible root	0.36 ± 0.02	Soil: 13.55 ± 0.8 mg kg^{-1}	Soil (monitoring survey)	As concentration in crops and soils to assess toxicity level	Pakistan	Husaini *et al.* (2011a)
Potato	Edible part	0.02 to 1.15	Irrigation water: < 1.0 to 243 $\mu g\ L^{-1}$ Soil: 2.55 to 5.82 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	Bangladesh	Saha and Ali (2010)
Potato	Edible part	0.021 to 0.133 (fresh weight)	Irrigation water: 70 to 280 $\mu g\ L^{-1}$ Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)

[a] concentration is in dry weight unless mentioned

Table 2.4 continued

Plant	Plant part	As in plant ($\mu g\ g^{-1}$) [a]	As in growing media	Growing medium	Purpose of Study (to determine)	Country	Reference
Radish	Edible root	0.43 ± 0.05	Soil: 13.55 ± 0.8 mg kg^{-1}	Soil (monitoring survey)	As concentration in crops and soils to assess toxicity level	Pakistan	Husaini *et al.* (2011a)
Radish	Tuber	0.43 to 1.95	Irrigation water: 19 to 104 µg L^{-1}	Soil (lysimeter experiment)	concentration and morphological changes in plants	Italy	Marconi *et al.* (2010)
Radish	Roots	35.5 ± 7.5	2 mg L^{-1} (As^{V})	Hydroponic	As uptake and species distribution in tissues	Australia	Smith *et al.* (2009)
Radish	Edible root	0.0037 to 0.0156 (fresh weight)	Soil: 1.29 to 25.28 mg kg^{-1}	Soil (monitoring survey)	uptake and risk assessment	China	Huang *et al.* (2006)
Radish	Roots	0.25 to 6.88	As spiked: 20 mg kg^{-1} as DMA (background soil As: 4.6 to 21.4 mg kg^{-1})	Soil (pot experiment)	As availability and distribution in radish	Czech Republic	Tlustos *et al.* (2004)
Radish	Tuber (skin) Tuber (peeled)	21.3 8.4	Soil: 748 mg kg^{-1}	Soil (field experiment)	As uptake and effect of Fe oxides amendment	UK	Warren *et al.* (2003)
Radish	Roots	0.25 to 5.50	As spiked: 20 mg kg^{-1} as As^{V}, As^{III} or DMA (background soil As: 15.1 ± 2.5 mg kg^{-1})	Soil (pot experiment)	uptake and distribution of As species in radish tissues	Czech Republic	Tlustos *et al.* (2002)
Radish	Roots	0.6 to 11.9	23.3 to 408 mg kg^{-1}	Mixture of soil and mine waste (pot experiment)	Heavy metal(loid)s concentration in vegetables	USA	Cobb *et al.* (2000)

[a] concentration is in dry weight unless mentioned

Table 2.4 continued

Plant	Plant part	As in plant ($\mu g\ g^{-1}$) [a]	As in growing media	Growing medium	Purpose of Study (to determine)	Country	Reference
Radish	Root skin Inner root	0.67 to 69.10 0.51 to 38.54	1-2-5 mg L^{-1} as As^{V}, As^{III}, MMA or DMA	Hydroponic	As concentration and distribution in plant tissues and risk assessment	Spain	Carbonell-Barrachina *et al.* (1999a)
Sorrel	Shoots	0.049 to 4.860	Soil: 31.4 to 184 mg kg^{-1}	Soil (pot experiment)	As uptake and risk assessment	Hungary	Sipter *et al.* (2009)
Spinach	Leaves	0.52 ± 0.02	Soil: 13.5 ± 0.8 mg kg^{-1}	Soil (monitoring survey)	As concentration in crops and soils to assess toxicity level	Pakistan	Husaini *et al.* (2011a)
Spinach	Leaves	1.48	Irrigation water: 5.92 ± 0.19 mg L^{-1} Soil: 9.62 ± 0.48 mg kg^{-1}	Soil (monitoring survey)	Metal(loid)s concentration in crops, water and soil	India	Tiwari *et al.* (2011)
Spinach	Leaves	0.425 to 6.744	As^{V} spiked at the rate of 25, 50, or 75 mg kg^{-1} to control soil (18 ± 1 mg As kg^{-1})	Soil (pot experiment)	As effect on amino acid and enzymes activities	Czech Republic	Pavlik *et al.* (2010)
Spinach	Leaves	0.21 to 0.90	Irrigation water: 35 to 157 µg L^{-1}; Soil: 8.7 to 46.2 mg kg^{-1}	Soil (monitoring survey)	As concentration in water, crops and soils and risk assessment	Pakistan	Arain *et al.* (2009)
Spinach	Leaves	0.065 to 1.475	Soil: < 3 to 50 mg kg^{-1}	Soil (pot experiment)	As uptake and risk assessment	USA	Rahman *et al.* (2004)

[a] concentration is in dry weight unless mentioned

Table 2.4 continued

Plant	Plant part	As in plant ($\mu g\ g^{-1}$) [a]	As in growing media	Growing medium	Purpose of Study (to determine)	Country	Reference
Spinach	Leaves	0.121 to 0.604 (fresh weight)	Irrigation water: 70 to 280 $\mu g\ L^{-1}$ Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Tomato	Fruit	0.27 to 1.70	Irrigation water: < 1.0 to 166 $\mu g\ L^{-1}$ Soil: 2.21 to 5.63 mg kg^{-1}	Soil (monitoring survey)	Uptake and risk assessment	Bangladesh	Saha and Ali (2010)
Tomato	Fruit	0.008 to 0.037 (fresh weight)	Irrigation water: 70 to 280 $\mu g\ L^{-1}$ Soil: 50 to 70 mg kg^{-1}	Soil (monitoring survey	Total and inorganic As concentration in plants and risk assessment	Chile	Munoz *et al.* (2002)
Tomato	Fruit	1.2 to 26.3	1-2-5 mg L^{-1} as As^{V}, As^{III}, MMA or DMA	Hydroponic	As concentration and distribution in plant tissues and risk assessment	Spain	Burlo *et al.* (1999)
Tomato	Fruit	0.12 to 0.41	2-5-10 mg L^{-1} (As^{III})	Hydroponic	As uptake and distribution in tissues	Spain	Carbonell-Barrachina *et al.* (1997)
Turnip	Edible root	0.22 ± 0.02	Soil: 13.5 ± 0.8 mg kg^{-1}	Soil (monitoring survey)	As concentration in crops and soils to assess toxicity level	Pakistan	Husaini *et al.* (2011a)
Turnip	Root skin Inner root	24.6 to 116 8.3 to 42.0	1-2-5 mg L^{-1} as As^{V}, As^{III}, MMA or DMA	Hydroponic	As concentration and distribution in plant tissues and risk assessment	Spain	Carbonell-Barrachina *et al.* (1999b)

[a] concentration is in dry weight unless mentioned

2.5.4 Risk assessment of arsenic

Assessing risk by comparing the concentration of As in food to a guideline value (as has been the case for most As studies) is not a correct approach to assess the risk. This is because the risk to an individual human, in addition to As concentration in plant tissues, depends on factors such as food intake, exposure time, exposure frequency, exposure duration, and target population and their body weight (USEPA, 1997). A second major limitation of the WHO and Australian MPC levels is that these guideline values are set for total As and therefore do not consider the differences in toxicity between organic and inorganic As species (Heikens, 2006). The As concentration in vegetables is majorly inorganic in nature (Smith *et al.*, 2009; Williams *et al.*, 2006).

Considering these shortfalls, the Chinese food safety standard for inorganic As (0.05 $\mu g\ g^{-1}$ fresh weight for vegetables) and the United States Environmental Protection Agency (USEPA) risk equations appear to be more robust approaches for risk assessment. The USEPA equations determine the carcinogenic and non-carcinogenic risk to humans (USEPA, 1997, 1998). These risks can be quantified using the USEPA defined parameters Hazard Quotient (HQ) for non-carcinogenic risk and Cancer Risk (CR) for carcinogenic risk assessment. The HQ can be calculated according to Equation 2.2. An HQ value greater than 1 defines an unacceptable non-carcinogenic risk to humans.

$$HQ = EDI / RfD \qquad \text{Equation 2.2}$$

Where EDI is Estimated Daily Intake (mg kg^{-1} day^{-1}) and RfD is a Reference dose for As (0.0003 mg As kg^{-1} body weight day^{-1}).

The parameter EDI can be calculated according to Equation 2.3

$$EDI = (C \times Fi \times Ef \times Ed) / (W \times Te) \qquad \text{Equation 2.3}$$

where C is the concentration of As in edible part of the plant (mg kg^{-1} fresh weight); Fi is Food intake (kg per person per day); Ef is Exposure frequency (days per year); Ed is Exposure duration (70 years, life expectancy for an individual); W is Average body weight (variable for adults and young, for example 60 kg for adults, aged 18 years and older WHO (1989)); Te is Average Exposure time (= Ed x 365 days)

The CR can be calculated according to Equation 2.4

$$CR = EDI \times CSF \qquad \text{Equation 2.4}$$

Where the parameter CSF is a cancer slope factor for As (1.5 mg kg^{-1} day^{-1}). The USEPA has proposed a range of 1 in 10, 000 (10^{-4}) to 1 in 1, 000, 000 (10^{-6}) as acceptable As cancer risk in humans (USEPA, 1998).

2.5.5 Arsenic distribution in vegetable tissues

Generally As occurs at higher concentrations in vegetables' roots compared to the aerial tissues. Tomato plants have been reported to accumulate 83 to 85% of total absorbed As in their roots (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1997; Miteva, 2002). Similarly, in radish 66%, and in turnip 75% of the total absorbed As remained in the plant roots (Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b). In another study, lettuce, radish, and chard roots accumulated considerably higher As concentrations than aerial tissues (Smith *et al.*, 2009). These researchers suggested that As may be complexed by phytochelatins (PCs) in plant roots which limit As translocation to aerial portions of the plants. Plants enhance PCs concentration in roots on exposure to As, induce As-PCs complexes and thereby tolerate As (Meharg & Hartley-Whitaker, 2002; Smith *et al.*, 2009). However, the apparent high concentration of As in the roots of edible root vegetables (for example radish, turnip) will present a greater toxicity risk to humans than leafy and fruit crops. In addition, the root surfaces of edible root vegetables will adsorb contaminated soil particles, and where inadequate washing is practiced, this may lead to direct ingestion of As by their end users (humans), even if the As concentration is low in plant parts (Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b).

Some vegetable species can, however, accumulate a higher concentration of As in their aboveground parts than in their roots. For example, bean plants have been reported to accumulate As mainly in shoots (87% of total absorbed As) with the order of accumulation recorded as shoots > leaves > roots (Carbonell-Barrachina *et al.*, 1997).

2.5.6 Factors affecting As accumulation and distribution in vegetables/crops

Arsenic accumulation in plants is a complex phenomenon and depends on many factors. In this section of the review, many of these factors are explored. Data relevant to vegetables is generally presented given the risk that As-contaminated vegetables may present to human health. Other food and agricultural crops are also discussed where data from vegetable studies is scarce.

Effect of As concentration

Across all reported studies, the As concentration in plants increases as a function of As concentration in the growing medium. This increase has been well documented in the tissues of many common vegetables including amaranth, radish, silverbeet, spinach, tomato, and turnip (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b; Pavlik *et al.*, 2010; Rahman & Naidu, 2009).

The As concentration of the growth media also affects its distribution in plant tissues. Lambkin and Alloway (2003) observed that at 40 mg As kg^{-1} soil, the distribution of As in barley plants was leaf > stalk > seed. This trend changed to stalk > leaf > seed at 80 mg As kg^{-1} soil. The lower concentration in leaf and seed at higher concentrations suggests the inhibition and/or reduction of stalk-leaf-seed transport mechanisms in plants (Lambkin & Alloway, 2003). Similarly, Tu and Ma (2002) reported that at low water-soluble As levels in soil (< 0.5 mg As kg^{-1}) the As concentration in *Pteris vittata* increased from old to young fronds while at moderate to high As levels (> 0.5 mg As kg^{-1}) the As concentration in fronds increased from young to old.

Effect of As species

Arsenic concentration, uptake and distribution in plants vary with the form of As present in the growth medium. However, it is difficult to identify which of the common As species (As^{V}, As^{III}, MMA or DMA) is preferred for uptake by plants. Numerous studies where these four As species have been applied to common vegetable species describe a variable uptake trend. For example in tomato plants, the total amount of As taken up followed the trend: DMA < MMA << $As^{V} \cong As^{III}$ (Burlo *et al.*, 1999). In turnip, the total amount of As taken up followed a trend of MMA < DMA < As^{III} < As^{V} whilst in radish the trend was DMA < As^{V} = As^{III} << MMA (Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b). Similarly, the amount of As absorbed by rice followed the trend of DMA < As^{V} < MMA < As^{III} (Marin *et al.*, 1992). A variable uptake trend has also been reported for white mustard and two grass species (Carbonell-Barrachina *et al.*, 1998; Jedynak *et al.*, 2010).

Arsenic translocation to plant aerial parts is also influenced by the As species present in the growth media. Organic species (MMA and/or DMA) have shown a higher extent of upward translocation to aerial parts than As^{V} and As^{III} in radish, turnip, tomato and rice (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b; Marin *et al.*, 1992).

Crop species and cultivars

Plants vary in their ability to concentrate As in their tissues because of genetic and morphological variation. For example, under similar growth conditions (hydroponic study- As^{III} levels 0 to 10 mg L^{-1}) tomato plants contained a many times higher As concentration than bean plants (Carbonell-Barrachina *et al.*, 1997).

The difference of As uptake in plants also exists among crop cultivars and their tissues. For instance, tomato cultivar Marmande accumulated significantly more As in its roots than the variety Muchamiel, while Muchamiel accumulated a significantly higher As concentration in its shoots and fruit than Marmande (Burlo *et al.*, 1999). Similarly, rice cultivar Mercury accumulated more As than Lemont in both roots and shoots (Marin *et al.*, 1992).

Effect of duration of crop cultivation

The uptake of As by plants is also dependent on the duration of crop cultivation. Jedynak *et al.* (2010) reported that white mustard plants when harvested after 6 weeks contained approximately two-to-three times more As in their tissues compared to plants which were harvested after 3 weeks. This trend indicates that the As uptake happens continuously over the lifetime of a plant.

Effect of soil properties

a) Soil pH

Soil pH has a profound effect on As chemistry by its influence on both soil chemical and biological processes (Anh *et al.*, 2013). Most studies that describe the effect of pH on As mobility in soil are laboratory batch studies, for example (Goh & Lim, 2004; Manning & Goldberg, 1997b; Smith *et al.*, 1999). All of these studies describe an increase in As^{V} mobility and decrease in As^{III} mobility as a function of an increase in soil pH level. This difference in mobility can be attributed to the effect of soil pH on surface charge of mineral constituents and protonation or deprotonation of As species (detail in section 2.3.3.2). Comparatively fewer studies have described the effect of pH on As mobility in the presence of plant species (Anh *et al.*, 2013; Tu & Ma, 2003). These studies describe a higher uptake of As by As hyper-accumulator species in an acidic environment.

The effect of pH on As mobility could be different where plant species are present, compared with soil only (laboratory batch studies), since plants are capable of altering the pH by producing organic exudates (Moreno-Jiménez *et al.*, 2012). An apparent limitation to available literature is a lack of studies where the effect of pH levels on the uptake of As by vegetables species is investigated.

b) Redox reactions

Arsenic uptake by plants is affected by soil redox which affects As speciation and mobility (Li *et al.*, 2009; Talukder *et al.*, 2012). Under oxic soil conditions, As mobility is low and As exists predominantly as the As^{V} ion; whereas under anoxic conditions, As mobility is high and the As^{III} ion is the dominant species in soil solution (Marin *et al.*, 1993; Masscheleyn *et al.*, 1991; Xu *et al.*, 2008). Increased As mobility has been attributed to the dissolution of As-binding minerals and/or reduction of As^{V} to more mobile As^{III} species under anoxic conditions.

Effect of Irrigation practice

Arsenic mobility and subsequent uptake by plants is significantly affected by irrigation practice. In recent years, water management has been adopted by many researchers to mitigate the As concentration in rice crop (Li *et al.*, 2009; Somenahally *et al.*, 2011; Talukder *et al.*, 2012; Xu *et al.*, 2008). All of these studies describe that flood irrigation (conventional irrigation practice for rice cultivation in South Asian countries) substantially increases As concentration in soil solution and rice tissues. Aerobic water management has been proposed by these researchers because this practice dramatically decreases the availability of As in soil and reduces the As concentration in rice tissues (Somenahally *et al.*, 2011; Talukder *et al.*, 2011; Xu *et al.*, 2008). The effect of water management has not been assessed for other food or agricultural crops. This is again a subject area that requires investigation.

Effect of Amendments

a) Effect of organic matter

The effect of organic matter (for example farm yard manure or poultry manure) on As mobility and plant uptake is inconsistent. In some studies, the addition of organic matter increased As mobility in soil (Cao *et al.*, 2003; Mench *et al.*, 2003). Increased mobility may be attributed to (i) competition between As and soluble organic compounds for adsorption sites in soils, (ii) reduction of As^{V} to As^{III}, and (iii) increased activity of soil microbes (Cao *et al.*, 2003; Turpeinen *et al.*, 1999; Weng *et al.*, 2009). Bhattacharya *et al.* (2010a) reported a positive correlation between As accumulation in rice plants and soil organic carbon. An increase in dissolved organic carbon can promote both As^{V} and As^{III} mobility in soils (Dobran & Zagury, 2006). In contrast, organic matter can significantly reduce As uptake by plants because of the formation of insoluble arseno-organic complexes and their adsorption onto organic colloids (Cao & Ma, 2004; Cao *et al.*, 2003; Das *et al.*, 2008). Fu *et al.* (2011) found a negative correlation between rice grain As concentration and soil organic matter content. Recently, it has also been claimed that soil organic amendments may have no significant effect on the As concentration in plant tissues in comparison to unamended treatment (McBride *et al.*, 2013).

b) Effect of biochar

In recent years, biochar has gained popularity as a soil amendment because of its beneficial effects on soil physical and chemical properties (Atkinson *et al.*, 2010). Biochars are carbon-rich combusted biological materials with large surface areas and cation exchange capacities, and have the potential to retain both contaminants and nutrients in soil (Beesley & Marmiroli, 2011; Beesley *et al.*, 2011). Studies investigating the effect of biochar on As mobility and/or retention are limited, complicated and inconsistent.

Biochar enhanced As mobility in soil pore water of a contaminated urban soil which was attributed to a rise in dissolved organic carbon and the pH of pore water (Beesley & Dickinson, 2011). Similarly, Namgay *et al.* (2010) found an increase in phosphate-extractable As concentration with biochar application. Interestingly, in Namgay's study, biochar application decreased the As concentration in maize shoots. In another study, biochar application increased the pore water As concentration in two out of three brownfield soils (Hartley *et al.*, 2009). These researchers proposed that this effect could be due to (i) competition between P and As for binding sites, and (ii) an increased soil pH as a function of biochar application. Nonetheless, there was no significant difference of As uptake by *Miscanthus x giganteus* with biochar and green waste compost application relative to untreated soils (Hartley *et al.*, 2009). Mohan *et al.* (2007) studied the sorption ability of various wood and bark biochars and found a removal of 70% of As by oak bark from aqueous solutions. Recently, Beesley *et al.* (2013) reported that biochar application increased the As concentration in pore water but decreased the As concentration in tomato tissues. These researchers proposed that leaching of As is a primary environmental risk when biochar is applied to contaminated soils.

c) Effect of phosphate

The effect of phosphate on As mobility and bioavailability is controversial and depends on various factors including growth media and As form. The effect of phosphate is more profound on As^V since both compete for the same uptake transporters and adsorption sites in soils (Esteban *et al.*, 2003; Meharg & Macnair, 1990; Qafoku *et al.*, 1999). The impact of phosphate on As bioavailability is different under hydroponic and soil medium. In general, under hydroponic conditions, phosphate decreases As influx in plants because of their direct competition for uptake (VandenBroeck *et al.*, 1997; Wang *et al.*, 2002). In contrast, phosphate increases As concentration and uptake in plants grown in soil because of their competition for adsorption sites and/or release of solid-phase As (Creger & Peryea, 1994; Jiang & Singh, 1994).

There is a negligible effect of phosphate on As^{III} influx in plants (Wang *et al.*, 2002). This insignificant effect may be attributed to a different uptake mechanism; As^{III} is taken up by plants through aquaporins (water channels) while phosphate and As^{V} share the same uptake system (Abedin *et al.*, 2002; Meharg & Jardine, 2003; Meharg & Macnair, 1990).

2.6 TOXICITY OF ARSENIC TO HUMANS

Humans are mainly exposed to arsenic toxicity through the ingestion of As-contaminated drinking water and food. The average human intake of As is 0.5 to 1.0 mg daily in the form of food and water (Cullen *et al.*, 1995). Drinking water is the major source of toxicity, and the presence of inorganic As species even at low concentration can be risky (Mahimairaja *et al.*, 2005; Ratnaike, 2003). Exposure to As causes many acute and chronic effects leading to various disorders in humans (Table 2.5). Toxicity symptoms may occur within 30 to 60 minutes after exposure, but may be delayed if ingested through food products (Cullen *et al.*, 1995). Extreme chronic effects of As poisoning in humans include cancer and death (Brammer & Ravenscroft, 2009). There are no proven treatments currently available to cure chronic As exposure (Ratnaike, 2003).

The toxicity of As varies with the As species and the amount ingested. Arsenite is considered more toxic to humans than other inorganic (As^{V}) and organic forms, because of the arsenite ion’s high lipid solubility (Crecelius, 1977; Cullen *et al.*, 1995; Ferguson & Gavis, 1972; Mahimairaja *et al.*, 2005). The reported lethal doses of As for humans have a wide range, ranging from 1.5 mg kg^{-1} body weight for As^{III} to 500 mg kg^{-1} body weight for DMA (Mahimairaja *et al.*, 2005).

Table 2.5 Effects of arsenic exposure to humans

Acute Effects
Nausea, vomiting, diarrhoea, multi-organ dysfunction, painful neuropathy (disease/injury affecting nerves/nerves cells), weakness, anaemia (decrease in number of red blood cells), burning sensation of eyes, swelling of legs, gastrointestinal disturbances, abdominal pain, metallic or garlic like taste associated with dry mouth, burning lips, excessive salivation, muscular pain, low blood pressure, high heartbeat, skin rashes, and jaundice
Chronic Effects
Skin • Lesions/hypo-pigmentation (loss of skin colour)/hyperkeratosis (darkening of skin and appearance of small corn or warts on palms, soles and torso) • Cancer Internal organ • Bladder cancer • Kidney cancer • Lung cancer • Prostate cancer Neurological effects Genetic disorders • Chromosomal breakage and aberration, and chromatid exchange • DNA damage Birth defects Diseases • Hypertension and cardiovascular disease • Pulmonary disease • Peripheral vascular disease • Respiratory disease • Diabetes

Source: (Cullen *et al.*, 1995; Hindmarsh & McCurdy, 1986; Mahimairaja *et al.*, 2005; Moore *et al.*, 1994; Peralta-Videa *et al.*, 2009; Ratnaike, 2003; Smith *et al.*, 2000)

Upon ingestion by humans, As is bound to hemoglobin, leukocytes, and plasma proteins (Cullen *et al.*, 1995). Within 24 hours it is cleared from the intravascular space and will start concentrating in liver, kidneys, spleen, lungs, gastrointestinal tract, muscles and nervous tissues (Cullen *et al.*, 1995; Mahimairaja *et al.*, 2005). Between 2 to 4 weeks after ingestion it can be detected in hair, nails, and skin due to its binding with sulfhydryl groups in keratin; and finally after 4 weeks of exposure, it accumulates in bones (Cullen *et al.*, 1995; Matschullat, 2000; Ratnaike, 2003; E. Smith *et al.*, 1998). In extreme cases, it may cause death within a few hours and/or few days (Armstrong *et al.*, 1984; Cullen *et al.*, 1995). On a cellular basis, As interferes with various metabolic systems by inactivating nearly 200 enzymes, especially those involved in cellular energy pathways, and DNA synthesis and repair (Ratnaike, 2003).

Arsenic may, however, be excreted from the body rapidly, mainly through urine. Excretion can occur within a few days as both organic and inorganic forms (Buchet *et al.*, 1981; Crecelius, 1977; Hindmarsh & McCurdy, 1986). The apparent biological half-life of inorganic arsenic (As^{III} and As^{V}) is 10 hours, but 30 hours for organic forms (MMA and DMA) (Crecelius, 1977).

Within the human body, As can be transformed into other forms; the main transformation pathway is the methylation of inorganic forms into MMA and DMA (Buchet *et al.*, 1981; Crecelius, 1977). Buchet *et al.* (1981) reported that 75% of ingested As^{III} was methylated into MMA and DMA in human body and then excreted. While 13% of ingested MMA was transformed to DMA, and the DMA was excreted unchanged. The conversion of inorganic forms of As into methylated forms seems to be a detoxification strategy within the human body (Crecelius, 1977). Arsenic can also be excreted in bile, faeces, hair, skin, lung, and sweat in small amounts (Cullen *et al.*, 1995).

2.7 OBJECTIVES OF THIS RESEARCH

1. A better understanding of the risk associated with irrigating vegetables with As-contaminated water in soil environment
2. The screening of vegetable species that may accumulate a higher concentration of As in their edible parts and therefore pose a greater risk to humans through their consumption

3. Determination of an acceptable level of As in irrigation water that can be used for vegetable cultivation
4. Improved understanding of the various As and soil factors, and soil management practices, that affect the As concentration in edible parts of vegetable(s)
5. Improved understanding of soil adsorption parameters and models, and elucidation of key soil properties which control As adsorption in soils of variable physical and chemical characteristics
6. Propose management strategies for South Asian horticulture where As-contaminated irrigation water is often used for vegetable cultivation

2.8 STAKEHOLDERS IN THIS RESEARCH

Understanding the response of vegetable crops to the As concentration of irrigation water has practical significance for South Asian countries (Bangladesh, India, Nepal, and Pakistan) where As-contaminated water is often used to irrigate food crops (Dahal *et al.*, 2008; Husaini *et al.*, 2011a; Saha & Ali, 2010; Tiwari *et al.*, 2011). Irrigation with As-contaminated water also occurs in Chile, Italy, Nigeria and Spain (Agbenin *et al.*, 2009; Marconi *et al.*, 2010; Moyano *et al.*, 2009; Munoz *et al.*, 2002). Therefore, the research described in this thesis will contribute to improve food safety in countries, mainly South Asia where As-enriched water is often used for irrigation.

A number of stakeholders have been considered in this work. Each of these parties may benefit from the outcomes of this research.

- People: Improved understanding of As risk could potentially save people of As affected areas from diseases and conditions that are caused by eating As-contaminated food crops. Decreased expenditure on medicines to treat As symptoms will improve the disposable incomes of these people. It is anticipated that the outputs of this research will lead to a healthier and prosperous quality of life for affected populations in South Asia.
- Farmers: Improved understanding of As uptake potential of vegetable species will help farmers to select the crops which limit As to their non-edible tissues.

- Non-governmental organizations (NGOs) & environmental organizations: This research will provide scientific evidence to NGOs and environmental organizations who promote sustainable development and a healthy environment in South Asia.
- Government (s): The governments of South Asian countries will benefit from improved health of humans. A direct financial benefit will come through a reduction in the need to purchase medicines that protect human health.
- Science: This research will make a contribution to scientific knowledge in terms of As uptake potential of various vegetable species as a function of As concentration in irrigation water, soil factors and management practices. Also, calculation of associated risk to humans upon ingestion of As-contaminated vegetables will be a significant addition to a currently limited vegetables risk assessment inventory.

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CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter comprises of two major sections. The first section describes the development of methodology used in this study to determine the As concentration in plants and soil. The second section details the laboratory methods adopted to determine soil physical and chemical properties. The relevance of experimental soil to South Asian soils is also described in this chapter.

3.2 METHODOLOGY TO ANALYZE TOTAL ARSENIC IN PLANTS

The total arsenic (As) concentration in plants can be determined using a range of sample preparation techniques and instruments. For plants, sample preparation is achieved through the use of two techniques, namely acid digestion and dry ashing (Baig & Kazi, 2012; Burlo *et al.*, 1999; Mayorga *et al.*, 2013; Munoz *et al.*, 2002). Acid digestion is used in preference to dry ashing due to ease of use, time, the chemical and energy saving advantages of this method, and mitigation of the risk of As volatilization under ashing conditions. The instruments commonly used to analyze the total As concentration in the resulting digest solutions include HGAAS (Hydride Generation Atomic Absorption Spectrometer), ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometer), GFAAS (Graphite Furnace Atomic Absorption Spectrometer), ICP-MS (Inductively Coupled Plasma Mass Spectrometer), and AFS (Atomic Fluorescence Spectrometer).

A review of published information describing the acid digestion technique for As shows a significant variability in methods, particularly regarding the amount of plant sample used, the type and amount of acid used, and the digestion temperature and duration (Table 3.1). Most commonly, 0.5 gram of plant material is digested with HNO_3 alone or HNO_3 in combination with H_2O_2. The digestion temperature and duration has a great variation in the available data, ranging from 60 ^{0}C to 230 ^{0}C for digestion temperature, and ranging from 3 minutes to 15 hours for duration. Therefore, a more robust methodology to determine the total As concentration in plant samples was developed for this research utilizing the analytical facilities available in the department of Soil and Earth Sciences, Massey University Palmerston North. The two instruments available for analyses were GFAAS and HGAAS.

Table 3.1 Acid digestion techniques to determine total As in vegetables tissues

Plant mass (g)	Acid for digestion	Digestion temperature and duration	Instrument used	Reference
0.2	HNO_3 and H_2O_2 (2 mL)	ND	GFAAS	Baig and Kazi (2012)
0.2	HNO_3 (6 mL), $HClO_4$ (1 mL), H_2O_2 (0.5 mL)	ND	HGAAS	Dahal *et al.* (2008)
0.2	HCl 6 mL	100 ^{0}C for 3 hours	HGAAS	Cobb *et al.* (2000)
0.2-0.5	HNO_3 (5 mL), $HClO_4$ (2 mL)	60 ^{0}C for 2 hours	HGAAS	Bhattacharya *et al.* (2010b)
~ 0.5	HNO_3 (10 mL), H_2O_2 (3 mL)	120 ^{0}C for 6 hours	ICP-MS	Smith *et al.* (2008)
0.5	H_2O_2 (2 mL), HNO_3 (8 mL), H_2SO_4 (0.5 mL)	230 ^{0}C for 2-3 hours	HGAAS	Mayorga *et al.* (2013)
0.5	HNO_3 (2 mL), H_2O_2 (1 mL)	120 ^{0}C for 8 hours	HGAAS	Samal *et al.* (2011)
0.5	HNO_3 7 mL	ND	ICP-AES	Marconi *et al.* (2010)
0.5	HNO_3 + H_2O_2 mixture (2:1, v/v) 5 mL	3 minutes	GFAAS	Arain *et al.* (2009)
0.5	HNO_3 10 mL	75-140 ^{0}C for 10 hours	ICP-MS	Smith *et al.* (2009)
0.5	HNO_3 5 mL	100-120 ^{0}C for 2 hours	ICP-MS/ ICP-OES	Gaw *et al.* (2008)
0.5	HNO_3 10 mL	60-110 ^{0}C for 9 hours	HGAAS	Warren *et al.* (2003)
0.5	HNO_3 4 mL	160 ^{0}C for 4 hours	GFAAS	Helgesen and Larsen (1998)
0.5	HNO_3 5 mL	120-130 ^{0}C	ICP-ES	Carbonell-Barrachina *et al.* (1997)
0.5-1.0	HNO_3 5 mL	75-140 ^{0}C for 15 hours	ICP-MS	Rahman *et al.* (2009)
0.5-1.0	HNO_3 (10 mL), H_2O_2 (2 mL)	120 ^{0}C for 2 hours	GFAAS	Al Rmalli *et al.* (2005)
1	HNO_3/H_2SO_4/$HClO_4$ (10:1:4, v/v)	ND	HGAAS	Gutierrez *et al.* (2010)
2	HNO_3 (25 mL), $HClO_4$ (10 mL)	3 hours	HGAAS	Saha and Ali (2010)
5	HNO_3 and H_2O_2	ND	GFAAS	Larsen *et al.* (1992)
ND	HNO_3 1 mL	180-200 ^{0}C	ICPS	Tiwari *et al.* (2011)
ND	HCl and H_2O_2	1 hour	AAS	Grant and Dobbs (1977)

ND: not described by researchers

3.2.1 DETERMINATION OF TOTAL ARSENIC IN PLANTS USING GFAAS

The determination of many elements using GFAAS requires addition of a matrix modifier. This modifier converts the analyte element into a phase with higher thermo-stability than the basic element, and allows removal of many contaminants in the aqueous phase during pyrolysis without losing the targeted element prior to the atomization stage (Schlemmer & Welz, 1986).

For arsenic, commonly used modifiers include nickel (Ni), palladium (Pd), and mixture of palladium and magnesium nitrates (Schlemmer & Welz, 1986). In this work nickel, magnesium nitrate [$Mg(NO_3)_2$], and mixture of nickel nitrate and magnesium nitrate were used at various pyrolysis temperatures (detail follows). Palladium was avoided due to the possibility that element may be the subject of future studies on the GFAAS. Recommended conditions for As analysis on GFAAS were adopted as per the Perkin Elmer Manual (*The THGA Graphite Furnace: Techniques and Recommended Conditions*) (Appendix I).

The methodology developed was tested through analysis of plants from the IPE collection (International Plant-analytical Exchange Program) of the Wageningen Evaluating Programs for Analytical Laboratories (WEPAL) organization, The Netherlands. The selected plant samples had a wide range of reported values for the total As concentration (ng g^{-1} equivalent to µg kg^{-1} or ppb) in each plant species (Table 3.2). This variation was due to analysis by a number of different techniques adopted by different laboratories that participated in the analysis program. These samples are not standard reference materials. For the purpose of method development in this study, the median value was selected with a variation of ± 20% for each plant species. This value was considered the threshold value for a decision on the reliability of a specified technique.

To digest plant samples, 10 mL of concentrated nitric acid (HNO_3, analytical grade) was added to a recorded mass of plant material (detail follows). Samples were kept overnight to provide sufficient time for the acid to pre-dissolve plant contents. Acid blanks were also included in the procedure. The following day samples were digested on a digestion block for 2 to 3 hours at 120 ^{0}C until the fumes vanished. The samples were then cooled to room temperature and diluted with 20 mL of deionized water. The samples were filtered with Whatman filter paper (42), and digestion tubes and filter were rinsed by several washings before the final volume was raised to 50 mL in volumetric flasks with deionized water. These samples were subsequently analyzed for As concentration using GFAAS.

In all laboratory analysis procedures, samples were prepared in clean glass wares and were stored in new polypropylene containers before analysis.

Table 3.2 Reported As concentration (ng g^{-1} dry weight) in plants selected for methodology development

Plant species	**Median (ng g^{-1})**	**Range (ng g^{-1})**
Carnation straw	128	40 to 200
TYM	776	211 to 965
Grass-GR 94	298	227 to 367
Broccoli	284	110 to 381
Pepper	315	241 to 392
Potato (bulb)	171	127 to 652
Potato (plant)	921	363 to 1384
Aubergine (plant)	280	250 to 344
Aubergine (plant)	292	136 to 370
French bean	307	172 to 661

Accuracy of analytical procedure as a function of pyrolysis temperature, modifiers, and plant mass

The accuracy of analytical technique was analyzed by various approaches which are discussed in following paragraphs.

At first, plant samples were analyzed using GFAAS at three pyrolysis temperatures 800, 1000 and 1200 ^{0}C with $Mg(NO_3)_2$ modifier (Table 3.3). Although the recommended pyrolysis temperature for GFAAS is 1200 ^{0}C (Appendix I), this temperature showed a great variability in the actual versus reported As concentration for each plant during the method development exercise (Table 3.3). For most plants (TYM, Grass-GR94, and Broccoli) the As concentration values in plants were very low in comparison to median reported values (IPE) when 1200 ^{0}C pyrolysis temperature and $Mg(NO_3)_2$ was used as a modifier. This may have been due to (i) the pyrolysis temperature being high enough to fully atomize As, and/or (ii) $Mg(NO_3)_2$ did not adequately stabilize As allowing for the removal of elements that can interfere with absorption during atomization. A similar trend of decreased value in most plants (TYM, Grass-GR 94 and Broccoli) was observed for a pyrolysis temperature of 800 and 1000 ^{0}C; though relatively greater accuracy was recorded at 800 ^{0}C.

Table 3.3 Arsenic concentration (ng g^{-1}) in selected plants on GFAAS as a function of pyrolysis temperatures with $Mg(NO_3)_2$ modifier

Plant Species [a]	**PT (^{0}C)** [b]	**As concentration (ng g^{-1})** [c]	**% age (+/-) over median value** [d]
Carnation straw (128) [e]	1200	361 ± 0.16	182
	1000	404 ± 0.15	216
	800	381 ± 0.24	197
TYM (776)	1200	504 ± 0.02	-35
	1000	584 ± 0.08	-25
	800	538 ± 0.04	-31
Grass-GR 94 (298)	1200	85 ± 0.04	-72
	1000	100 ± 0.05	-66
	800	145 ± 0.02	-51
Broccoli (284)	1200	27 ± 0.03	-91
	1000	96 ± 0.07	-66
	800	155 ± 0.03	-45

[a] Plant mass used: 0.2 grams; [b] Pyrolysis Temperature; [c] values are mean ± SE (n = 3); [d] % age (+/-) over median value was calculated as the difference between mean As concentration in vegetable tissues (column 3) and vegetable median value (column 1), multiplied by 100 and divided by vegetable median value (column 1); [e] values in parenthesis represent median value (ng g^{-1}) of each plant species

A further experiment was performed using a pyrolysis temperature of 800 ^{0}C and the three modifiers namely Ni, $Mg(NO_3)_2$, and Ni+$Mg(NO_3)_2$. The results of this experiment are presented in Table 3.4. A trend of reduced values was again found at this temperature with each modifier relative to the reported value. Overall, Ni modifier (Ni 2000 mg L^{-1} as nickel nitrate) resulted in less deviation of the analyzed As concentration from the reported median values for most plant species (except carnation straw) while $Mg(NO_3)_2$ yielded the greatest deviation (except carnation straw). This indicates that Ni was relatively more suitable modifier for As analysis on GFAAS.

Table 3.4 Arsenic concentration (ng g^{-1}) in selected plants on GFAAS as a function of modifiers at 800 ^{0}C pyrolysis temperature

Plant Species [a]	Modifier used	As concentration (ng g^{-1}) [b]	% age (+/-) over median value [c]
Carnation straw (128) [d]	Ni [e]	23 ± 0.11	-82
	$Mg(NO_3)_2$ [f]	66 ± 0.04	-48
	Ni + $Mg(NO_3)_2$ [g]	28 ± 0.09	-78
TYM (776)	Ni	714 ± 0.88	-8
	$Mg(NO_3)_2$	210 ± 0.17	-73
	Ni + $Mg(NO_3)_2$	748 ± 2.13	-4
Grass-GR 94 (298)	Ni	186 ± 0.12	-38
	$Mg(NO_3)_2$	63 ± 0.05	-79
	Ni + $Mg(NO_3)_2$	140 ± 0.11	-53
Broccoli (284)	Ni	133 ± 0.16	-53
	$Mg(NO_3)_2$	33 ± 0.02	-88
	Ni + $Mg(NO_3)_2$	71 ± 0.03	-75

[a] Plant mass used: 0.4 grams; [b] values are mean ± SE (n = 3); [c] % age (+/-) over median value was calculated as the difference between mean As concentration in vegetable tissues (column 3) and vegetable median value (column 1), multiplied by 100 and divided by vegetable median value (column 1). [d] values in parenthesis represent median value (ng g^{-1}) of each plant species; [e] Ni 2000 mg L^{-1} as nickel nitrate; [f] $Mg(NO_3)_2$ as 0.3% solution; [g] mixture of nickel nitrate (Ni 2000 mg L^{-1}) and magnesium nitrate (0.3% solution)

In the experiments described above, the mass of plant material used in the analysis was 0.2 grams (Table 3.3) and 0.4 grams (Table 3.4). An experiment was conducted to observe whether mass of plant material used for digestion and analysis affects the As concentration and performance of GFAAS (Plant mass used: 0.4 to 1.0 gram; Table 3.5). The results indicate that an increase in plant mass improved the As concentration values using a pyrolysis temperature of 800 ^{0}C and Ni modifier (2000 mg L^{-1}). Arsenic concentration (ng g^{-1}) values in tested species (Broccoli and Aubergine) were found to be close to the median value (variation ± 10% to median) when 1.0 gram plant material was used (Table 3.5).

Table 3.5 Arsenic concentration (ng g^{-1}) in vegetable species as a function of plant mass (g) under pyrolysis temperature 800 ^{0}C and Ni modifier (2000 mg L^{-1}) on GFAAS

Vegetable Species	Mass of plant used (g)	As concentration (ng g^{-1}) [a]	%age (+/-) over median value [b]
Broccoli (284) [c]	0.4	132 ± 0.10	-54
	0.6	235 ± 0.30	-17
	0.8	325 ± 0.09	14
	1.0	296 ± 0.05	4
Aubergine (plant) (292)	0.4	109 ± 0.04	-63
	0.6	178 ± 0.11	-39
	0.8	224 ± 0.10	-23
	1.0	261 ± 0.27	-11

[a] values are mean ± SE (*n* = 3); [b] % age (+/-) over median value was calculated as the difference between mean As concentration in vegetable tissues (column 3) and vegetable median value (column 1), multiplied by 100 and divided by vegetable median value (column 1). [c] values in parenthesis represent median value (ng g^{-1}) of each plant species

Based on the above results, another experiment was designed with different vegetable species using the same analytical conditions (1.0 gram plant mass, PT 800 ^{0}C and Ni modifier 2000 mg L^{-1}) to validate the proposed technique. The results were found acceptable for most plant species (except French beans) with variation of ± 20% from reported median values (Table 3.6).

Table 3.6 Arsenic concentration (ng g^{-1}) in vegetable species with plant mass (1.0 g) under pyrolysis temperature 800 ^{0}C and Ni modifier (2000 mg L^{-1}) on GFAAS

Vegetable Species	As concentration (ng g^{-1}) [a]	%age (+/-) over median value [b]
Pepper (315) [c]	374 ± 0.43	19
Potato (bulb) (171)	179 ± 0.87	5
Aubergine (Plant) (280)	254 ± 0.54	-9
French bean (307)	168 ± 0.61	-45

[a] values are mean ± SE (*n* = 3); [b] % age (+/-) over median value was calculated as the difference between mean As concentration in vegetable tissues (column 2) and vegetable median value (column 1), multiplied by 100 and divided by vegetable median value (column 1). [c] values in parenthesis represent median value (ng g^{-1}) of each plant species

3.2.2 DETERMINATION OF TOTAL ARSENIC IN PLANTS USING HGAAS

Hydride Generation Atomic Absorption Spectrometer (HGAAS) is specifically designed for metalloids analysis (for example, As, Hg, Se, Bi) and is more widely used in the scientific community than GFAAS (Table 3.1) because of better sensitivity, minimal matrix interferences and inexpensive requirements (Foster & Sumar, 1996). Experiments were conducted to check if the accuracy of plant analysis was improved by using HGAAS.

The HGAAS, also known as Flow Injection Analysis-Mercury Hydride System (FI-MH-AAS), involves reaction of an acidified aqueous solution with a reducing agent (sodium borohydride, $NaBH_4$), to generate volatile hydrides of As, Se, Hg or Bi. These hydrides are transported to a quartz cell by an inert carrier gas (such as argon or nitrogen). In the quartz cell, the hydrides are converted to gaseous metal atoms, and, being in the path of the relevant element source lamp, absorb light. Element concentrations are proportional to the amount of light absorbed.

As part of the method development procedure, a Flow Injection Analysis System (FIAS) was coupled to the Atomic Absorption Spectrometer to determine the As concentration in digested sample solutions. Further details of HGAAS installation and optimization are reported in Appendix II. For As analysis of the plant samples (and soil samples), the recommended and adopted analytical parameters for HGAAS are shown in Appendix III.

Subsamples of various vegetable species (potato, pepper, aubergine, and broccoli) were weighed to 1.0 gram, and digested with 10 mL concentrated HNO_3 as described in section 3.2.1.

The sensitivity of HGAAS is greater for As^{III} than As^{V}. This is because HGAAS allows the determination of hydride forming elements; hydrides are generated during the reaction of acidified aqueous samples with reducing agent which are then transported to a quartz cell by means of an inert gas. The As^{III} is the species which forms gaseous hydride, AsH_3. Therefore, the standard procedure for HGAAS is to pre-reduce all samples prior to analysis. Reduction is achieved by taking 1 mL of sample aliquot and adding 1 mL of concentrated HCl, and 1 mL solution of 5% (w/v) Potassium Iodide (KI) plus 5% (w/v) ascorbic acid. The treated samples were allowed to stand for 1 hour at room temperature and finally diluted to 10 mL with 10% HCl. At this point the samples can be analyzed for As concentration using HGAAS.

Working standards were prepared from an As^{III} standard solution (1000 mg L^{-1}). Working standards and $NaBH_4$ (reducing agent) were prepared fresh each time before analysis. Working standard solutions were run during analysis as sample, to ascertain precision of analysis.

The As concentration (ng g^{-1}) determined in the various plant digests and As solutions (prepared and run as samples in analysis) is summarized in Table 3.7. The As concentration in two vegetable species (Aubergine and Broccoli) and in tested As solutions (0.5, 1.0 and 10.0 µg L^{-1}) was close to the median/ standard value with a variation of ± 10%. The values of Pepper and Potato (plant) were found with a variation in between ± 30 to 40% of the median values. However, the value of potato (bulb) was higher than the reported median value (> 50%). Each of these Wageningen samples is not a Standard Reference Material (SRM), so the accuracy of analysis can only be inferred, not quantified.

Table 3.7 Arsenic concentration (ng g^{-1}**)** in various plant species and As standard solutions on HGAAS

Substrate	**As concentration (ng g^{-1})** [a]	**% age (+/-) over median/standard value** [b]
Potato (bulb) (171) [c]	264 ± 0.01	54
Pepper (315)	404 ± 0.01	28
Aubergine (Plant) (280)	296 ± 0.02	6
Broccoli (284)	276 ± 0.03	-3
Potato (Plant) (921)	572 ± 0.06	-38
0.5 µg L^{-1}	0.54 ± 0.02	9
1.0 µg L^{-1}	1.09 ± 0.04	9
10.0 µg L^{-1}	10.02 ± 0.10	0.2

[a] values are mean ± SE (*n* = 3); [b] % age (+/-) over median value was calculated as the difference between As concentration in vegetable tissues (column 2) and vegetable median value (column 1), multiplied by 100 and divided by vegetable median value (column 1); [c] values in parenthesis represent median value (ng g^{-1}) of each vegetable species

Analysis of Plant SRM using HGAAS

The accuracy of the HGAAS to analyze As was ascertained using the standard reference material (SRM, 1573a, Tomato leaves, National Institute of Standards and Technology, USA). A 0.5 gram of SRM was digested in 5 mL of concentrated HNO_3 (the ratio of mass to volume was the same i.e. 1:10 as for the Wageningen plant samples). The analyzed concentration of the SRM (0.116 ± 0.007 µg g^{-1}, $n = 3$) was in good agreement with the certified reference value (0.112 ± 0.004 µg g^{-1}).

Choice of Instrument

The results of the technique adopted to determine As in the Wageningen plant samples show that both the instruments (GFAAS and HGAAS) can be used for As analysis. However, considering the satisfactory reading of SRM sample by HGAAS, this instrument was used for subsequent analytical work.

3.3 METHODOLOGY FOR ANALYZING TOTAL ARSENIC IN SOIL USING HGAAS

Based on the method development protocol for plant samples, HGAAS was selected as the preferred instrument for analysis of total As in soil samples. To test the accuracy of this technique, analysis of a reference soil material (CRM - GBW 07403 of the National Research Center for CRMs of China, Beijing) was carried out.

To digest soil samples, the *aqua-regia* (HCl: HNO_3, 3:1) method was used. One gram of soil sample was weighed and dissolved in 10 mL of *aqua-regia*. The samples were kept overnight to allow time for pre-digestion of the soil. The next day the samples were placed on a digestion block at 120 ^{0}C for 2 to 3 hours. Subsequently the samples were cooled at room temperature and filtered with Whatman filter paper (42) and diluted to a final volume of 50 mL with deionized water. The samples were pre-reduced to As^{III}, as described in the plant reduction procedure in section 3.2.2 before analysis using HGAAS.

The analyzed As concentration of the soil reference material (4.2 ± 0.06 µg g^{-1} $n = 4$) was within the range of reported values for the reference material (4.4 ± 0.6 µg g^{-1}). This analytical protocol for soil was subsequently adopted throughout the study described in this thesis.

3.4 QUALITY CONTROL

In subsequent analytical work, the reported plant and soil SRMs and sample blanks were included in the procedure. The precision was maintained at 10% relative standard deviation (RSD) and any sample value higher than the defined RSD value was repeated. The SRM samples were tested after every 15 samples to check for analytical drift with time.

3.5 CHOICE OF SOIL FOR EXPERIMENTS AND ITS RELEVANCE TO SOUTH ASIAN SOILS

Soils in many of the agricultural areas throughout South Asian countries (Bangladesh, India and Pakistan) are alkaline (pH greater than 7) and low in organic matter content (≤ 1%) (Bhattacharya *et al.*, 2010a; Lal, 2004; Panaullah *et al.*, 2009). Many of the essential macro and micro nutrients (P, Fe, Zn, Cu and Mn) have low bioavailability in these soils because of the decreased solubility of many nutrients at pH above 7.0 (Brady & Weil, 2008; Rashid *et al.*, 1997). These essential nutrients are therefore supplemented to crops using chemical fertilizers and organic matter amendments. The texture of South Asian soils is variable and ranges from light to heavy in nature. Soil texture is one of the important soil characteristics that determine the optimal crop species for cultivation on any area of land. For example, clay rich soils are preferred for rice cultivation because these soils can hold water for longer periods as is required for rice growth. In contrast, light textured soils (silt loam, sandy loam, loams) are used for vegetable cultivation because they drain more readily and vegetables do not require excess water for growth.

Low soil content of Fe and relatively high pH is expected to increase the bioavailability of As in these soils. A low Fe content of soils creates limited opportunities for the adsorption of As to these soils. A pH greater than 7 further increases the As bioavailability through an increase in (i) the magnitude of net negative charge on Fe, Al and Mn oxides and silicate minerals, and (ii) the amount of negatively charged As^V species in soil solution because of the deprotonation of As^V compounds (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{-2}$). Furthermore, the addition of

P fertilizers to soils will displace any adsorbed As from soil surfaces, and will increase the As concentration either in soil solution or groundwater by As leaching.

Despite this discussion, there are, however, exceptions to this general nature of South Asian soils. The soils of some areas of India and Bangladesh are lateritic with very high Fe content and acidic pH (5.3 to 6.5) (Bhuiya & Walker, 1977; Hossain *et al.*, 2009; Rautaray *et al.*, 2003). Arsenic bioavailability in these soils may be limited because of adsorption of As onto Fe mineral surfaces; the net positive charge of Fe surfaces that can be expected under a low pH environment which will adsorb the dominant As^{V} species. There appears to be no existing literature about the presence of lateritic soils in Pakistan.

Environmental situation and soils of Karachi, Pakistan

Karachi is the largest, and the most populated metropolitan city of Pakistan, with an estimated population of 23.5 million as of April 2013 (Amer, 2013). The city is also the industrial and financial hub of Pakistan. The food requirement of the people of Karachi is met by the crops grown by farmers in surrounding areas. Unfortunately most of the locally produced vegetables are irrigated with untreated wastewater (combination of household and industrial water). This wastewater contains excessive concentrations of many elements, for example Fe, Cu, Zn, Mn, Cd, Cr, Ni and Pb (Saif *et al.*, 2005). As a result, irrigated vegetables and soils has become contaminated with these elements (Huq *et al.*, 2005a; Huq *et al.*, 2005b; Huq *et al.*, 2005c). Arsenic, however, is generally ignored and almost no data is available regarding its concentration in the plants, soils and wastewater of Karachi. It is important to mention that Karachi produces the highest amount of wastewater in the country i.e. 604 million m^3 $year^{-1}$, which is 26.3% of the total wastewater of the country (WWF, 2007). Unfortunately only 16% of the total produced wastewater of the city is treated.

Soils of Karachi are alkaline, moderately to strongly calcareous and low in organic matter content (Table 3.8). Soils are majorly used for the cultivation of horticultural crops (vegetables and orchards) by groundwater and wastewater. Understanding the fate of As in these soils has great significance for food safety and health of the people of Karachi.

Table 3.8 Characteristics of soils of Karachi, adopted from Jaffri *et al.* (1976)

Soil properties	**Description**
pH	7.9 to 8.4
Organic matter content	≤ 1%
Lime content	Moderately to strongly calcareous
Texture	Sandy loam, silt loam, loam, clay loam, silty clay, and clay
Structure	Sub angular blocky
Moisture holding capacity	Low to high
Parent materials	Most soils: Piedmont alluvium Some soils: River alluvium and river sands
Main rocks	Sandstones, shale, limestone and conglomerate

Selection of a NZ soil to model intensively cultivated soils of South Asia

Considering the chemical nature of South Asian soils (Bangladesh, India and Pakistan) and in particular those used for vegetable cultivation in the area surrounding Karachi, a Rangitikei silt loam soil was selected for the current study. The chemical properties of Rangitikei soil are within the range of South Asian soils (Table 3.8 and 3.9). In brief, the Rangitikei soil was low in available P, and amorphous and crystalline Fe content which is the common characteristic of South Asian soils particularly of Pakistan. The light texture of Rangitikei soil (Silt loam) also make this soil a reasonable choice for the current study, since light textured soils (silt loams, sandy loams, loams) are often used for vegetables cultivation in South Asian countries, particularly in Pakistan (Jaffri *et al.*, 1976; Khoso, 1994). The results obtained from this study may therefore be confidently related to South Asian horticulture, specifically that for the areas surrounding Karachi.

Table 3.9 Comparison of experimental soil with selected South Asian soils where As contamination is reported

Properties	**Rangitikei Soil** [a]	**South Asian soils** [b]	**Reference**
pH (H_2O)	6.1 ± 0.05	5.5 to 7.9	(Baig & Kazi, 2012; Bhattacharyya *et al.*, 2003; Panaullah *et al.*, 2009)
Olsen P (µg P g^{-1})	7.05 ± 0.05	3 to 131	(Panaullah *et al.*, 2009; Rahman *et al.*, 2007)
Total Fe (%)	2.3 (*n* = 1)	0.2 to 4.0	(Panaullah *et al.*, 2009; Rahman *et al.*, 2007)
Amorphous Fe (%)	0.38 ± 0.01	0.25 to 0.98	(Panaullah *et al.*, 2009; Sahu *et al.*, 2011)
Crystalline Fe (%)	0.65 ± 0.03	0.73 to 2.10	(Hossain *et al.*, 2009; Sahu *et al.*, 2011)

[a] values are mean ± SE (*n* = 2, unless mentioned); [b] values represent range

3.6 METHODS ADOPTED TO DETERMINE PHYSICAL AND CHEMICAL PROPERTIES OF SOIL

The general physical and chemical properties of the soil used in this study were determined at two laboratories; the Soil and Earth Sciences Group Laboratory, Massey University Palmerston North New Zealand, and the Soil Laboratory of LandCare Research Limited Palmerston North New Zealand. The soil properties were determined by the technical staff of these laboratories.

After field collection, soil samples were air dried, and sieved through a 2 mm stainless steel sieve prior to various laboratory analysis (Table 3.10). The parameters soil pH, Olsen P, sulfate, CEC, amorphous and crystalline Fe and Al were analyzed according to the methods of Blakemore *et al.* (1987). Total C and N were analyzed using a Leco furnace. Soil texture was determined by the pipette method (Claydon, 1989), and soil mineral identification was carried out using XRD (Whitton & Churchman, 1987).

Table 3.10 Methods adopted to determine soil properties

Soil property	Brief method description	Analysis by	Reference
Soil pH	10 gram (g) soil + 25 mL water + Overnight stand	Digital pH meter	Blakemore *et al.* (1987)
Olsen P	1 g soil + 20 mL $NaHCO_3$ (0.5 M) + shake for 30 minutes + filtration	Spectro-photometer	Blakemore *et al.* (1987)
Sulfate	5 g soil + 25 mL extracting solution (0.01 M KH_2PO_4) + shake for 1 hour then centrifuge at 2000 rpm for 15 minutes	Auto Analyzer	Blakemore *et al.* (1987)
CEC	1 g soil + 2 g acid washed silica sand, pack into a semi-micro leaching tube + percolate with 50 mL ammonium acetate (1 M, pH 7.0) + several washes with ethanol + leach the soil with 45 mL NaCl (1 M)	Ca, Mg, K and Na by Atomic Absorption Spectrometer	Blakemore *et al.* (1987)
Total C and N	0.1 g soil	LECO Furnace (FP- 2000)	LandCare Research New Zealand Ltd. (2008)
Soil texture	20 g soil + pre-treatment to remove $CaCO_3$ and organic matter by HCl (1 M) and H_2O_2 (30%) respectively + dispersion by 25 mL sodium hexametaphosphate and ultrasonic probe for 5 minutes	Pipette	Claydon (1989)
Amorphous Fe and Al	1 g soil + 100 mL acid oxalate reagent + shake for 4 hours (in the dark) + filtration	Atomic Absorption Spectrometer	Blakemore *et al.* (1987)
Crystalline Fe and Al	1 g soil + 1 g sodium dithionite and 50 mL sodium citrate solution (22%) + shake overnight (16 hours) + add 5 drops superfloc (0.2%) + filtration	Atomic Absorption Spectrometer	Blakemore *et al.* (1987)
Clay minerals	40 g soil + addition of water to raise volume to 500 mL + stir and leave to stand for 8 hours to settle down sand and silt particles. Afterwards, centrifuge at 3000 g for 5 minutes + decant the water + scoop out the clay from centrifuge tube and spread on a metallic sample holder + leave for air drying before analysis	X-Ray Diffraction (GBC, Emma)	Whitton and Churchman (1987)

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CHAPTER 4

RISK ASSESSMENT OF VEGETABLES IRRIGATED WITH ARSENIC CONTAMINATED WATER [1]

4.1 INTRODUCTION

Arsenic (As) contaminated water is used in South Asian countries to irrigate food crops, but the subsequent uptake of As by vegetables and associated human health risk is poorly understood (Chapter 1 and Chapter 2). This chapter comprises a glasshouse experiment where four vegetable species (carrot, radish, tomato and spinach) were tested for their uptake potential against a range of As concentrations in irrigation water. These crops are commonly grown around the world and show a high potential for As uptake when grown under hydroponic and soil conditions (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999a; Munoz *et al.*, 2002; Rahman *et al.*, 2004).

Understanding the As uptake response of vegetable species to irrigation with As-contaminated water has practical significance for South Asian countries where As-contaminated water is often used to irrigate food crops. A common irrigation technique used in South Asia is flood irrigation (also known as surface irrigation) where a field is divided into small plots surrounded by earth banks. Water is applied to plots by an adjacent channel and flows over the soil surface by gravity. When the plot is saturated/flooded (defined as about 2-3 cm water head), the water inflow is diverted to irrigate an adjacent plot. The surface water moves downward slowly in the field over a period of 2-3 days, during which time the soil remains in a flooded state. Aerobic conditions are re-instated once the surface water has drained. The effect of flood irrigation on As chemistry in soil where irrigation water is contaminated with this metalloid has not been previously considered and may affect both As mobility and uptake by plants.

The research reported in this chapter directly addresses the identified lack of information on As uptake by vegetables as a function of the As concentration of the irrigation water and

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[1] Components of this chapter have been published as Bhatti, S. M., Anderson, C. W. N., Stewart, R. B., & Robinson, B. H. (2013). Risk assessment of vegetables irrigated with arsenic-contaminated water. Environmental Science: Processes & Impacts, 15, 1866–1875

water management technique used, and the risk to humans that would be associated with the ingestion of potentially contaminated vegetables. The specific objectives of the current study were to determine: (i) the extent of arsenic accumulation and distribution in tissues of four vegetable species cultivated using a range of As concentration levels in irrigation water; (ii) the critical As concentration in water that may be acceptable for irrigation; (iii) the effect of irrigation techniques (flood versus non-flood irrigation) on As accumulation by vegetables; and (iv) the human risk associated with the ingestion of the vegetable specie(s) which accumulate the most arsenic.

4.2 MATERIALS AND METHODS

Four commonly grown vegetables, four concentrations of As in irrigation water, and two irrigation management techniques were used in the current study. The two irrigation techniques were (i) non-flooded water management where soil moisture was maintained at 70% field capacity (Fc) of the soil throughout the plant growth period and (ii) flooded water management where an alternating regime of saturation to 110% Fc of soil for three days followed by draining to attain aerobic conditions until the next irrigation event was used.

4.2.1 Crops

Four commonly grown vegetable species, carrot (*Daucus carota*, cv. All Year Round), radish (*Raphanus sativus,* cv. Champion), spinach (*Spinacia oleracea*, cv. Perpetual), and tomato (*Solanum esculentum,* cv. Italian Dwarf Romandore F1 hybrid) were selected for the current research. Carrot and spinach were grown under both non-flooded and flooded water management, while radish and tomato were only cultivated under non-flooded water management. Spinach and carrot were grown under both water management techniques because of their more extensive use in a range of cuisines (main dish, salad, and desserts) compared to radish and tomato. The experiment was conducted in a glasshouse at the Plant Growth Unit of Massey University Palmerston North, New Zealand with one healthy plant per pot. The experiment was laid down in a complete randomized design with three replications per treatment. The glasshouse temperature was maintained at 12 ± 2 ^{0}C minimum (night) and 22 ± 2 ^{0}C maximum (day).

4.2.2 Pot Preparation and Soil

Plastic pots (16.5 x 16.5 x 19 cm) were prepared to allow for both irrigation techniques. For the non-flood irrigation, the pots were drilled at the bottom (5 holes per pot) to provide aeration to plants. For the flood irrigation, a hole was drilled at one side of the pot (2 cm from the base) and a silicone rubber pipe (0.5 cm x 4 cm) was inserted. The portion of the silicone rubber which was inside the pot was attached to a PVC pipe (0.8 cm x 14 cm). This internal PVC pipe was cut through the upper side and wrapped with Coolaroo non-woven mulch mat (made of polypropylene material) to drain water. The portion of the silicone rubber which was outside the pot was attached to a PVC pipe (0.3 cm x 28 cm), and clamped to the top edge of the pot (Figure 4.1). Both types of pots were filled with a basal layer (500 grams) of gravel (> 2 mm) to facilitate water drainage and aeration. Each pot was filled with 4 kg of Rangitikei Silt Loam soil collected from a quarry adjacent to the Manawatu River near Palmerston North, New Zealand. The soil was irrigated with distilled water (to 50% field capacity) and fertilized with P and K prior to seeding. Nitrogen was applied to plants in two splits, prior to seeding and with the second irrigation. The rate of NPK used for the experiment was 50 mg N kg^{-1} (equivalent to 100 kg N ha^{-1} applied as DAP and urea), 22.5 mg P kg^{-1} (equivalent to 45 kg P ha^{-1} applied as DAP), and 67.5 mg K kg^{-1} (equivalent to 135 kg K ha^{-1} applied as K_2SO_4) according to the fertilizer guidelines of Wallace (2000).

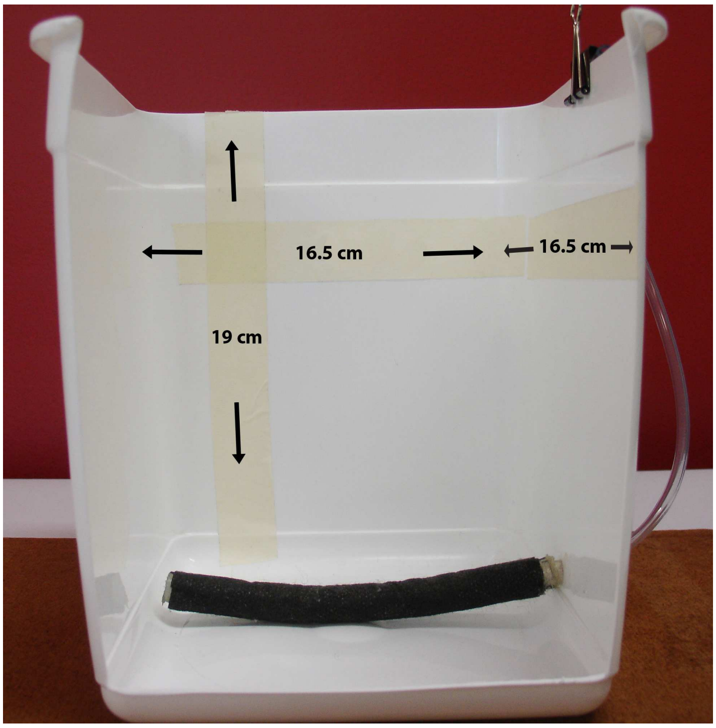


Figure 4.1 Image of the pot designed to model flood irrigation

4.2.3 Irrigation

Four arsenic concentrations in water (50, 100, 200, and 1000 µg L^{-1}) and a control (distilled water) were used as treatments. Arsenic irrigation waters were prepared from a stock solution of 1000 mg L^{-1} of sodium arsenate heptahydrate ($Na_2HAsO_4.7H_2O$). The irrigation was initiated once all the plants were germinated (10 days after sowing). On the day of irrigation, each pot was weighed and adjusted to 70% and/or 110% Fc with treatment water for non-flooded and flooded water management respectively. Flooded conditions were maintained for three days by clamping the external/exhaust pipe. On day four, the clamp was removed and the pots were drained. The irrigation frequency was every ten days for the non-flooded plants and fortnightly for the flooded plants. Immersion of the basal leaves was observed in the flooded plants, and we assume that some surface absorption of As would have been apparent for these plants while water remained standing on the soil surface (approximately 2-3 cm head).

4.2.4 Plant Harvest and Analysis

Plants were harvested at maturity (defined as the point of human consumption) and divided into various parts; spinach (leaves and roots), carrot and radish (leaves and taproot), and tomato (fruit, shoot, and roots). Carrot and radish taproots were further divided into peel (skin) and the edible root. The removal of radish and carrot skin before eating or cooking is a usual practice in South Asia. These plant parts were washed with distilled water; surface dried using paper towels, and weighed to determine the fresh biomass yield. Plants were then oven dried at 70 ^{0}C for four days and re-weighed for dry biomass. The dried plant parts were homogenized to a powder using a Cyclotec herbage mill (Model 1093, Salmond Smith Biolab Ltd.).

Subsamples (0.5 g) were digested in 5 mL of concentrated nitric acid (HNO_3) and analyzed using HGAAS as described in Chapter 3. A standard reference material (1573a, Tomato leaves, NIST) and sample blanks were included in the procedure. The limit of detection (LOD) for As in HGASS was 0.145 µg L^{-1} and the sample blanks were below this LOD value. The analyzed As concentration of the standard reference material (0.112 ± 0.007 µg g^{-1} n = 13) was in good agreement with certified reference value (0.112 ± 0.004 µg g^{-1}).

4.2.5 Soil Analysis

The experimental soil was analyzed for its physical and chemical properties (Table 4.1) by the methods described in Chapter 3. Total As concentration was determined in soil before and after vegetables cultivation. After vegetables harvest, the soil from each treatment pot was sampled using a vertical corer (1.76 cm x 11.25 cm) to determine the extent of As accumulation in soil as a function of irrigation.

For the determination of total As concentration in soil, 1.0 gram soil was digested in 10 mL *aqua-regia* (HCl: HNO_3, 3:1) and analyzed using HGAAS as described earlier in Chapter 3.

A reference soil material (CRM-GBW 07403, National Research Center for CRMs of China, Beijing) and sample blanks were analyzed in parallel with the soil samples and the latter were found to be below the instrument detection limit. The analyzed As concentration of the reference soil material (4.2 ± 0.05 $\mu g\ g^{-1}$ $n = 7$) was within the range of reported values (4.4 ± 0.6 $\mu g\ g^{-1}$).

The soil was an acidic silt loam with low levels of available P, SO_4 and CEC, and very low to medium contents of amorphous and crystalline Fe and Al (Table 4.1) (Blakemore *et al.*, 1987). The As concentration in the experimental soil was below the New Zealand soil limit for total As which is 20 mg kg^{-1} (NZWWA, 2003).

4.2.6 Statistical Analysis

The data for As concentration in plant tissues and soil after vegetable harvest was analyzed by ANOVA (One way and Two-way) using Minitab 16.1. The Tukey's test was used to determine the significant differences among treatment means using a probability value of 0.05.

All the graphs were prepared using Grapher 9.6 (Golden Software Inc. Colorado).

Table 4.1 Physical and chemical properties of the Rangitikei silt loam soil used for the experiment

Soil properties	**Values** [a]
pH (H_2O)	6.1 ± 0.05
Olsen P (µg P g^{-1})	7.05 ± 0.05
SO_4 (µg S g^{-1})	4.40 ± 0.40
Total C (%)	0.50 ± 0.15 (*n* = 3)
Total N (%)	0.02 ± 0.01 (*n* = 3)
Amorphous Fe (%)	0.38 ± 0.01
Crystalline Fe (%)	0.65 ± 0.03
Amorphous Al (%)	0.10 ± 0.01
Crystalline Al (%)	0.11 ± 0.01
CEC (me/100g)	10.50 ± 1.50
Texture	Silt loam [b]
Total As (µg g^{-1})	4.9 ± 0.02 (*n* = 3)

[a] values are mean ± SE (*n* = 2, unless mentioned); [b] Sand 14%, Silt 65%, and Clay 21%

4.2.7 Human health risk assessment

To evaluate the risk presented to humans through ingestion of edible parts of the vegetables in the current study, the As concentration in each vegetable was re-expressed on a fresh weight and assumed to be present in an inorganic form. The fresh weight concentrations were then compared to the Chinese food safety standard for inorganic As in foods, 0.05 mg kg^{-1} fresh weight (Heikens, 2006). Vegetables with an As concentration below this guideline value present an acceptable risk to humans while vegetables with an As concentration above the guideline value represent a potentially unacceptable risk to consumers.

To further quantify this potential risk, the USEPA defined Hazard Quotient (HQ) and Cancer Risk (CR) was calculated using following equations.

$HQ = EDI / RfD$ Equation 4.1, and

$CR = EDI \times CSF$ Equation 4.2

The detailed description of these equations can be referred in Chapter 2, Section 2.5.4.

4.3 RESULTS AND DISCUSSION

4.3.1 Arsenic concentration in vegetables

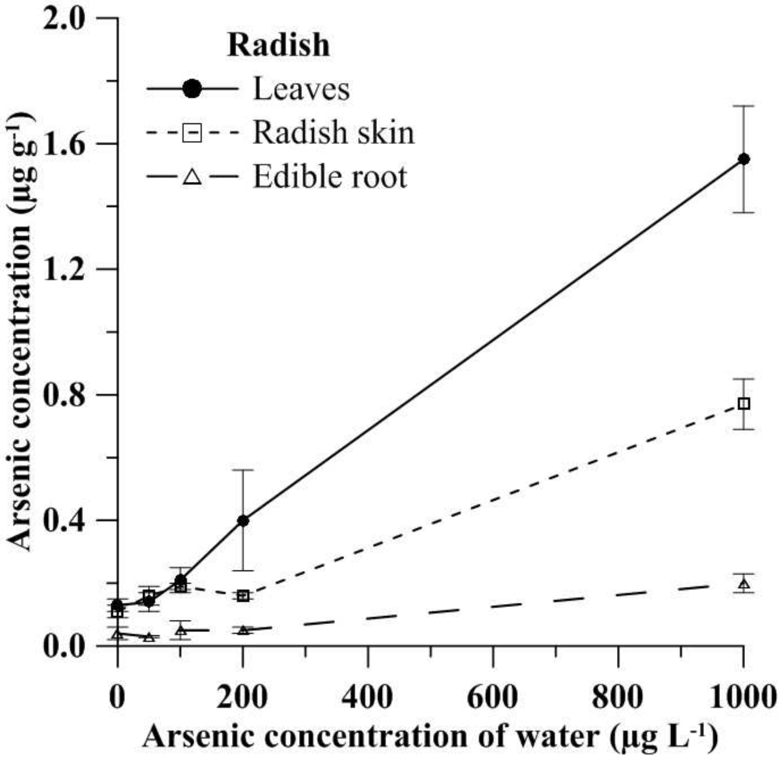
Arsenic accumulation and distribution within plant tissues and among plant species varied as a function of the As treatment and the irrigation technique used. The results for each species are reported separately, followed by comparison of As bioaccumulation among the studied vegetables and possible factors affecting the accumulation of As by the plants.

Radish

The As concentration in radish tissues (leaves, radish skin and edible root) was significantly higher in the plants irrigated with 1000 µg L^{-1} when compared to all other treatments ($P < 0.05$, Figure 4.2). An increase in As concentration in radish as a function of As exposure has been reported by other researchers (Cobb *et al.*, 2000; Marconi *et al.*, 2010). Among the various plant tissues, leaves accumulated the highest concentration of As, followed by radish skin and the edible root. A relatively higher concentration of As in leaves in current study is in agreement with those previous hydroponic and soil studies where a higher concentration of As has also been observed in leaves (Cobb *et al.*, 2000; Liu *et al.*, 2006; Smith *et al.*, 2008). However, our findings of a higher concentration of As in leaves contradicts some other previous hydroponic and soil studies where As was mostly retained in the roots (Carbonell-Barrachina *et al.*, 1999a; Smith *et al.*, 2009; Tlustos *et al.*, 2002a). Among the components of the taproot, skin accumulated a higher concentration of As by a factor of 2.7 to 5.3 relative to the edible root. A higher concentration of As in radish skin relative to edible root observed in this study is consistent with previous hydroponic and soil studies (Carbonell-Barrachina *et al.*, 1999a; Warren *et al.*, 2003).

Tomato

The As concentration in shoots and roots of tomato was significantly higher at an irrigation water concentration of 1000 µg As L^{-1} relative to the other treatments ($P < 0.05$, Figure 4.2). In contrast, there was no significant difference in As concentration in tomato fruit among the various treatments.



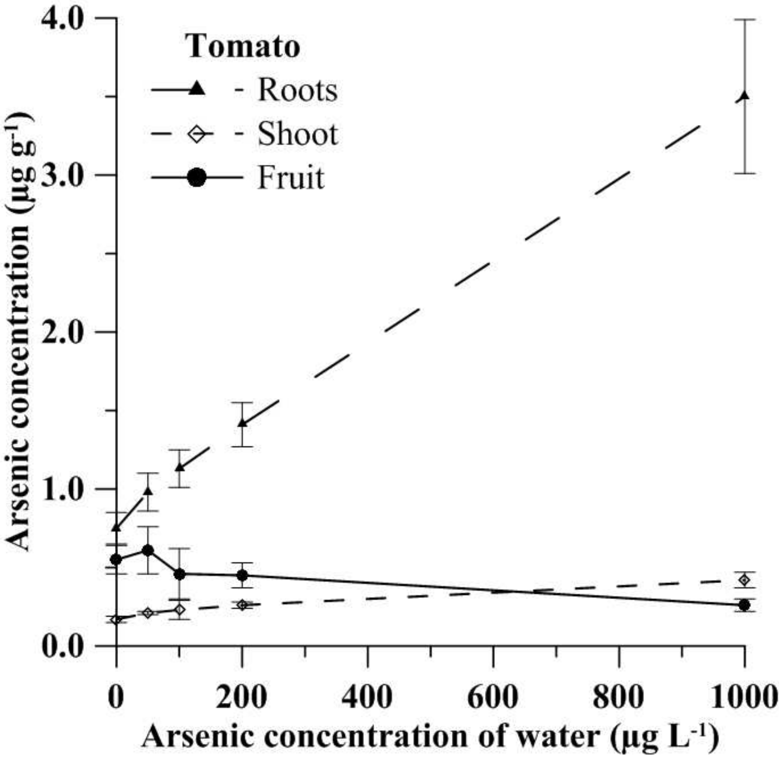


Figure 4.2 Total arsenic concentration (µg g^{-1} dry weight) in various parts of radish (top) and tomato (bottom) grown under non-flooded irrigation. Data are means ± SE (*n* = 3)

An increase in the As concentration of tomato roots and shoots as a function of As concentration in various growth media (soil, mixtures of soil and As-contaminated mine tailings and nutrient solution) has been reported (Burlo *et al.*, 1999; Cobb *et al.*, 2000; Miteva, 2002). Among the various plant tissues, the As concentration of roots was higher than in fruit and shoots under all treatments. Roots accumulated an As concentration that was 1.4 to 5.1 times higher than that recorded for the fruit and shoot. This higher concentration in roots relative to other tissues of tomato is consistent with previous studies that have investigated the response of tomato to arsenic (Carbonell-Barrachina *et al.*, 1997; Cobb *et al.*, 2000; Miteva, 2002). Burlo *et al.* (1999) reported that As in tomato plants was mainly accumulated in roots (85% of total As), followed by shoots (14%) and fruit (1%) when grown in nutrient solution. Carbonell-Barrachina *et al.* (1997) suggested that tomato plants tolerate As by exclusion, limiting its transport to shoots by increasing the As concentration in the roots. Among the upper plant parts, the As concentration in tomato fruit was significantly higher than in shoots for all the treatments, with the exception of the 1000 µg L^{-1} treatment ($P < 0.05$). This indicates that shoots may transport a large quantity of As to fruit at low As levels (< 1000 µg As L^{-1}), but transfer is inhibited or restricted above this level. Carbonell-Barrachina *et al.* (1997) suggested that when As is above a threshold level, the growth and transport function of a plant is affected, resulting in limited As translocation.

Spinach

Spinach was grown under both non-flooded and flooded irrigation. The response of spinach to As in water under both irrigation techniques is presented in Figure 4.3. The effect of the 1000 µg As L^{-1} irrigation treatment was significant under both irrigation techniques and resulted in a higher concentration of As in the leaves and roots of plants relative to the other treatments ($P < 0.05$). An increased As concentration in leaves of spinach with increasing As concentration in soil has been documented previously (Pavlik *et al.*, 2010). Among the plant tissues, roots had a higher As concentration than in leaves under both irrigation techniques. The As concentration in roots was 2.7 to 3.1 times higher than in leaves for plants under non-flooded water management, and 1.1 to 7.7 times higher under flooded water management. Tlustos *et al.* (2002b) also demonstrated a higher concentration of As in spinach roots relative to the aerial biomass. Nonetheless, a comparison of the As concentration in roots shows no significant difference for the same concentration of irrigation water under the two irrigation techniques.

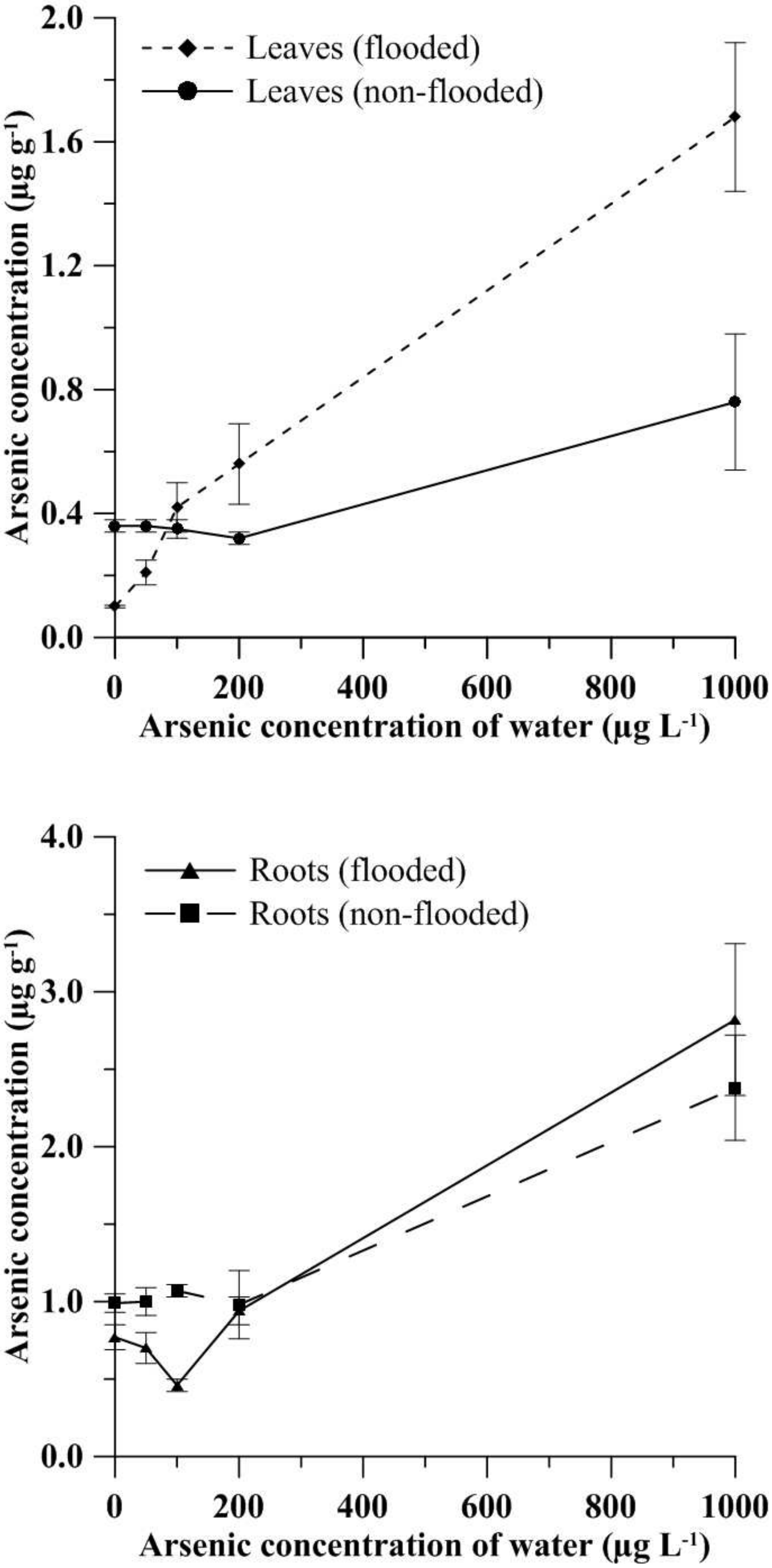


Figure 4.3 Total arsenic concentration (µg g^{-1} dry weight) in various parts of spinach grown under variable As treatments and irrigation techniques. Data are means ± SE (*n* = 3)

For leaves, a relatively higher As concentration was observed in flood irrigated spinach plants for the 100, 200 and 1000 µg As L^{-1} treatments relative to the plants subject to non-flood irrigation. There could be three possible explanations for such an increase, (i) a higher amount of As was introduced in the flooded pots due to an increase in the total volume of irrigation water (110% Fc vs. 70% Fc), (ii) direct absorption of As by spinach leaves from standing water during the initial phase of the flood irrigation event, and (iii) more translocation of As to aerial parts. An increased translocation of As in the current study is in agreement with the findings of Talukder *et al.* (2012), who reported that As is more easily translocated to the aboveground biomass of rice plants under anaerobic conditions than aerobic conditions.

Carrot

Carrot was also grown under both non-flooded and flooded irrigation. Among the various As treatments, plants irrigated with 1000 µg As L^{-1} had a significantly ($P < 0.05$) higher As concentration in their leaves than for other treatments under both irrigation techniques (Figure 4.4). A similar effect at this treatment level was also observed for edible roots subject to flood irrigation. Among the plant tissues, the trend of As concentration as a function of As in irrigation water differed between the two irrigation techniques. Under non-flood irrigation, carrot skin accumulated more As than leaves, while under flood irrigation, leaves accumulated more As than carrot skin. The reported higher concentration of As in leaves subject to flood irrigation may be attributed to enhanced translocation from roots under this water management, and surface absorption by leaves. Liu *et al.* (2006) found a higher concentration of As in carrot leaves than in edible parts and suggested that carrot leaves are efficient bio-accumulators of heavy metals/metalloids. Considering the components of the taproot, the skin accumulated more As than the edible root under both irrigation techniques. This phenomenon is consistent with other studies where a higher concentration of As in carrot peel (approximately 3 times) than the edible root has been reported (Helgesen & Larsen, 1998; Munoz *et al.*, 2002). Zandstra and De Kryger (2007) reported a higher concentration of As in the root shoulder and peel than the peeled root and attributed this to direct contact with soil particles. Overall, the As concentration in plant tissues (leaves, carrot skin and edible root) was higher in plants grown under flood irrigation relative to those grown under non-flood irrigation.

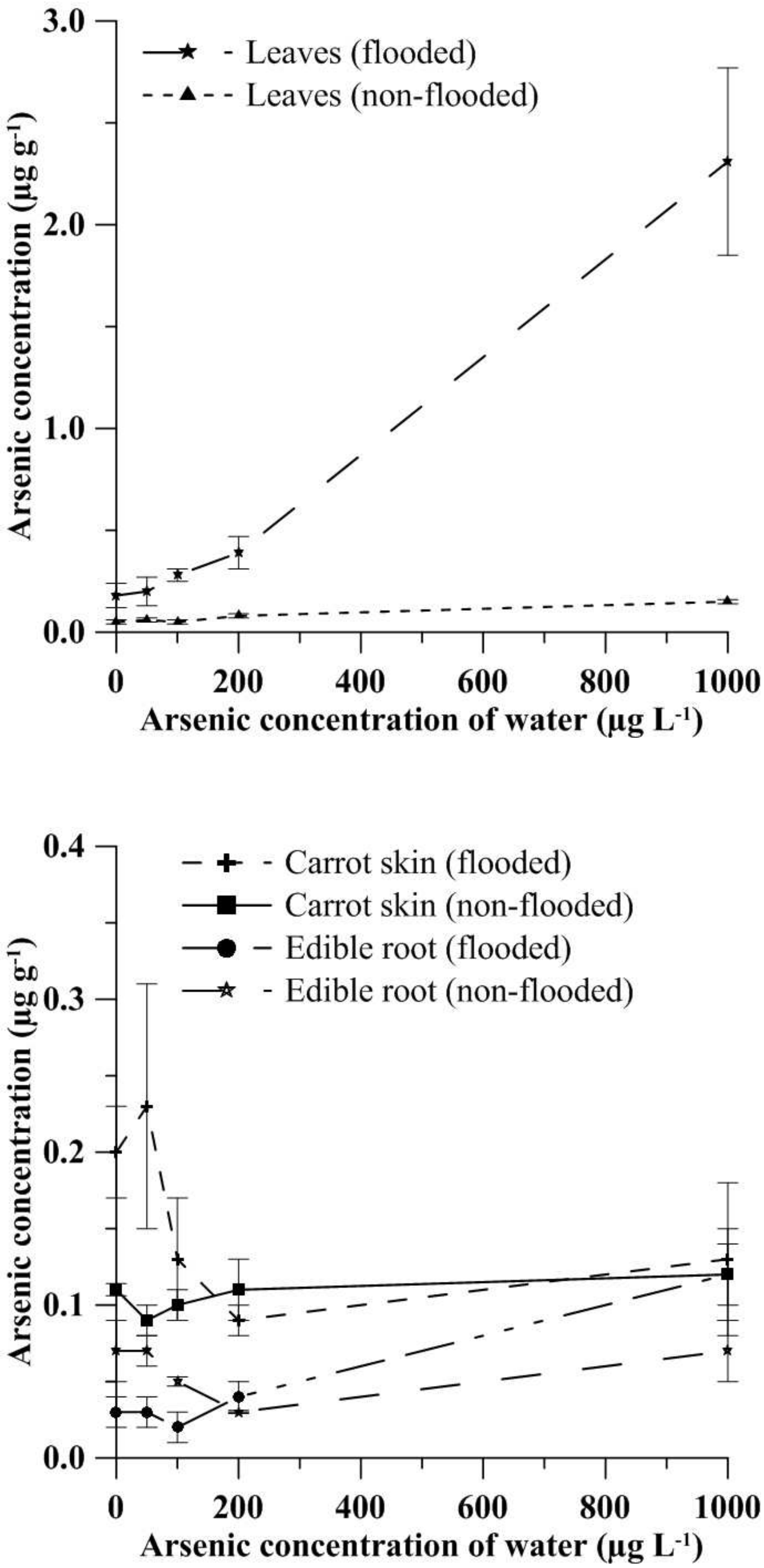


Figure 4.4 Total arsenic concentration (µg g^{-1} dry weight) in various parts of carrot grown under variable As treatments and irrigation techniques. Data are means ± SE (*n* = 3)

4.3.2 Arsenic content in vegetables and possible factors affecting accumulation in plants

Arsenic is a non-essential element for plants. The uptake of As by plants is a complex phenomenon and depends on various As-plant-soil factors, including As concentration and species, crop species, and soil redox conditions (Marin *et al.*, 1992; Onken & Hossner, 1995; Smith *et al.*, 2009; Wang *et al.*, 2002; Xu *et al.*, 2008). The results show that As accumulation by plants was dependent on the As concentration of irrigation water, the irrigation technique used, and the vegetable species tested. Figure 4.5 depicts the As content of the studied vegetables where content is defined as the As concentration (µg g^{-1}) x dry matter yield (g). The As content in plants varied among species, and can be ranked from high to low as spinach (flooded) > tomato > spinach (non-flooded) > radish > carrot (flooded) > carrot (non-flooded). This variation in As uptake among the vegetables may be attributed to genetic differences among these plants together with the irrigation technique used. Husaini *et al.* (2011) reported As accumulation in the order spinach > tomato > carrot > radish when these plants were irrigated with untreated industrial effluent. Similarly, Arain *et al.* (2009) reported higher As levels in leafy vegetables compared to root vegetables and grain crops collected from an agricultural field irrigated with As-contaminated lake water.

A second factor controlling As accumulation in plant species is the concentration of As present in irrigation water. In general, each species showed an increase in As content as a function of the As concentration in irrigation water (Figure 4.5). Such an increase in plant tissues with As concentration in growth media is well documented in literature (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999b; Pavlik *et al.*, 2010). Among the various As irrigation water levels, the 1000 µg As L^{-1} treatment promoted the highest As concentration in these plant species. This is consistent with the findings of Dahal *et al.* (2008), who found a positive correlation between the As concentration in plants and the As concentration of irrigation water. These researchers also reported the highest As concentration in all parts of the studied vegetables in samples collected from plots irrigated with the highest As concentration of this study (1.014 mg As L^{-1} in groundwater).

A third possible factor affecting the As concentration in plants of the current study is the irrigation technique used. The flood and non-flood irrigation techniques could differentially affect soil redox potential which subsequently affect As mobility, speciation and uptake by plants (Li *et al.*, 2009; Talukder *et al.*, 2012; Xu *et al.*, 2008).

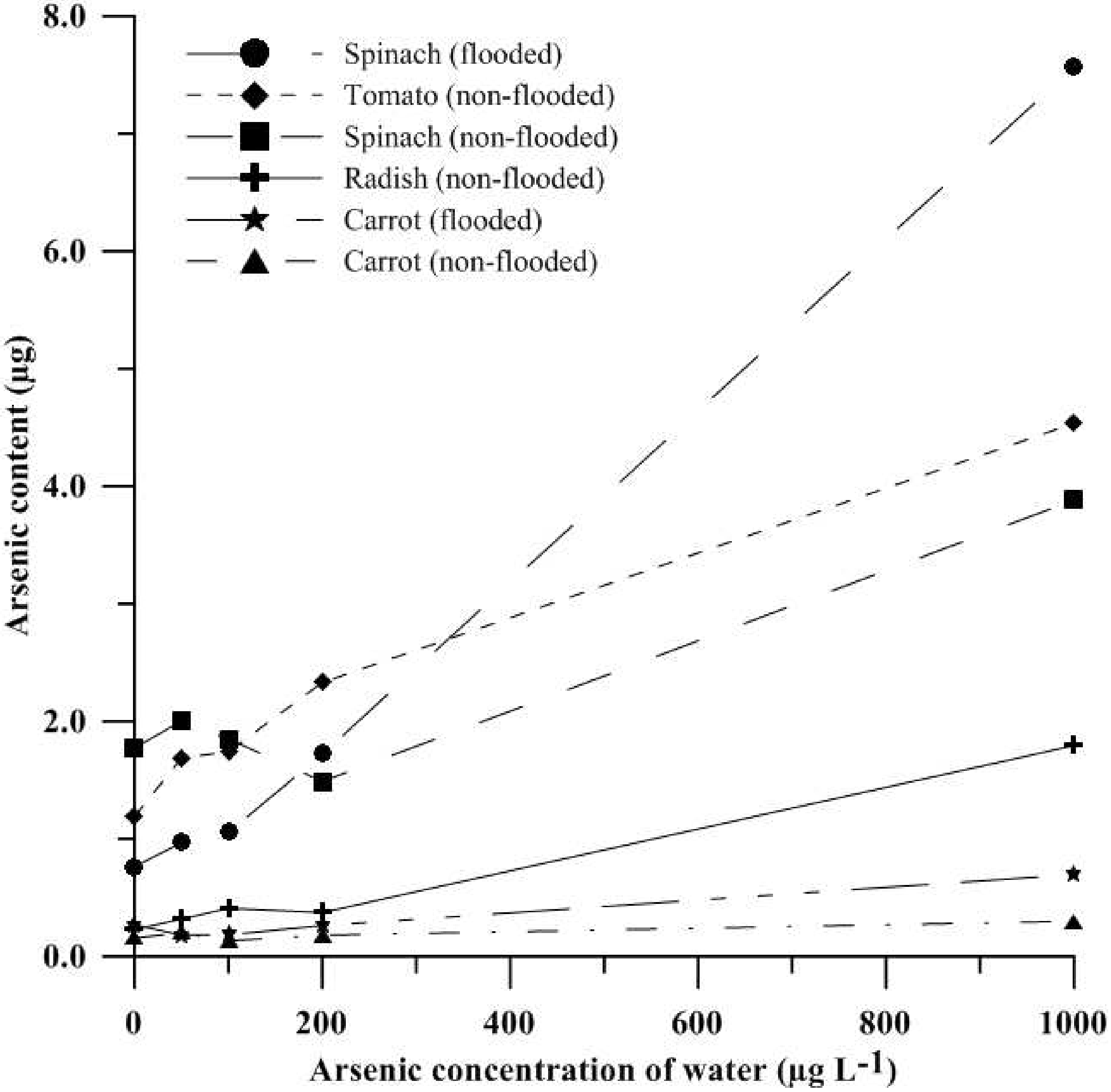


Figure 4.5 Arsenic content (µg) in four vegetables as a function of As treatments and irrigation techniques. Data points represent the mean value for each species calculated as a weighted function of the relative mass of each plant organ. *n* was therefore variable among crops. For radish, carrot and tomato *n* = 9 (for example in radish, triplicate values of each of leaves, radish skin and edible root). For spinach *n* = 6 (triplicate values of each of leaves and roots).

The soil redox potential was not measured in the current work because the system was in a state of flux and measuring redox would have disturbed the system. However, we assume that the soil subject to flood irrigation will have had a periodically lower redox potential than the soil subject to non-flood irrigation. Li *et al.* (2009) reported that flooded water management significantly reduced soil redox potential compared with aerobic treatment for an acidic silty clay loam soil. Similarly, Talukder *et al.* (2012) reported a highly reduced redox potential (-41 to -76 mV) under flooded water management relative to aerobic water management (+135 to +138 mV) for an acidic sandy loam soil.

In this study, there was a lower As content in the vegetables grown under non-flood irrigation than the plants under flood irrigation. A similar finding for rice was reported by Xu *et al.* (2008) who found a 10-15 fold higher concentration of As in the grain of paddy rice relative to dry land rice. Lower As content under non-flooded conditions may be due to sorption of As with hydrous oxides minerals effecting a reduction in As mobility and uptake by plants (Brammer & Ravenscroft, 2009; Masscheleyn *et al.*, 1991; Xu *et al.*, 2008). In contrast, for soils subject to flood irrigation management, As mobility may have been higher due to (i) reduction of arsenate (As^{V}) to arsenite (As^{III}), and (ii) dissolution of metal hydrous oxides which releases the adsorbed As, leading to increased uptake by plants (Abedin *et al.*, 2002; Masscheleyn *et al.*, 1991; Xu *et al.*, 2008).

4.3.3 Risk Assessment

The As concentration in both the edible portion and taproot of carrot (µg g^{-1} fresh weight, Table 4.2) was less than the Chinese maximum permissible concentration (MPC) for all irrigation treatments. Ingestion of carrot cultivated under the conditions of this study therefore presents an acceptable risk to human health. The edible portion of radish was also acceptable for consumption; however for the 1000 µg As L^{-1} irrigation treatment, the As concentration in taproot was above the MPC. In tomato fruit, the As concentration was equivalent to the MPC level for As concentrations in irrigation water less than 1000 µg L^{-1}. In contrast, for spinach leaves, the As concentration was above the MPC level for most treatments under both irrigation techniques. The As concentration in spinach leaves for the 100, 200, and 1000 µg L^{-1} treatments under flood irrigation and for the 1000 µg L^{-1} treatment under non-flood irrigation was of concern. For these treatments, As was 1.6 to 6.4 times higher than the MPC level. These values were further explored to determine potential risk to humans using the USEPA hazard quotient (HQ) and cancer risk (CR) calculations.

Table 4.2 Total arsenic concentration (µg g^{-1} fresh weight) in the edible parts of four common vegetables

	Radish		Tomato	Spinach		Carrot			
As in water (µg L^{-1})	**non-flooded**		**non-flooded**	**non-flooded**	**flooded**	**non-flooded**		**flooded**	
	Edible root	**Taproot**	**Fruit**	**Leaves**	**Leaves**	**Edible root**	**Taproot**	**Edible root**	**Taproot**
0	0.003	0.011	0.055	0.058	0.019	0.008	0.022	0.004	0.032
50	0.002	0.014	0.061	0.058	0.040	0.008	0.019	0.004	0.036
100	0.004	0.018	0.046	0.056	0.080	0.006	0.019	0.003	0.021
200	0.004	0.015	0.045	0.051	0.106	0.004	0.018	0.006	0.017
1000	0.014	0.068	0.026	0.122	0.319	0.008	0.022	0.017	0.034

Mean fresh weight As concentration was calculated as a product of mean As concentration (µg g^{-1} dry weight) and mean water content of each vegetable species according to the formula: Fresh weight µg g^{-1}= (Dry weight µg g^{-1}) x (1- % moisture/100)

Use of the HQ and CR risk assessment model requires quantification of the daily vegetable consumption for the target population. The average daily consumption of vegetables per person varies among countries (Table 4.3). Considering the eating habit of people of West Bengal India, where vegetables are eaten with each of three meals a day (Roychowdhury *et al.*, 2003; Samal *et al.,* 2011), the intake of 500 grams per day was used for further calculation to consider the worst case scenario. Only adults (> 18 years old; body weight 60 kg) and adolescents (12-18 years old; body weight 50 kg) are considered in this discussion to provide a conservative picture of exposure. Sufficient vegetable intake data for children (< 12 years) in South Asian countries is not available and was therefore not considered in this discussion.

Assuming a scenario where 500 grams of spinach (Fi) is consumed on 52 days in a year (Ef), the HQ value ranged from 0.32 to 1.26 for adults and 0.38 to 1.51 for adolescents (Table 4.4 and Appendix IV). A higher HQ value for adolescents compared to adults infers greater risk for this demographic. The Hazard Quotient exceeded 1 and defines unacceptable non-carcinogenic risk for both adults and adolescents consuming spinach cultivated under flood irrigation with water containing an As concentration of 1000 µg L^{-1}. Calculation of the parameter CR shows that there is an increased probability of cancer through ingestion of spinach leaves cultivated under flood irrigation with water containing 100 µg As L^{-1} or greater (probability in excess of 1 in 10,000). The cancer risk also exceeded 1 in 10,000 for spinach cultivated using non-flood irrigation with water containing 1,000 µg L^{-1} As. This risk from spinach consumption may be higher in areas where vegetables consumption is higher, and where As-contaminated water is also used for drinking and cooking.

4.3.4 Arsenic concentration in soil after vegetable harvest

An increase in the As concentration of soil as a function of As concentrations in irrigation water was observed for soils of all vegetable species (Table 4.5). However, the As concentration in soil was significantly higher where 1000 µg L^{-1} water was applied relative to all other treatment water levels. Arsenic concentration for 1000 µg L^{-1} increased the As concentration in soil by 5 to 32% relative to the control treatment at the time of vegetable harvest. Increase in soil As concentration for current study is consistent with previous studies where As-contaminated water was applied to soil (Baig & Kazi, 2012; Moyano *et al.,* 2009; Saha & Ali, 2010).

Table 4.3 Daily average vegetable intakes (gram fresh weight) per capita around the world

Region/ Country	**Vegetables consumption**	**References**
Bangladesh	130 to 205	Alam *et al.* (2003); Khan *et al.* (2009); Rahman *et al.* (2012)
USA	162	Tao and Bolger (1999)
Republic of Croatia	275	SapunarPostruznik *et al.* (1996)
Santiago, Chile	327	Munoz *et al.* (2005)
Denmark	376	Helgesen and Larsen (1998)
India	450 to 500	Samal *et al.* (2011); Roychowdhury *et al.* (2003)

Table 4.4 Hazard Quotient (HQ) and Cancer Risk (CR) for the ingestion of spinach leaves as a function of the concentration of As in irrigation water and the irrigation technique used

As in water ($\mu g\ L^{-1}$)	**Irrigation Technique**	**^{a}C ($\mu g\ g^{-1}$ fresh weight)**	**Hazard Quotient (HQ)**		**Cancer Risk (CR)**	
			Adults ^{b}	**Adolescent ^{c}**	**Adults**	**Adolescent**
100	flooded	0.080	0.32	0.38	1.4×10^{-4}	1.7×10^{-4}
200	flooded	0.106	0.42	0.50	1.9×10^{-4}	2.3×10^{-4}
1000	non-flooded	0.122	0.48	0.58	2.2×10^{-4}	2.6×10^{-4}
	flooded	0.319	1.26	1.51	5.7×10^{-4}	6.8×10^{-4}

^{a} Concentration of arsenic in spinach leaves; ^{b} greater than 18 years old; ^{c} 12-18 years old; Parameters have been calculated where the As concentration in spinach leaves exceeds the MPC of 0.05 $\mu g\ g^{-1}$ fresh weight

Table 4.5 Total arsenic concentration (µg g^{-1}) in soil after vegetable harvest under variable As treatments and irrigation techniques

As in water (µg L^{-1})	**Radish**	**Tomato**	**Spinach** (non-flooded)	**Spinach** (flooded)	**Carrot** (non-flooded)	**Carrot** (flooded)
0	4.81 ± 0.06 ^{d}	4.48 ± 0.06 ^{b}	4.84 ± 0.02 ^{B}	^{b} 5.18 ± 0.10 ^{A}	^{b} 5.11 ± 0.05 ^{A}	^{b} 4.49 ± 0.05 ^{B}
50	5.02 ± 0.07 ^{cd}	4.46 ± 0.05 ^{b}	4.80 ± 0.05 ^{A}	^{b} 5.08 ± 0.10 ^{A}	^{b} 5.21 ± 0.07 ^{A}	^{b} 4.58 ± 0.08 ^{B}
100	5.33 ± 0.06 ^{bc}	4.52 ± 0.04 ^{b}	5.01 ± 0.08 ^{A}	^{b} 5.26 ± 0.08 ^{A}	^{b} 5.09 ± 0.05 ^{A}	^{b} 4.61 ± 0.03 ^{B}
200	5.34 ± 0.03 ^{b}	4.65 ± 0.03 ^{b}	4.81 ± 0.05 ^{B}	^{b} 5.37 ± 0.10 ^{A}	^{b} 5.28 ± 0.06 ^{A}	^{b} 4.71 ± 0.01 ^{B}
1000	6.01 ± 0.09 ^{a}	5.76 ± 0.10 ^{a}	5.08 ± 0.10 ^{B}	^{a} 5.92 ± 0.01 ^{A}	^{a} 6.09 ± 0.03 ^{A}	^{a} 5.91 ± 0.14 ^{A}

Data are means ± SE ($n = 3$); Mean in each column followed by different letters (in lower case) are significantly different among As treatments ($P < 0.05$); Mean in each row followed by different letters (in upper case) are significantly different between irrigation techniques at a particular As treatment ($P < 0.05$)

The effect of irrigation technique was significant for As concentration in soil where spinach and carrot were grown ($P < 0.05$). However, the trend was different for both crop species. Where spinach was grown, the soil subjected to flood irrigation retained more As in soil relative to soil under non-flood irrigation. Where carrot was grown, the soil subjected to non-flood irrigation technique had more As than flood irrigation technique. A high As retention in soil where spinach was grown with flood irrigation may be attributed to higher amount of As introduced to flooded pots during each irrigation event (110% Fc versus 70% Fc). For soils where carrot was grown, this generalization was not satisfied. One possibility is that, less As retention in soil is due to high As uptake by carrot under flood irrigation technique relative to the carrot under non-flood irrigation. However, this hypothesis is invalid because there appears no significant difference in As uptake in carrot grown in flooded versus non-flooded irrigation (Figure 4.5). It appears that As may have been leached from the soil where carrot was grown under flood irrigation, however, this proposition requires further investigation.

4.4 CONCLUSIONS

The effect of irrigation with 1000 µg As L^{-1} was significant in all vegetable species (carrot, radish, spinach and tomato) relative to the other treatments and enhanced the As concentration of each plant. A similar trend of increased As concentration with 1000 µg L^{-1} was observed in soil after vegetables harvest. The distribution of As among vegetables tissues varied for the species used. Tomato and spinach accumulated a higher As concentration in roots relative to aerial biomass, while radish and carrot accumulated a higher As concentration in leaves and skin relative to edible root. Among the studied vegetables, As uptake increased in the order carrot < radish < tomato < spinach. The effect of irrigation technique was significant on the As concentration in the studied vegetables. Spinach and carrot grown under flood irrigation had a higher As concentration in aerial biomass relative to non-flood irrigation, possibly due to the increased mobility and bioavailability of As. In terms of risk to human health from consuming the edible parts of these vegetables, these findings indicate that spinach leaves accumulate a significant level of As under the treatments used, ranging from 1.6 to 6.4 times higher than the Chinese maximum permissible level of As in vegetables (0.05 µg g^{-1} fresh weight). Spinach leaves also pose an unacceptable carcinogenic and non-carcinogenic risk to humans upon their consumption. This risk is quantified by calculated HQ and CR values (USEPA), where an HQ value greater than 1 represents an unacceptable non-carcinogenic risk and a CR value greater than 10^{-4} represents an unacceptable carcinogenic risk. The HQ value for spinach ranged from 0.32 to 1.26 for adults and 0.38 to 1.51 for adolescents while the CR value ranged from 1.4 x 10^{-4} to 5.7 x 10^{-4} for adults and 1.7 x 10^{-4} to 6.8 x 10^{-4} for adolescents. Irrigation water with an As concentration greater than 50 μg L^{-1} should be avoided for spinach cultivation where flood irrigation is practiced.

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CHAPTER 5

ARSENIC ADSORPTION IN SOILS

5.1 INTRODUCTION

Arsenic (As) is a toxic element and its behaviour at the soil-plant interface is important for biota and ecosystem functioning. The bioavailability and mobility of As in soils is controlled by adsorption-desorption reactions (Bolan *et al.*, 2012; Zhang & Selim, 2005). Understanding such reactions is therefore prerequisite to determine the fate and behavior of As in soil.

Arsenic adsorption varies among soils and depends on both As speciation and soil factors; a detailed description of these factors is presented in Chapter 2. In brief, the oxidation state of As (As^{V} or As^{III}) is the overriding factor that will dictate the extent to which As will be retained by soil and mineral surfaces. Under equivalent As concentrations, As^{V} is adsorbed in higher quantity than As^{III} (Naidu *et al.*, 2009; Smith *et al.*, 1999). However, the adsorption of As^{V} and As^{III} is highly pH dependent (Pierce & Moore, 1980; Xu *et al.*, 1991). An increase in soil pH decreases As^{V} adsorption but increases As^{III} adsorption (Smith *et al.*, 1999). Other soil properties that influence As adsorption include the type and nature of minerals and organic constituents, clay content, and the presence of competing ions (Elkhatib *et al.*, 1984a; Grafe *et al.*, 2001; Manning & Goldberg, 1996; Redman *et al.*, 2002). In general, soils with a higher concentration of Fe, Al and Mn hydrous oxides, and a higher clay content, retain more As than those with less of these mineral constituents (Deschamps *et al.*, 2003; Manning & Goldberg, 1997b; Smith *et al.*, 1999).

The adsorption of As to soil surfaces can be modeled by Langmuir and/or Freundlich isotherm equations (Elkhatib *et al.*, 1984a; Manning & Goldberg, 1997b; Nam *et al.*, 2010; Sahu *et al.*, 2011). The values of these models' parameters (b, K, n and K_f) vary between published studies, depending on many factors that include soil mineralogy, reaction time, solution pH, As concentration and its oxidation state (Zhang & Selim, 2008). The Langmuir and Freundlich isotherms are empirical models with specific assumptions. The Langmuir equation is based on three assumptions: (i) a constant energy of adsorption that is independent of the extent of surface coverage (a homogenous surface); (ii) adsorption on specific sites, with no interaction between solute (adsorbate) molecules; and (iii) maximum adsorption equal to a complete monolayer on all reactive adsorbent surfaces (Bohn *et al.*, 1985). The Langmuir equation has an advantage of providing an adsorption maximum of a soil or mineral component. In contrast, the Freundlich equation is based on the assumption that the energy of adsorption decreases with an increase in surface coverage due to surface heterogeneity (Bohn *et al.*, 1985). This model quantifies the partitioning of a solute (As) between soil and liquid phases, expressed by K_f (Sahu *et al.*, 2011; Zhang & Selim, 2008). A relatively low K_f value indicates that a significant portion of a solute (As) will be potentially bioavailable (Naidu *et al.*, 2009).

Elevated levels of As (> 100 mg kg^{-1}) in agricultural soils of New Zealand have been reported due to long term use of agrichemicals and timber preservation compounds (Gaw *et al.*, 2006; Robinson *et al.*, 2006). Sheep and cattle dip sites are of major concern, where a typical concentration of 1000 to 3000 mg kg^{-1} is common, and at some sites the concentration has reached 11,000 mg kg^{-1} (Ministry for the Environment, 2006). Elevated concentrations of As in the Waikato River (0.001 to 0.153 mg L^{-1}) and geothermal waters (0.008 to 9.8 mg L^{-1}) from the Taupo Volcanic Zone New Zealand have also been reported (Lord *et al.*, 2012; Robinson *et al.*, 2003). Elevated levels of As in water can be a function of the release of either anthropogenic or geogenic contamination into aquatic systems. Despite the apparent concern, limited data is available that describes As adsorption to New Zealand soils. Moreover, the reported adsorption studies have been limited to As^V with As concentration levels many orders of magnitude higher (up to 500 mg L^{-1}) than those naturally found in soil solution and/or soil irrigated with As-contaminated water (Bolan *et al.*, 2012; Carey *et al.*, 1996; Naidu *et al.*, 2009). New Zealand soils are generally young, less weathered, and rich in organic matter, short-range order aluminosilicates (allophane and imogolite) and oxyhydroxides of Fe and Al (McLaughlin *et al.*, 2000).

The specific objectives of this study were to (i) determine As adsorption kinetic (time required for equilibration of As) in the selected soils, (ii) model As adsorption behavior of selected soils through Langmuir and Freundlich isotherms, and (iii) define the soil properties that control As adsorption parameters in the studied soils.

5.2 MATERIALS AND METHODS

5.2.1 Soils

The Rangitikei silt loam, Korokoro silt loam and Tokomaru silt loam soils were selected for the current experiment. These soils vary in their chemical properties and represent 3 of the soil orders recognized in New Zealand (Recent, Brown, and Pallic) that cover 61% of New Zealand land area (Hewitt, 2010; NZ Soils Portal Landcare Research Ltd, 2013). These soils were selected since they have similar texture (light textured) as soils of South Asia where vegetables are grown; for example in Pakistan, soils commonly used for vegetable cultivation are silt loams, sandy loams and loams (Jaffri *et al.*, 1976; Khoso, 1994).

Surface samples (0-10 cm) of Korokoro silt loam (Brown soil) and Tokomaru silt loam (Pallic soil) were collected from Palmerston North New Zealand by Jeyakumar for his PhD research (Jeyakumar, 2010). Rangitikei silt loam (Recent soil) was collected from a quarry adjacent to the Manawatu River near Palmerston North by the author. This soil was also used for glasshouse experiment described in this thesis (Chapter 4). The Rangitikei soil was further amended with calcium hydroxide to achieve a pH level of 7.5 to model the pH level of South Asian soils which is generally in the range of 7.0 to 7.9 (Baig & Kazi, 2012; Bhattacharya *et al.*, 2010; Panaullah *et al.*, 2009). To achieve the target soil pH level of 7.5, calcium hydroxide (analytical grade) was added to Rangitikei silt loam soil and allowed to equilibrate for 14 days. This amended soil was included in the adsorption isotherm experiment as a fourth soil.

Selected soils were air dried and sieved through a 2 mm stainless steel mesh before analysis and/or adsorption studies.

5.2.2 Soil Analysis

A detailed description of soil analysis is given in Chapter 3. In brief, Olsen P, SO_4, pH, amorphous and crystalline Fe and Al concentrations in the selected soils were determined by the methods of Blakemore *et al.* (1987). Total C was measured using a Leco furnace. Soil texture was determined by the pipette method (Claydon, 1989). Total As in soil was analyzed using HGAAS after digestion of the soils in *aqua-regia* (HCl: HNO_3, 3: 1). Mineral content of soils was determined by XRD (X-Ray Diffraction, GBC, Emma).

5.2.3 Arsenic adsorption kinetics

To investigate the As adsorption kinetic behavior of the selected soils, a subsample (1.0 g) of each soil (in duplicate) was weighed into polypropylene centrifuge tubes. To each tube, 20 mL of 1.0 mg L^{-1} As^{V} or As^{III} solution was added. Arsenate (As^{V}) and arsenite (As^{III}) were included in this experiment since these both As species are mainly present in irrigation water (Baig & Kazi, 2012; Norra *et al.*, 2005). The As solutions were prepared in 0.01 M $Ca(NO_3)_2.4H_2O$ background solution. Calcium as a background electrolyte is commonly used in batch studies and is believed to increase As adsorption (more of As^{V} than As^{III}) by enhancing surface positive charge (Carey *et al.*, 1996; Smith *et al.*, 2002; Wenzel, 2012). Addition of calcium electrolyte was also used to represent the soil solution composition of South Asian soils where calcium is a dominant element. The desired concentration of 1.0 mg L^{-1} was prepared by dilution of 1000 mg L^{-1} stock solutions of sodium arsenate ($Na_2HAsO_4.7H_2O$) or sodium meta-arsenite ($NaAsO_2$) for As^{V} and As^{III} respectively.

Soil suspensions were shaken for a specific length of time using an end-over-end shaker. The time points were (hours): 0.25, 0.50, 1, 3, 6, 12, 24, and 36. After each defined time point, the tubes were centrifuged at 10,000 rpm for ten minutes and the supernatant were filtered through a cellulose nitrate filter of 0.45 µm pore size. This filtration technique is rapid and minimizes the adsorption of element onto apparatus (Campbell & Davies, 1995). The aliquot was analyzed for As using a HGAAS, as described in Chapter 3. The amount of As adsorbed by each soil was calculated as the difference between the amount of As added initially and the amount remaining in the solution after each reaction time.

The As adsorption kinetic studies were limited to three soils, Rangitikei silt loam (unamended), Korokoro silt loam and Tokomaru silt loam soil.

5.2.4 Arsenic adsorption isotherms

One gram subsample of each soil (in triplicate) was equilibrated with 20 mL of As^V or As^{III} solution at one of the five As concentrations (mg L^{-1}): 0.05, 0.1, 0.2, 0.5, and 1.0. Calcium nitrate (0.01 M $Ca(NO_3)_2.4H_2O$) was used as the background electrolyte. The soil samples were shaken for 48 hours. The samples were centrifuged, filtered and analyzed for As, as described above in the adsorption kinetic section.

Adsorption isotherms studies were conducted on four substrates i.e. the three previously described soils used for adsorption kinetic study, and the Rangitikei soil that was amended with calcium hydroxide to achieve a pH level of 7.5.

5.2.5 Arsenic adsorption modeling

The adsorption data were modeled using Langmuir and Freundlich equations. The linear form of the Langmuir equation used for calculation was:

$C/(x/m) = 1/Kb + C/b$ Equation 5.1 (Bohn *et al.*, 1985)

Where C (µg mL^{-1}) is the concentration of As left in the solution after reaction time, x/m (µg g^{-1}) is the amount of As adsorbed per unit mass of soil, K (mL µg^{-1}) is Langmuir bonding energy coefficient, and b (µg g^{-1}) is the adsorption maxima per unit mass of soil . The Langmuir isotherm was constructed by plotting C/(x/m) against C, and the model was considered appropriate if the resulting plot was linear (Campbell & Davies, 1995). Using least squares linear regression, b was calculated as reciprocal of the slope and K as the slope divided by the intercept (Bohn *et al.*, 1985; Campbell & Davies, 1995).

The linear form of the Freundlich equation used was:

$\log x/m = 1/n \log C + \log K_f$ Equation 5.2 (Bohn *et al.*, 1985)

The terms x/m and C (expressed in units of ng g^{-1} and ng mL^{-1} respectively) are the same as defined previously for the Langmuir equation. The Freundlich isotherm was obtained by plotting the natural log of x/m against the natural log of C. The parameter K_f (mL g^{-1}) is the Freundlich partition coefficient (Sahu *et al.*, 2011; Zhang & Selim, 2008). The parameter n is a unitless measure of isotherm nonlinearity with values commonly less than 1 (Campbell & Davies, 1995; Nam *et al.*, 2010; Yolcubal & Akyol, 2008). The parameters K_f and n were defined from least square linear regression where n is the slope and K_f is the intercept.

5.2.6 Statistical analysis

A relationship between soil properties and the Langmuir-Freundlich parameters (b and K_f) was determined by Pearson's correlation. Since correlation does not imply causation, a stepwise regression analysis (backward elimination, $\alpha = 0.10$) was performed on the data set to determine the most significant soil properties that control Langmuir and Freundlich parameters (b and K_f). All the statistical analyses were performed using Minitab 16.1 software.

5.3 RESULTS AND DISCUSSION

5.3.1 Soil characteristics

Table 5.1 shows that the experimental soils were naturally acidic (pH 5.3 to 6.1), varied from low to high Olsen P (7.05 to 37.15 $\mu g\ g^{-1}$), and very low to medium extractable sulphate (4.40 to 16.25 $\mu g\ S\ g^{-1}$) (Blakemore *et al.*, 1987). The background total As concentration in these soils ranged from 1.7 to 4.9 $\mu g\ g^{-1}$ which is below the New Zealand soil limit for total As, defined as 20 $\mu g\ g^{-1}$ (NZWWA, 2003). The soils contain very low to medium amorphous and crystalline Fe and Al contents (Blakemore *et al.*, 1987).

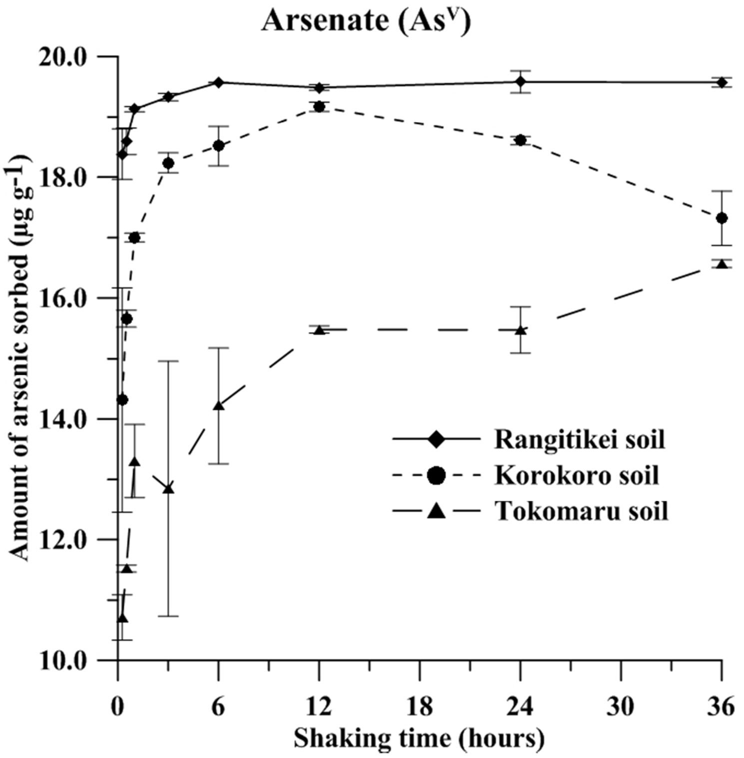
5.3.2 Arsenate and arsenite adsorption kinetics

Figure 5.1 shows the kinetic behavior of selected soils to As^{V} and As^{III} species. The amount of As adsorbed onto each soil was dependent on the reaction time, soil type and As species applied. For As^{V}, the greatest rate of As adsorption was observed in the first 3 hours. For the Rangitikei silt loam soil, 97% of As was adsorbed within 3 hours, and reached to 98 percent in 36 hours. In Korokoro silt loam soil, 91% of As was adsorbed within 3 hours, and adsorption reached a maximum at 12 hours (96%) and decreased thereafter. The reason for this decrease is unknown. For the Tokomaru silt loam soil, 64% of added As was adsorbed within 3 hours, followed by a slow increase to 83% in 36 hours.

Table 5.1 Characteristics of the experimental soils

Properties	**Rangitikei Soil**	**Korokoro Soil**	**Tokomaru Soil**
pH (H_2O)	6.1 ± 0.05 [a]	5.3 ± 0.05	5.9 ± 0.01
Olsen P (µg P g^{-1})	7.05 ± 0.05	13.80 ± 1.80	37.15 ± 0.85
SO_4 (µg S g^{-1})	4.40 ± 0.40	13.00 ± 1.00	16.25 ± 3.75
Total C (%)	0.50 ± 0.15 (n = 3)	5.70 ± 0.07	3.54 ± 0.02
Amorphous Fe (%) [b]	0.38 ± 0.01	0.76 ± 0.04	0.39 ± 0.03
Crystalline Fe (%) [b]	0.65 ± 0.03	0.40 ± 0.01	0.22 ± 0.03
Amorphous Al (%) [b]	0.10 ± 0.01	0.43 ± 0.02	0.20 ± 0.01
Crystalline Al (%) [b]	0.11 ± 0.01	1.03 ± 0.09	0.15 ± 0.05
Texture	Silt loam (Sand 14%, Silt 65%, and Clay 21%)	Silt loam (Sand 5%, Silt 72.5%, and Clay 22.5%)	Silt loam (Sand 28%, Silt 59.5%, and Clay 12.5%)
Total As (µg g^{-1})	4.9 ± 0.02 (n = 3)	3.7 ± 0.07 (n = 3)	1.7 ± 0.05 (n = 3)
Dominant minerals	Quartz, Mica, Feldspar	Quartz, Feldspar	Quartz, Feldspar
Soil collection site	Quarry adjacent to Manawatu River Palmerston North	Tuapaka farm Palmerston North	No.4 dairy farm Massey University Palmerston North
Soil order	Recent soil	Brown soil	Pallic soil
NZ land cover (%) [c]	6	43	12

[a] values are mean ± SE (n = 2, unless mentioned); [b] amorphous and crystalline Fe and Al content were determined by extraction as described by Blakemore *et al.* (1987). The employed method will extract all Fe and Al associated with each phase regardless of mineralogy, including (but not limited to) allophane minerals, and hydrous oxides; [c] NZ Soils Portal Landcare Research Ltd (2013)



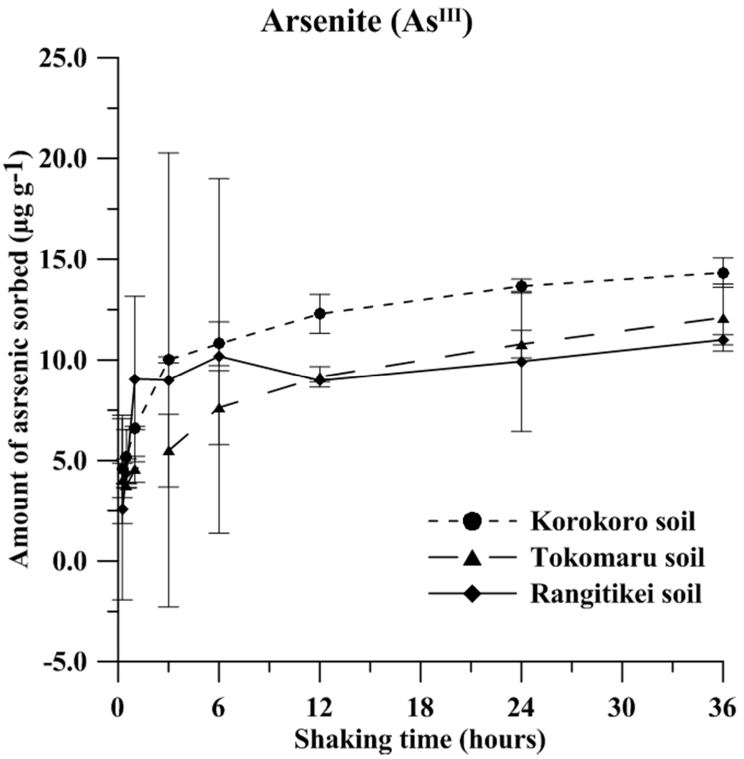


Figure 5.1 Adsorption kinetic of As^V (top) and As^{III} (bottom) for studied soils (each value is mean of duplicates ± SE)

The rate of As^{III} adsorption onto experimental soils was slower than that for As^{V}. A rapid rate of As^{III} adsorption was observed over the first 6 hours followed by a constant increase at a slower rate. After 36 hours, only 72, 61, and 55 percent of added As^{III} was adsorbed by Korokoro silt loam, Tokomaru silt loam and Rangitikei silt loam soil respectively.

An initial rapid adsorption of As^{V} and As^{III} followed by a decrease in adsorption rate with time has been well documented (Barrachina *et al.*, 1996; Elkhatib *et al.*, 1984b; Lin *et al.*, 2002). Pierce and Moore (1982) suggested that a rapid adsorption of As could be because of electrostatic processes which transition to slow adsorption through specific adsorption or the formation of chemical bonds between As species and the adsorbent surface. Zhang and Selim (2005) suggested that a diphasic kinetics of As adsorption in soils may be due to (i) heterogeneity of adsorption sites, (ii) slow precipitation at the mineral surface, and (iii) slow diffusion to less accessible sites within the soil matrix. Slow adsorption of As on adsorbent surfaces may continue for days to months (O'Reilly *et al.*, 2001; Zhang & Selim, 2005).

Since the soils showed evidence for slow but continual adsorption in 36 hours, the reaction time was extended to 48 hours for subsequent adsorption isotherm studies.

5.3.3 Arsenate and arsenite adsorption: Langmuir and Freundlich Isotherms

Langmuir and Freundlich isotherm plots for the four soils at both As species, are displayed in Figure 5.2 and 5.3. The corresponding model-derived parameters are presented in Table 5.2. All soils showed a good fit ($R^2 \geq 0.88$) to the Langmuir model when As^{V} or As^{III} was added, with the exception of Korokoro silt loam soil for As^{III} ($R^2 = 0.137$). Arsenic adsorption maximum (b) was higher for As^{V} than As^{III} in the soils, with the exception of Korokoro silt loam soil. A greater adsorption affinity of As^{V} than As^{III} has been reported previously and attributed to greater electrostatic interactions between the soil surface and the anionic As^{V} species relative to the neutral As^{III} species (Manning & Goldberg, 1997b; Naidu *et al.*, 2009; Smith *et al.*, 1999).

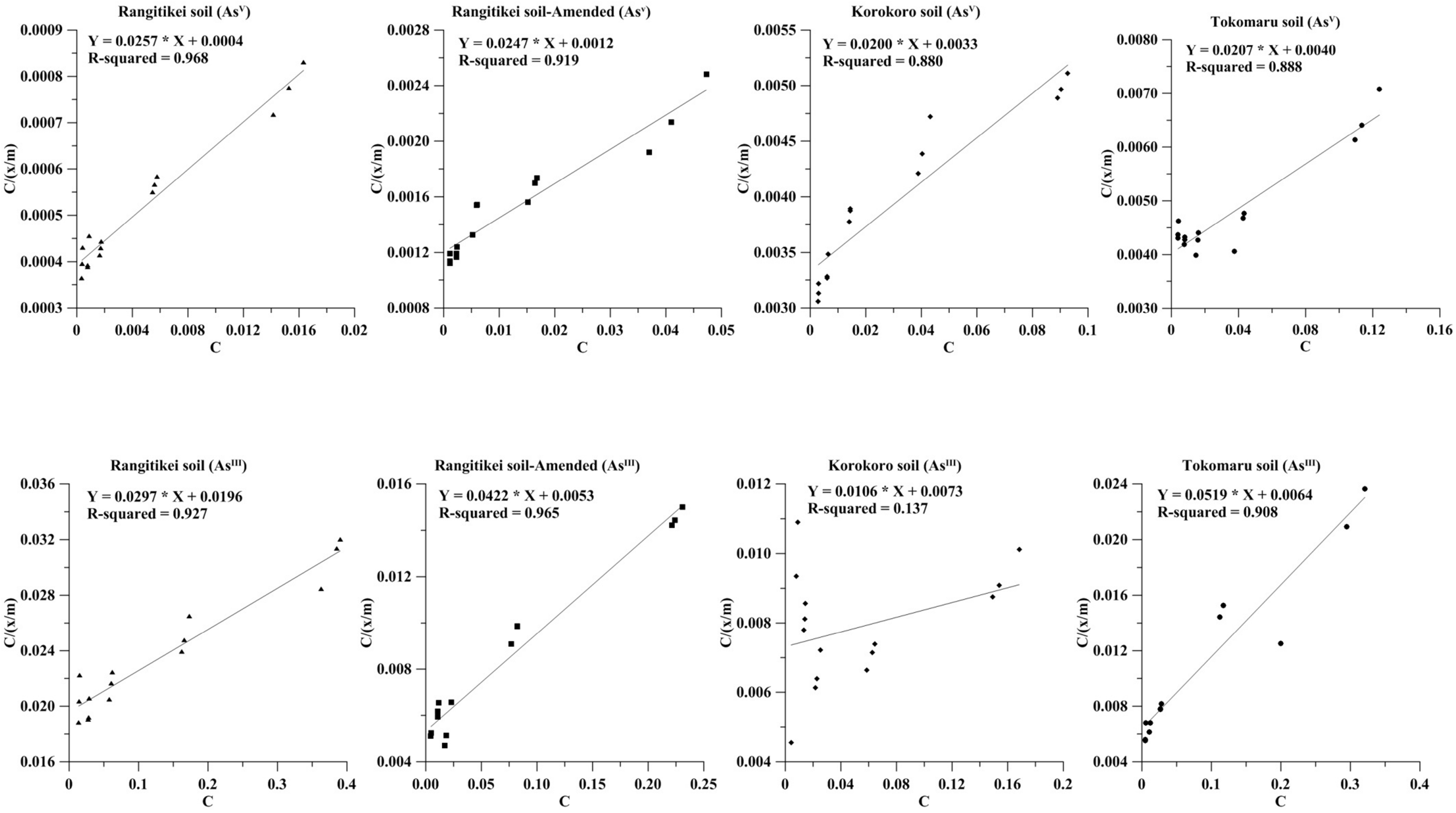


Figure 5.2 Langmuir isotherm plots for the adsorption of As species onto experimental soils (15 data points used in each graph)

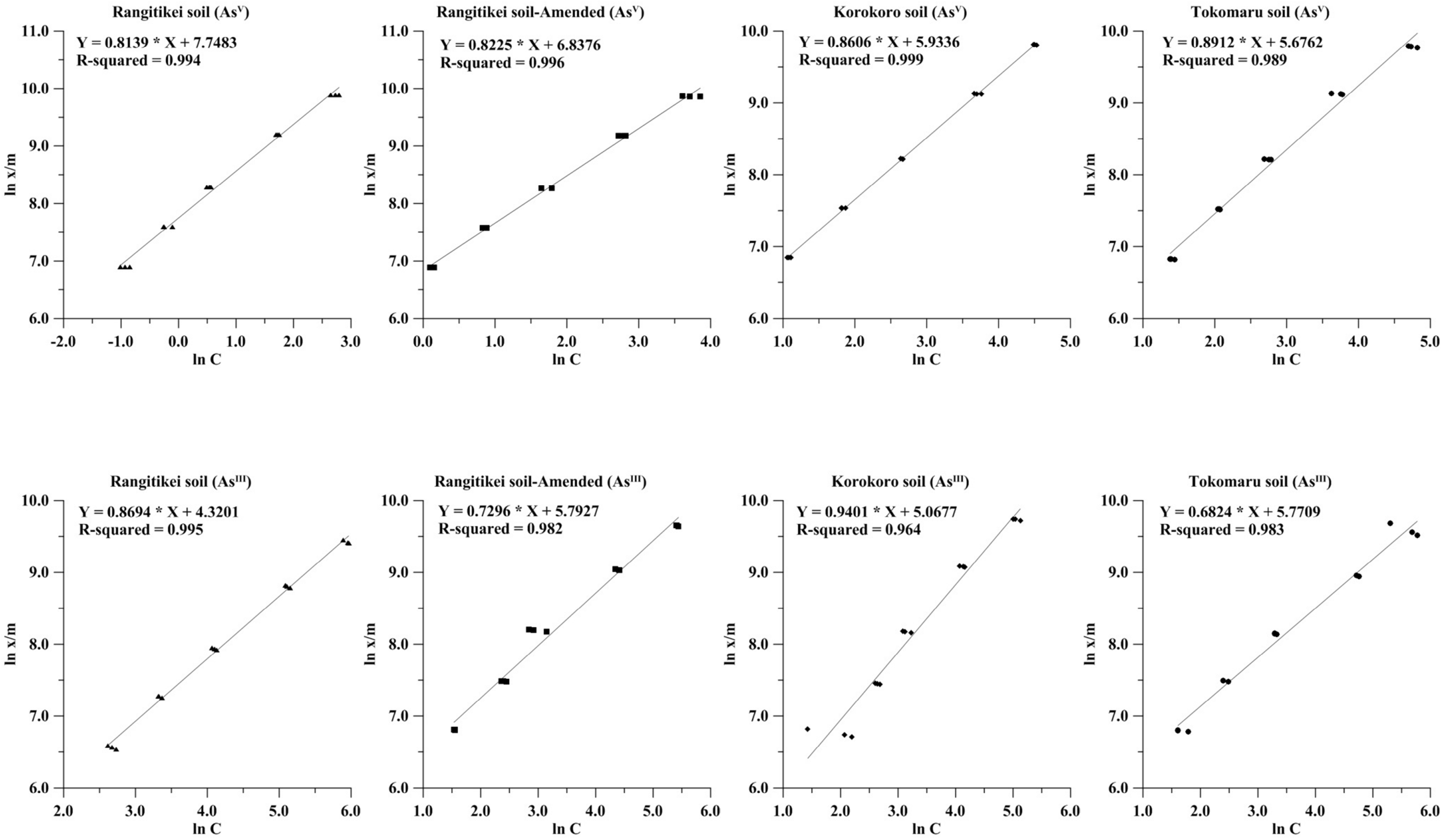


Figure 5.3 Freundlich isotherm plots for the adsorption of As species onto experimental soils (15 data points used in each graph)

Table 5.2 Langmuir and Freundlich isotherm parameters for As adsorption onto experimental soils

Soil type	As species added	Langmuir isotherm parameters			Freundlich isotherm parameters		
		R^2	b (µg g^{-1})	K (mL µg^{-1})	R^2	n (unitless)	K_f (mL g^{-1})
Rangitikei soil (Control)	As^V	0.968	38.91	64.25	0.994	0.814	7.748
	As^{III}	0.927	33.67	1.52	0.995	0.869	4.320
Rangitikei soil (Amended)	As^V	0.919	40.49	20.58	0.996	0.823	6.838
	As^{III}	0.965	23.70	7.96	0.982	0.730	5.793
Korokoro soil	As^V	0.880	50.00	6.06	0.999	0.861	5.934
	As^{III}	0.137	94.34	1.45	0.964	0.940	5.068
Tokomaru soil	As^V	0.888	48.31	5.18	0.989	0.891	5.676
	As^{III}	0.908	19.27	8.11	0.983	0.682	5.771

b: Langmuir adsorption maxima; K: Langmuir bonding energy coefficient; n: Freundlich adsorption nonlinearity factor; K_f: Freundlich partition coefficient

There was a significant decrease in As^{III} adsorption when the pH of the Rangitikei silt loam soil was raised to 7.5 (33.67 µg g^{-1} for the control and 23.70 µg g^{-1} for amended soil). In contrast, there was a slight increase in As^{V} adsorption when the pH was raised to 7.5 (38.91 µg g^{-1} for control and 40.49 µg g^{-1} for amended soil). These findings are in contrast to the results of Smith *et al.* (1999) who reported an increase in As^{III} adsorption with increasing pH of experimental soils. However, many researchers who have studied the adsorption of As^{III} on mineral compounds (amorphous Fe and Al hydroxide, hematite, alumina, clay minerals) found a maximum adsorption of As^{III} at pH 7.0 to 9.5 followed by a decrease in adsorption with any further increase in pH (Manning & Goldberg, 1997a; Pierce & Moore, 1980; Singh *et al.*, 1988; Xu *et al.*, 1991). According to Xu *et al.* (1991) adsorption of As^{III} onto adsorbent surfaces may decrease under alkaline condition as the amount of negative surface charge increases as a function of pH. This proposition may not, however, be valid for our results, since an increase in negative surface charges should also decrease As^{V} (a negatively charged species) adsorption which is not evident in the current study.

There appear to be three possible explanations for the observed results (i) the presence of preferential adsorption sites for As^{III} in the Rangitikei soil which may have decreased in abundance with an increase in soil pH, (ii) an oxidation of As^{III} to As^{V} as a function of pH which promoted As mobility, and (iii) formation and lower stability of As^{III}-oxides with an increase in soil pH value. The preferential adsorption of As^{III} onto mineral compounds, and the oxidation of As^{III} to As^{V} in soil solution and on mineral surfaces under alkaline pH has been reported (Manning & Goldberg, 1997a, b; Sun & Doner, 1996). Sadiq *et al.* (1983) reported that oxides of As^{III} are less stable at pH 7.0 and above.

Langmuir bonding energy coefficient (K) values ranged from 1.45 to 64.25 mL µg^{-1}, with higher values for As^{V} than As^{III} in most soils. The Tokomaru silt loam soil was an exception where the K value was higher for As^{III} than As^{V}. The K values for As^{V} in Rangitikei soil (control and amended) were higher than all other soils.

Adsorption of As (As^{V} or As^{III}) for each of the soils was better described by the Freundlich adsorption model ($R^2 \geq 0.96$) than the Langmuir model. A better fit to the Freundlich isotherm model indicates that the bonding energy of adsorbing sites in each of these soils is heterogeneous in nature (Campbell & Davies, 1995; Petruzzelli *et al.*, 1985).

Values of n were less than 1 in all soils (0.682 to 0.940), with no consistent pattern among soils and As species. An n value less than one describes non-linear (concentration dependent) adsorption behavior of As^V and As^{III} over the range of concentrations in each soil. Values of n less than one have been widely reported for adsorption studies investigating the relationship between As and soil medium (Manning & Goldberg, 1997b; Nam *et al.*, 2010; Zhang & Selim, 2005).

Freundlich partition coefficient (K_f) values ranged from 4.320 to 7.748 mL g^{-1} in the soils. The K_f values were higher for As^V relative to As^{III} in most soils. These K_f values are less than most previous studies (Table 5.3) and indicate that much of the As will be bioavailable and leachable in selected New Zealand soils. However, As^{III} will be more mobile in selected soils than As^V because of lower K_f values.

5.3.4 Soil properties affecting arsenic adsorption parameters

Arsenate (As^V) adsorption maximum (b) had a significant positive relationship with Olsen P, sulphate, amorphous Fe and Al, crystalline Al and total C content of each soil (r = 0.616 to 0.964; Table 5.4). There was a significant negative relationship of b with pH and crystalline Fe content (r = -0.755 to -0.828). A significant positive relationship of As^V adsorption with Al and Fe oxides, and soil C content has been previously described (Livesey & Huang, 1981; Sahu *et al.*, 2011; Zhang & Selim, 2005). A significant positive relationship of As^V adsorption with Olsen P is in contrast to many studies where phosphate significantly reduced As^V retention in soils (Livesey & Huang, 1981; Manful *et al.*, 1989; Manning & Goldberg, 1996). There is some evidence that certain binding sites are uniquely available for adsorption of either phosphate or arsenate (Liu *et al.*, 2001; Manning & Goldberg, 1996). The presence of specific adsorption sites for phosphate in experimental soils may be responsible for this positive relationship between these anions. A negative relationship of As^V adsorption in experimental soils as a function of soil pH is consistent with many studies (Manful *et al.*, 1989; Smith *et al.*, 1999; Xu *et al.*, 1991). However, among all the reported soil properties, a stepwise regression analysis determines that amorphous Al and total C (R^2 = 99.6%) are the main soil properties affecting As^V adsorption maximum.

Table 5.3 Selected studies detialing Langmuir and Freundlich isotherm parameters for As adsorption, and soil properties influencing these parameters

Soil		**As species added**	**Langmuir isotherm parameters**		**Freundlich isotherm parameters**		**Soil properties controlling adsorption parameters**	**References**
Collected from	**pH range**		**b (µg g^{-1})**	**K (mL µg^{-1})**	**n (unitless)**	**K_f (mL g^{-1})**		
Palmerston North New Zealand	5.3 to 6.1	As^V	38.91 to 50.00	5.18 to 64.25	0.814 to 0.891	5.676 to 7.748	Amorphous Al, total C, and Olsen P	Current study
		As^{III}	23.70 to 94.34	1.45 to 8.11	0.682 to 0.940	4.320 to 5.793		
Guava orchard, West Bengal India	7.5 to 7.9	As^V	1.6 to 8.6	0.69 to 15	1.2 to 2.7	0.89 to 6.7	Organic C, clay & Al_2O_3	Sahu *et al.* (2011)
Agricultural soils, 500 m from mine mouth Korea	5.9 to 6.8	As^V	ND [1]	ND	0.56 to 0.77	211 to 1150	Fe & Al oxides, pH	Nam *et al.* (2010)
Louisiana and New Hampshire, USA	5.8 to 6.1	As^V	64.6 to 742.0	0.106 to 0.830	0.087 to 0.368	36.2 to 217	Fe and Al oxides [2]	Zhang and Selim (2005)
West Virginia USA	4.1 to 7.0	As^{III}	ND	ND	0.339 to 0.958	19.2 to 102.0	Fe oxide and pH	Elkhatib *et al.* (1984a)
Saskatchewan Canada	5.9 to 7.5	As^V	248 to 335	ND	ND	ND	Al and Fe [3], and clay %	Livesey and Huang (1981)

ND [1] not determined by researchers; [2] Citrate-bicarbonate-dithionite extracted; [3] ammonium oxalate-extractable

Table 5.4 Regression equation and Pearson's correlation coefficient (r) between soil properties and Langmuir-Freundlich equation parameters

Soil properties	**Langmuir parameter (b)**		**Freundlich parameter (K_f)**	
	As^V	**As^{III}**	**As^V**	**As^{III}**
pH (H_2O)	-0.755 **	-0.921 ***	0.566 *	-0.202 ^{NS}
Olsen P	0.616 *	-0.399 ^{NS}	-0.794 ***	0.961 ***
SO_4	0.773 **	-0.079 ^{NS}	-0.875 ***	0.914 ***
Amorphous Fe	0.633 *	0.967 ***	-0.419 ^{NS}	0.037 ^{NS}
Crystalline Fe	-0.828 ***	0.091 ^{NS}	0.938 ***	-0.982 ***
Amorphous Al	0.813 ***	0.888 ***	-0.640 *	0.291 ^{NS}
Crystalline Al	0.643 *	0.961 ***	-0.431 ^{NS}	0.052 ^{NS}
Clay	-0.236 ^{NS}	0.745 **	0.474 ^{NS}	-0.778 **
Total C	0.964 ***	0.684 **	-0.864 ***	0.600 *

Regression equation

As^V

b = 40.0 - 24.6 Amorphous Al + 3.69 Total C (R^2 = 99.6%)

K_f = 5.55 + 0.274 pH - 0.0275 Olsen P + 4.60 Amorphous Al + 0.784 Crystalline Fe - 0.619 Crystalline Al - 0.413 Total C (R^2 = 99.9%)

As^{III}

b = 12.2 - 1.17 Olsen P - 33.3 Amorphous Fe + 157 Amorphous Al + 1.20 Clay + 5.09 Total C (R^2 = 99.9%)

K_f = 4.39 + 0.0245 Olsen P - 3.70 Amorphous Al + 0.340 Total C (R^2 = 99.5%)

* Significant at $P < 0.05$; ** Significant at $P < 0.001$; *** Significant at $P < 0.0001$; ^{NS} Not significant ($P > 0.05$)

There was a significant positive relationship of As^{III} maximum with amorphous Fe and Al, crystalline Al, clay and total C. A significant negative relationship was found between soil pH and As^{III} adsorption maximum. An increase in As^{III} adsorption in the presence of Fe and Al oxides and clay minerals is well documented (Manning & Goldberg, 1997a, b; Pierce & Moore, 1980). According to stepwise regression analysis, As^{III} adsorption maximum can be modeled by Olsen P, amorphous Fe and Al, clay and total C ($R^2 = 99.9\%$).

The Freundlich partition coefficient (K_f) for As^V had a significant positive relationship with soil pH and crystalline Fe, while a significant negative relationship with Olsen P, sulphate, amorphous Al, and total C. The K_f for As^V can be predicted by soil pH, Olsen P, amorphous Al, crystalline Fe and Al, and total C ($R^2 = 99.9\%$). In contrast, the K_f for As^{III} was positively related with Olsen P, sulphate and total C whilst negatively related with crystalline Fe and clay content of selected soils. The K_f values for As^{III} can be modelled by regression equation using Olsen P, amorphous Al and total C ($R^2 = 99.5\%$).

In summary, there is a significant relationship (positive or negative) between the soil properties amorphous Al, total C, and Olsen P, and Langmuir-Freundlich parameters for As^V and As^{III}. Moreover, according to stepwise regression analysis, these soil properties were the main variables controlling the As adsorption behavior of selected New Zealand soils. This is a significant finding and indicates that managing these soil properties can be helpful to mitigate As risk in selected soils. The findings of this research are in accordance with previous studies where oxide/hydroxides minerals, clay content, organic C, and pH have been the main soil properties affecting adsorption parameters (Table 5.3). The effect of phosphate on As adsorption is also well documented (Livesey & Huang, 1981; Smith *et al.*, 2002).

5.4 CONCLUSIONS

Arsenate and arsenite adsorption kinetic vary among selected New Zealand soils. The adsorption kinetic was biphasic where major fraction of As was adsorbed within a few hours (3-6 hours) followed by a continual slow increase in adsorption reaction. The Freundlich isotherm better modeled the adsorption behavior of selected soils than the Langmuir isotherm model, based on higher coefficient of determination values ($R^2 \geq 0.96$). The soils showed a greater affinity for As^V than As^{III} as defined by high adsorption maxima, bonding energy and

As partition coefficient values. Overall, it appears that the adsorption capacity of selected New Zealand soils for As is limited and much of the As (As^{III} greater than As^{V}) will be bioavailable and leachable. Adsorption behavior of the soils can be predicted by defined soil properties, however, mainly by amorphous Al, total C and Olsen P contents. These findings are useful and indicate that As risk can be managed by the defined soil properties.

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CHAPTER 6

RESPONSE OF SPINACH TO ARSENIC CONTAMINATED WATER IN SOILS AMENDED WITH BIOCHAR AND CATTLE MANURE [1]

6.1 INTRODUCTION

Chapter 4 shows that flood irrigation of spinach (*Spinacia oleracea*, cv. Perpetual) with irrigation water containing an As concentration greater than 50 µg L^{-1} resulted in an As concentration in spinach leaves that exceeded the critical limit for As in food and therefore presents a human health risk. However, the As uptake by vegetables is a complex phenomenon and is affected by many As-plant-soil factors. The research described in Chapter 6 was designed to further explore the factors which can promote As concentration into spinach.

Factors affecting As concentration in plants

There are a number of factors that play a significant role in the process of As accumulation by crop species. These factors include the As concentration and speciation in irrigation water or growth media, soil pH, redox conditions, and soil amendments (for example organic matter amendment and biochar). Although the influence of each of these factors on As accumulation in plants is described in Chapter 2, the key points are reviewed here.

The As concentration in vegetables increases as a function of the As concentration in soil or solution culture (Burlo *et al.*, 1999; Pavlik *et al.*, 2010). The effect of As species is variable among vegetable species and therefore a general model to describe the behaviour of plants to organic or inorganic species cannot be reliably described. Soil pH has a significant effect on As mobility; however, there are few studies that investigate the effect of pH on As uptake by plants (Anh *et al.*, 2013; Tu & Ma, 2003). The effect of soil organic matter and biochar on As

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bioavailability is limited and inconsistent, with reports of both an increase and decrease of the As concentration in plants (Beesley *et al.*, 2013; Cao *et al.*, 2003; Das *et al.*, 2008).

Chapter 6 describes a greenhouse experiment that was conducted to better understand the As uptake behaviour of spinach in a complex soil environment. The experimental conditions investigated in this work include a range of As^V or As^{III} concentrations in irrigation water (up to 1000 µg L^{-1}). Both As^V and As^{III} were included in this experiment because both of these inorganic As species are found in irrigation water (Baig & Kazi, 2012; Norra *et al.*, 2005). A soil pH of 7.5 was included in the experiment to represent the soil pH of South Asian countries, which is generally in the range of 7.0 to 7.9 (Baig & Kazi, 2012; Bhattacharya *et al.*, 2010a; Panaullah *et al.*, 2009). Chapter 5 shows that As mobility in soil can be managed by total C content of soil. Therefore, the soil was amended with two forms of carbon. In South Asia, the addition of organic matter (farm yard manure or poultry manure) to agricultural soils is a common agronomic practice. Recently, application of biochar to agricultural soil in South Asia has also been reported (Kumar *et al.*, 2013; Mankasingh *et al.*, 2011). These two amendments were therefore included as treatments in the experimental design to quantify their effect on As uptake by spinach.

Chapter 6 focuses on spinach as this is the crop that presents the highest risk to human health, as described in Chapter 4. Detailed consideration of spinach revealed that most As-spinach studies are monitoring field surveys, where the concentration in spinach ranged from 0.085 to 1.48 µg g^{-1} dry weight (Arain *et al.*, 2009; Baig & Kazi, 2012; Tiwari *et al.*, 2011) or 0.121 to 0.604 µg g^{-1} wet weight (Munoz *et al.*, 2002). In contrast, there are limited data where spinach is grown in contaminated soils. In such soil studies, the reported As concentration in spinach ranged from < 0.08 to 6.74 µg g^{-1} dry weight (Pavlik *et al.*, 2010; Rahman *et al.*, 2004; Warren *et al.*, 2003). With the exception of the work presented in Chapter 4, no As irrigation studies appear to have been conducted where spinach is tested for its As uptake potential from contaminated water under controlled conditions.

The objectives of this study were to (i) identify which of the experimental factors (concentration, species, pH and amendments) promote As concentration in spinach tissues, and (ii) to calculate the effect of these parameters on risk to humans that is associated with the ingestion of As-enriched spinach leaves.

6.2 MATERIALS AND METHODS

Spinach (*Spinacia oleracea*, cv. Perpetual) was grown in pots under controlled conditions and exposed to four concentrations (50, 200, 500 and 1000 µg L^{-1}) of As^{V} or As^{III} in irrigation water, two soil pH levels (6.1 and 7.5), and two soil amendments (biochar and cattle manure). Control treatments with no As in irrigation water and no amendment were also included. The response of spinach to these factors was investigated under flood irrigation. The experiment was conducted in a glasshouse at the Plant Growth Unit of Massey University Palmerston North, New Zealand in two phases because of intense labour and logistical requirements. In the first phase, a no soil amendment (control/unamended soil) experiment (include concentration, species and pH factors only) was conducted during March to May 2012. In the second phase, an experiment was conducted with an addition of biochar or cattle manure amendments during September to November 2012. The results of both experiments are comparable because (i) the glasshouse temperature was maintained at 12 ± 2 ^{0}C minimum (night) and 22 ± 2 ^{0}C maximum (day), (ii) the atmospheric temperature was similar in both experiments (temperature range for 1st experiment was 18.1 ± 3.2 ^{0}C to 8.2 ± 4.7 ^{0}C whilst for 2nd experiment the range was 16.3 ± 2.3 ^{0}C to 7.4 ± 3.6 ^{0}C; http://cliflo.niwa.co.nz) and (iii) the amount of water applied to each pot (on average) among trials was in acceptable range of < 10% variation (control trial: 1.28 ± 0.01 litre; biochar trial: 1.37 ± 0.01 litre; cattle manure trial: 1.39 ± 0.01 litre; Appendix V). The experiments were laid in a complete randomized design with five replications per treatment.

6.2.1 Pot preparation and soil amendments

Flood irrigation was achieved using the model system for plastic pots (16.5 x 16.5 x 19 cm), defined for the first glasshouse experiment (Chapter 4). Briefly, the pots were drilled at one side (2 cm from the base) and inserted with silicone and PVC materials to hold and drain water. A basal layer (500 grams) of gravel (> 2 mm) was placed in each pot to facilitate water drainage and aeration.

The Rangitikei silt loam soil (used in glasshouse experiment 1, Chapter 4 and laboratory batch experiment, Chapter 5) was again used in this experiment. This soil is acidic in its natural state (pH 6.1). To achieve the target soil pH level of 7.5, calcium hydroxide (analytical grade) was added to Rangitikei silt loam soil and allowed to equilibrate for 14 days.

Biochar was obtained from the New Zealand Biochar Research Centre (NZBRC). The biochar was produced from pine wood chips, heated at 350 to 450 ^{0}C for 4 hours using a 25 L gas-fired rotating drum Kiln Bioenergy Pyrolysis Unit. The biochar was ground using a food processor and then sieved to 2 mm prior to incorporation into soil. Cattle manure was collected from No. 4 dairy farm Massey University Palmerston North. The cattle manure was sun dried for one month, chopped by a chipper (Al-ko Garden Shredder) and sieved through a 5 mm garden sieve. These carbon amendments were amended to soil at a rate of 5 g kg^{-1} soil (equivalent to 10 tons ha^{-1}). The calculated amount of these amendments was thoroughly mixed by hand and added to each treatment pot soil (4 kg air dried soil) to keep uniformity among pots. The pots were irrigated with distilled water (to 50% field capacity of soil) and fertilized with NPK fertiliser at recommended rate (50-22.5-67.5 mg NPK kg^{-1} soil), as executed in glasshouse experiment 1. Three seeds were sown in each pot, which were thinned to one healthy plant after germination (10 days after sowing).

6.2.2 Irrigation

Arsenate or arsenite enriched waters were prepared from sodium arsenate heptahydrate ($Na_2HAsO_4.7H_2O$) and sodium (meta) arsenite ($NaAsO_2$) salts respectively prior to each irrigation event. A control treatment (distilled water) was also included in the experiment. The irrigation was initiated after spinach germination (10 days after sowing). At each irrigation event, pots were weighed and adjusted to 110% Fc with treatment water. The pots were maintained at flooded condition for two days, drained on the third day, and left to dry for seven days before the next irrigation. A complete irrigation cycle was therefore 10 days. This irrigation frequency is different from the earlier glasshouse experiment (Chapter 4) where the plants were irrigated every fortnight. This increase in irrigation frequency was made since spinach is a short duration crop (2 months) and watering every fortnight would only include four irrigations. We assume that some surface absorption of As would have happened through spinach basal leaves which were immersed in standing water (approximately 2-3 cm head) after each irrigation event.

6.2.3 Plant harvest and analysis

Spinach leaves and roots were harvested at maturity (defined as the point of human consumption). These plant parts were washed with running tap water to remove soil particles, followed by washing with distilled water and deionized water, then surface dried using paper towels and stored in paper bags. Spinach leaves and roots were oven dried at 70 ^{0}C for four days and weighed for dry biomass. The dried plant tissues were homogenized to a powder using a Cyclotec herbage mill (Model 1093, Salmond Smith Biolab Ltd.).

Subsamples of spinach leaves and roots were analyzed according to protocol described in Chapter 3. A standard reference material (1573a, Tomato leaves, NIST) and sample blanks were included in the analytical procedure. The sample blanks were found to have an As concentration below the detection limit of 0.145 µg L^{-1} in HGAAS. The analyzed As concentration of the SRM (0.111 ± 0.002 µg g^{-1}, n = 29) was in good agreement with the certified reference value (0.112 ± 0.004 µg g^{-1}).

6.2.4 Soil analysis

After spinach harvest, the soil from each treatment pot was sampled using a vertical corer (1.76 x 11.25 cm) to determine the extent of As accumulation in soil as a function of irrigation. Soil analysis for total As was achieved according to the protocol described in Chapter 3.

A standard reference material (CRM-GBW 07403, National Research Center for CRMs of China, Beijing), was used in parallel with the experimental soil samples. The analyzed As concentration of the standard reference material (4.1 ± 0.1 µg g^{-1} n = 18) was within the reported range of the certified reference value (4.4 ± 0.6 µg g^{-1}).

6.2.5 Biochar and cattle manure analysis

Biochar samples were finely ground to < 500 µm prior to laboratory analysis. The pH was determined in water at a 1:20 ratio of biochar : deionized water after 1.5 hours shaking (IBI, 2012; Rajkovich *et al.*, 2012). Total C and N were measured by Leco analyzer (TruSpec

CHNS, LECO Corp. St. Joseph, MI). Ash content was determined by thermal analysis using thermogravimetry analyzer (SDT Q 600, TA Instruments, Melbourne, Australia) (Pereira *et al.*, 2011). Total As was determined by nitric/hydrochloric acid digestion using ICP-MS.

The pH of cattle manure samples (5 mm sized) was determined in water at a 1:10 ratio (cattle manure: deionized water) using a digital pH meter. For total C and N analysis, cattle manure samples were homogenized to powder by Cyclotec herbage mill (Model 1093, Salmond Smith Biolab Ltd.) and analyzed using an Elementar (Vario Macro Cube, Analysensysteme GmbH, Germany).

The properties of biochar and cattle manure are presented in Table 6.1. The pH of biochar and cattle manure was slightly alkaline (7.4 and 7.6 respectively). Total C was higher in biochar (851 g kg^{-1}) than cattle manure (364 g kg^{-1}). The trend was inverse for total N which was higher in cattle manure (24 g kg^{-1}) compared to biochar (14 g kg^{-1}). The biochar had a four times higher C:N ratio than cattle manure; the C:N ratio was 61 and 16 for biochar and cattle manure respectively.

Table 6.1 Properties of biochar and cattle manure used in the experiment

Properties	**Biochar**	**Cattle manure**
pH (H_2O)	7.4	7.6
Total C (g kg^{-1})	851	364
Total N (g kg^{-1})	14	24
C/N	61	16
Ash content (%)	2.9	ND
Total As (mg kg^{-1} dry weight)	< 2	ND

ND Not determined

6.2.6 Statistical analysis

The As concentration in spinach tissues (leaves and roots) and soil (after spinach harvest) was statistically analysed against four experimental factors (General linear model, 4 way ANOVA) and interpreted to generate an overall picture of As behaviour in a complex soil environment. Secondly, the As concentration in spinach tissues and soil was assessed as a function of the two factors (General linear model, 2 way ANOVA) in order to provide mechanistic understanding of the process of As uptake by spinach plants. These statistical analyses were conducted using Minitab 16.1 software. The Tukey's test was used to determine significant differences among treatment means using a probability value of 0.05.

All the graphs were prepared using Grapher 9.6 (Golden Software Inc. Colorado) and Microsoft Excel 2010.

6.2.7 Human health risk assessment

An assessment of the risk to humans that might be associated with the ingestion of As-enriched spinach leaves was calculated as described previously in Chapter 2 and Chapter 4. Briefly, the As concentration in spinach leaves was re-expressed on a fresh weight and compared with the Chinese food safety standard for inorganic As in vegetables (GB 2762-2005) which is 0.05 µg g^{-1} fresh weight (Heikens, 2006). The analysis of potential risk associated with this food standard may, however, be overly conservative. A new proposed standard value of 0.5 µg g^{-1} fresh weight for total As in vegetables is currently under development (Bugang & Woolsey, 2010). These authors state that *On August 12, 2010, China's Ministry of Health, under the guidance of the Chinese Centre for Disease Control Nutrition and Food Safety (CCDCNFS), notified the World Trade Organization (WTO) that it is replacing GB 2762-2005, the National Standard for the Maximum Levels of Contaminants in Foods, with a new standard to address the maximum levels of many contaminants (including arsenic). This standard, which is yet to be given a numerical identification, will replace the previous standard, GB 2762-2005.* Since this new standard is still in a phase of approval and implementation, the previous food standard value of 0.05 µg g^{-1} fresh weight was again used for risk calculations in this study. Despite the possibly conservative nature of using a 0.05 µg g^{-1} standard value, the purpose of using this standard value was a first level screening tool to identify cases which require further investigation. In this sense the actual value used is relatively unimportant.

An arsenic concentration in leaves above this critical value (0.05 µg g^{-1} fresh weight) was considered potentially risky to consumers, and was further quantified using a series of more accurate risk assessment models.

The first model used was based on the USEPA defined hazard quotient (HQ) and cancer risk (CR) equations (USEPA, 1997, 1998).

$HQ = EDI / RfD$ Equation 6.1, and

$CR = EDI \times CSF$ Equation 6.2

A detailed description of these equations is presented in Chapter 2, Section 2.5.4. In brief, an HQ value greater than 1 defines an unacceptable non-carcinogenic risk to humans while a CR value greater than 1 in 10,000 propose an unacceptable cancer risk in humans.

The USEPA defined parameters (HQ and CR) merely describe the risk to an individual from consumption of As-enriched foods and whether this risk is acceptable or not. This risk assessment model does not describe the possible health effects which may develop in an individual as a consequence of As exposure. Arsenic exposure can lead to numerous cancerous and non-cancerous effects to humans; there is no level above zero concentration which does not present some risk to humans. Understanding As-induced health effects is essential in formulating a strategy for immediate response to As poisoning and the mitigation of its effects on human health.

Health effects to an individual from exposure to various levels of As can be explored by calculating the As intake per day (mg As kg^{-1} body weight) from the source of exposure (generally either drinking water or food). These As intake values can be compared with proposed ATSDR (Agency for Toxic Substances and Disease Registry) intake values to explore the type of poisoning (acute or chronic) and the potential health effects that an individual may experience through As exposure (ATSDR, 2007; Ministry for the

Environment, 2011). The ATSDR has proposed As intake values based on clinical observations of people affected from ingestion of As-contaminated drinking water and foods (ATSDR, 2007).

The As intake values (mg As kg^{-1} body weight day^{-1}) from food source (spinach in this study) may also be compared with As intakes from drinking water, as proposed by Piper and Kim (2006). The type of As poisoning (acute and chronic) and effects that can be expected as a function of a range of As concentrations in drinking water has been described by these authors. The As drinking water data was used for comparison in the current risk assessment as the observed toxicological effects of As primarily come from epidemiological investigations of humans exposed to As in drinking water (Ministry for the Environment, 2011). A further advantage of these comparisons (As intake from food versus As intake from drinking water) is the opportunity to estimate the As concentration in drinking water.

To calculate the As intake (mg kg^{-1} body weight day^{-1}) from spinach leaves and compare these values with proposed ATSDR and drinking water intake values the following equation was used:

Daily As Intake (mg kg^{-1} body weight) = As concentration in spinach leaves (mg kg^{-1}) x mass of spinach eaten per day (kg) / body weight (kg) of an individual Equation 6.3

In summary, the risk that might be associated with the ingestion of As-contaminated spinach leaves is explored in this chapter through consideration of the Chinese food safety standard, the USEPA risk assessment model, and the ATSDR and drinking water oral reference intake values.

6.3 RESULTS

6.3.1 Overall Picture

The overall picture refers to the scenario where all (four) experimental factors are statistical analysed together to understand the As uptake response of spinach in a complex soil environment.

6.3.1.1 Arsenic concentration in spinach tissues (leaves and roots)

The response of spinach leaves and roots to the experimental factors is presented in Table 6.2 and 6.3 respectively. The As concentration in spinach leaves and roots increased significantly as a function of the As concentration in the irrigation water ($P < 0.05$).

The effect of pH on As concentration was significant for spinach roots only ($P < 0.05$) where the plants grown at pH 6.1 had significantly higher As concentration in their roots than the plants grown at pH 7.5.

The effect of As species was significant for the As concentration in spinach leaves and roots ($P < 0.05$), although the trend was variable between plant tissues. The plants irrigated with As^{III} had a significantly higher concentration of As in their leaves but a significantly lower concentration of As in their roots when compared to the plants irrigated with As^{V} species.

The effect of soil amendments was significant for the As concentration in leaves and roots ($P < 0.05$). The plants grown in biochar and cattle manure amended soil had a significantly higher As concentration in their leaves than those which were grown in the control (unamended) soil. For roots, the trend was slightly different and there was a significantly higher concentration of As for plants grown in cattle manure amended soils than the plants of other amendments (biochar and control).

6.3.1.2 Arsenic concentration in soil after spinach harvest

The As concentration in soil at the end of the experiment was significantly affected by the As concentration in the irrigation water ($P < 0.05$, Table 6.4). The soil As concentration was significantly increased where the concentration of As in the irrigation water was 200 µg L^{-1} or greater relative to the control and 50 µg L^{-1}. There was no significant effect of soil pH on As retention in soil ($P > 0.05$). The effect of As species was significant ($P < 0.05$). The As concentration in soil at the end of the experiment was greater where the As^{III} species was applied relative to As^{V} species. The soil amendments also had a significant effect on the soil As concentration at the end of the experiment ($P < 0.05$). The soil As concentration was reduced where soil was amended with carbon amendments relative to the control. The trend of As retention was control > biochar > cattle manure amended soil (all significantly different from each other at $P < 0.05$).

Table 6.2 Total As concentration (μg g^{-1} dry weight) in spinach leaves as influenced by the As concentrations and species in irrigation water, soil pH and amendments (Data are means ± SE, *n = 5*)

As in water (μg L^{-1})	**As^{V}**						**As^{III}**					
	pH 6.1			**pH 7.5**			**pH 6.1**			**pH 7.5**		
	control	**biochar**	**cattle manure**	**control**	**biochar**	**cattle manure**	**control**	**biochar**	**cattle manure**	**control**	**biochar**	**cattle manure**
0	0.08 ± 0.01	0.07 ± 0.004	0.10 ± 0.02	0.04 ± 0.005	0.06 ± 0.005	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.004	0.10 ± 0.02	0.04 ± 0.005	0.06 ± 0.005	0.07 ± 0.01
50	0.14 ± 0.04	0.13 ± 0.01	0.18 ± 0.02	0.09 ± 0.02	0.20 ± 0.02	0.32 ± 0.07	0.13 ± 0.03	0.22 ± 0.06	0.19 ± 0.02	0.06 ± 0.01	0.16 ± 0.02	0.13 ± 0.01
200	0.29 ± 0.04	0.33 ± 0.04	0.38 ± 0.05	0.26 ± 0.06	0.63 ± 0.05	0.58 ± 0.07	0.28 ± 0.04	0.48 ± 0.06	0.47 ± 0.08	0.27 ± 0.04	0.53 ± 0.03	0.55 ± 0.13
500	0.59 ± 0.10	1.65 ± 0.28	0.84 ± 0.16	0.50 ± 0.17	1.42 ± 0.30	1.22 ± 0.15	0.41 ± 0.04	1.21 ± 0.18	2.12 ± 0.23	0.41 ± 0.02	1.25 ± 0.11	2.46 ± 0.27
1000	0.67 ± 0.14	2.52 ± 0.67	1.45 ± 0.21	0.64 ± 0.20	2.07 ± 0.14	2.77 ± 0.23	1.02 ± 0.19	2.53 ± 0.27	2.10 ± 0.13	1.20 ± 0.24	3.25 ± 0.80	3.22 ± 0.27

Analysis of Variance (ANOVA)

Source of variation	***P* value**[†]	**Tukey's Studentized Range Test**							
		Concentration		**pH**		**Species**		**Amendment**	
Concentration	< 0.0005								
pH	0.309	1000	1.95 ^{A} [††]	7.5	0.82 ^{A}	As^{III}	0.84 ^{A}	cattle manure	0.97 ^{A}
Species	0.042	500	1.17 ^{B}	6.1	0.70 ^{A}	As^{V}	0.68 ^{B}	biochar	0.94 ^{A}
Amendment	< 0.0005	200	0.42 ^{C}					control	0.36 ^{B}
Conc*pH [¶]	< 0.0005	50	0.16 ^{D}						
Conc*Species	0.006	0	0.07 ^{E}						
Conc*Amendment	< 0.0005								
pH*Amendment	< 0.0005								

[†] *P* value significant at < 0.05. [¶] Interaction is only shown for the factors where *P* value is ≤ 0.05. [††] Treatment means obtained from ANOVA; values followed by different letters within the same source of variation are significantly different (*P* < 0.05).

Table 6.3 Total As concentration ($\mu g\ g^{-1}$ dry weight) in spinach roots as influenced by the As concentrations and species in irrigation water, soil pH and amendments (Data are means ± SE, *n = 5*)

As in water ($\mu g\ L^{-1}$)	**As^{V}**						**As^{III}**					
	pH 6.1			**pH 7.5**			**pH 6.1**			**pH 7.5**		
	control	**biochar**	**cattle manure**	**control**	**biochar**	**cattle manure**	**control**	**biochar**	**cattle manure**	**control**	**biochar**	**cattle manure**
0	0.79 ± 0.07	0.96 ± 0.22	1.16 ± 0.12	0.49 ± 0.04	0.59 ± 0.07	0.62 ± 0.11	0.79 ± 0.07	0.96 ± 0.22	1.16 ± 0.12	0.49 ± 0.04	0.59 ± 0.07	0.62 ± 0.11
50	0.92 ± 0.12	1.43 ± 0.22	1.39 ± 0.05	0.55 ± 0.03	0.92 ± 0.09	1.33 ± 0.18	1.23 ± 0.12	1.34 ± 0.17	1.93 ± 0.23	0.90 ± 0.09	0.79 ± 0.12	0.71 ± 0.13
200	2.39 ± 0.33	1.93 ± 0.33	2.15 ± 0.25	1.43 ± 0.04	0.93 ± 0.08	1.21 ± 0.12	1.61 ± 0.21	1.70 ± 0.31	2.30 ± 0.41	1.28 ± 0.04	1.01 ± 0.12	1.11 ± 0.25
500	2.52 ± 0.34	2.74 ± 0.28	5.57 ± 0.52	2.71 ± 0.34	1.92 ± 0.21	3.70 ± 0.35	2.36 ± 0.16	2.92 ± 0.32	2.92 ± 0.37	1.37 ± 0.13	1.10 ± 0.14	1.87 ± 0.40
1000	5.59 ± 0.98	4.60 ± 0.77	6.27 ± 0.81	3.55 ± 0.05	2.77 ± 0.15	2.78 ± 0.37	3.25 ± 0.77	5.27 ± 1.35	5.68 ± 0.91	2.59 ± 0.10	1.83 ± 0.38	2.90 ± 0.61

Analysis of Variance (ANOVA)

Source of variation	***P* value**[†]	**Tukey's Studentized Range Test**							
Concentration	< 0.0005	**Concentration**		**pH**		**Species**		**Amendment**	
pH	< 0.0005	1000	3.92 ^{A††}	6.1	2.53 ^{A}	As^{V}	2.20 ^{A}	cattle manure	2.37 ^{A}
Species	< 0.0005	500	2.64 ^{B}	7.5	1.49 ^{B}	As^{III}	1.82 ^{B}	control	1.84 ^{B}
Amendment	< 0.0005	200	1.59 ^{C}					biochar	1.82 ^{B}
Conc*Species[¶]	< 0.0005	50	1.12 ^{D}						
Conc*Amendment	0.011	0	0.77 ^{E}						
pH*Amendment	0.004								

[†] *P* value significant at < 0.05. [¶] Interaction is only shown for the factors where *P* value is ≤ 0.05. [††] Treatment means obtained from ANOVA; values followed by different letters within the same source of variation are significantly different (*P* < 0.05).

Table 6.4 Total As concentration (µg g^{-1}) in soil after harvest of spinach as influenced by the As concentrations and species in irrigation water, soil pH and amendments (Data are means ± SE, $n = 5$)

As in water (µg L^{-1})	**As^{V}**						**As^{III}**					
	pH 6.1			**pH 7.5**			**pH 6.1**			**pH 7.5**		
	control	**biochar**	**cattle manure**	**control**	**biochar**	**cattle manure**	**control**	**biochar**	**cattle manure**	**control**	**biochar**	**cattle manure**
0	5.11 ± 0.04	3.98 ± 0.28	3.68 ± 0.15	4.99 ± 0.07	3.84 ± 0.03	3.43 ± 0.05	5.11 ± 0.04	3.98 ± 0.28	3.68 ± 0.15	4.99 ± 0.07	3.84 ± 0.03	3.43 ± 0.05
50	5.07 ± 0.05	3.92 ± 0.09	3.82 ± 0.04	4.78 ± 0.21	3.49 ± 0.11	3.86 ± 0.09	5.23 ± 0.06	3.81 ± 0.06	3.65 ± 0.06	4.81 ± 0.04	3.92 ± 0.10	3.62 ± 0.07
200	4.77 ± 0.11	4.09 ± 0.04	3.70 ± 0.02	5.17 ± 0.14	4.15 ± 0.09	3.75 ± 0.08	5.31 ± 0.02	4.38 ± 0.12	3.54 ± 0.07	5.18 ± 0.09	4.31 ± 0.04	3.53 ± 0.07
500	5.18 ± 0.13	4.04 ± 0.10	3.92 ± 0.04	5.15 ± 0.12	4.50 ± 0.35	4.11 ± 0.08	5.83 ± 0.07	4.47 ± 0.14	4.42 ± 0.12	5.60 ± 0.08	4.62 ± 0.08	4.66 ± 0.11
1000	6.04 ± 0.14	4.83 ± 0.13	4.37 ± 0.12	5.76 ± 0.21	5.08 ± 0.21	4.41 ± 0.19	6.10 ± 0.08	4.70 ± 0.12	4.26 ± 0.10	6.25 ± 0.17	4.43 ± 0.11	4.78 ± 0.14

Analysis of Variance (ANOVA)

Source of variation	***P* value†**	**Tukey's Studentized Range Test**							
		Concentration		**pH**		**Species**		**Amendment**	
Concentration	< 0.0005	1000	5.08 $^{A \dagger\dagger}$	6.1	4.50 ^{A}	As^{III}	4.55 ^{A}	control	5.32 ^{A}
pH	0.526	500	4.71 ^{B}	7.5	4.48 ^{A}	As^{V}	4.43 ^{B}	biochar	4.22 ^{B}
Species	0.002	200	4.32 ^{C}					cattle manure	3.93 ^{C}
Amendment	< 0.0005	50	4.17 ^{D}						
Conc*pH ¶	0.004	0	4.16 ^{D}						
Conc*Species	< 0.0005								
Conc*Amendment	< 0.0005								

† P value significant at < 0.05. ¶ Interaction is only shown for the factors where P value is ≤ 0.05. †† Treatment means obtained from ANOVA; values followed by different letters within the same source of variation are significantly different ($P < 0.05$).

6.3.2 Detailed assessment of treatments effect on the As concentration in spinach tissues and soil after spinach harvest

The detailed assessment refers to the scenario where two experimental factors were statistically analysed together to provide mechanistic understanding of the process of As accumulation by spinach.

6.3.2.1 Effect of the As concentrations in irrigation water and soil amendments for a particular species-pH combination

In this scenario, the data of As concentration in spinach tissues and soil after spinach harvest was statistically analysed as a function of the As concentration levels in irrigation water and soil amendments for a particular species-pH combination. This approach was adopted to determine the effect of individual factors (concentration or amendments) and their interaction (concentration x amendment) on As accumulation in spinach tissues and soil for a particular species-pH combination.

Spinach tissues (leaves and roots)

The As concentration in spinach leaves was significantly affected by the As concentration in irrigation water for all soil amendments and species-pH combinations ($P < 0.05$; Figure 6.1). Plants irrigated with water containing 500 and 1000 µg L^{-1} As had a significantly higher As concentration in their leaves for each species-pH combination relative to the lower As concentration treatments. The biochar and cattle manure amendments increased the concentration of As in spinach leaves compared to the plants with no amendment ($P < 0.05$). However, the effect of these amendments was dependent on As concentration level in the irrigation water and soil pH level (irrespective of As^{V} or As^{III} species). At pH 6.1, there was a significantly higher As concentration in spinach leaves when grown with 500-1000 µg L^{-1} water in cattle manure and/or biochar amended soil than the control soil at these concentration levels. At pH 7.5, there was a significant difference in leaves As concentration for 50 to 1000 µg L^{-1} treatment water where cattle manure and/or biochar was applied to the soil than the control soil at these concentration levels.

In roots, an increase in the As concentration was also observed as a function of the As concentration in irrigation water (Figure 6.2). Plants irrigated with water containing ≥ 200 µg As L^{-1} had a significantly higher root As concentration than the plants irrigated with no As for all species-pH combinations ($P < 0.05$). The amendment effect was significant for most of the species-pH combinations ($P < 0.05$) with the exception of As^{III}-pH 7.5. For As^{V} water at both pH levels, plants grown in the cattle manure amended soil had a significantly higher concentration of As in the roots relative to plants grown in the control and biochar amended soil. For the As^{III}-pH 6.1 combination, the plants grown in cattle manure amended soil concentrated more As only relative to the plants with no amendment.

Soil after spinach harvest

There was an increase in the As concentration in soil as a function of As concentrations in irrigation water for all soil amendments (Figure 6.3). However, the As concentration in irrigation water that resulted in the highest As concentration in soil was variable for As^{V} and As^{III}. For As^{V} (both As^{V}-pH 6.1 and As^{V}-pH 7.5), there was a significantly higher As concentration in soil for the 1000 µg L^{-1} treatment relative to the other irrigation concentrations ($P < 0.05$). For As^{III} (As^{III}-pH 6.1 and As^{III}-pH 7.5), both the 500 and 1000 µg L^{-1} treatments led to a significantly higher As concentration in soil when compared with other irrigation concentrations. The effect of the amendments was significant for As retention in soil ($P < 0.05$). For all species-pH combinations, the soil As concentration was significantly lower for the carbon amended soil relative to the control soil. The soil amended with biochar generally had a higher concentration of As at the end of the experiment when compared to the soil amended with cattle manure. The trend of amendment effect on As retention was therefore control > biochar > cattle manure soil (all amendments significantly different from each other at $P < 0.05$).

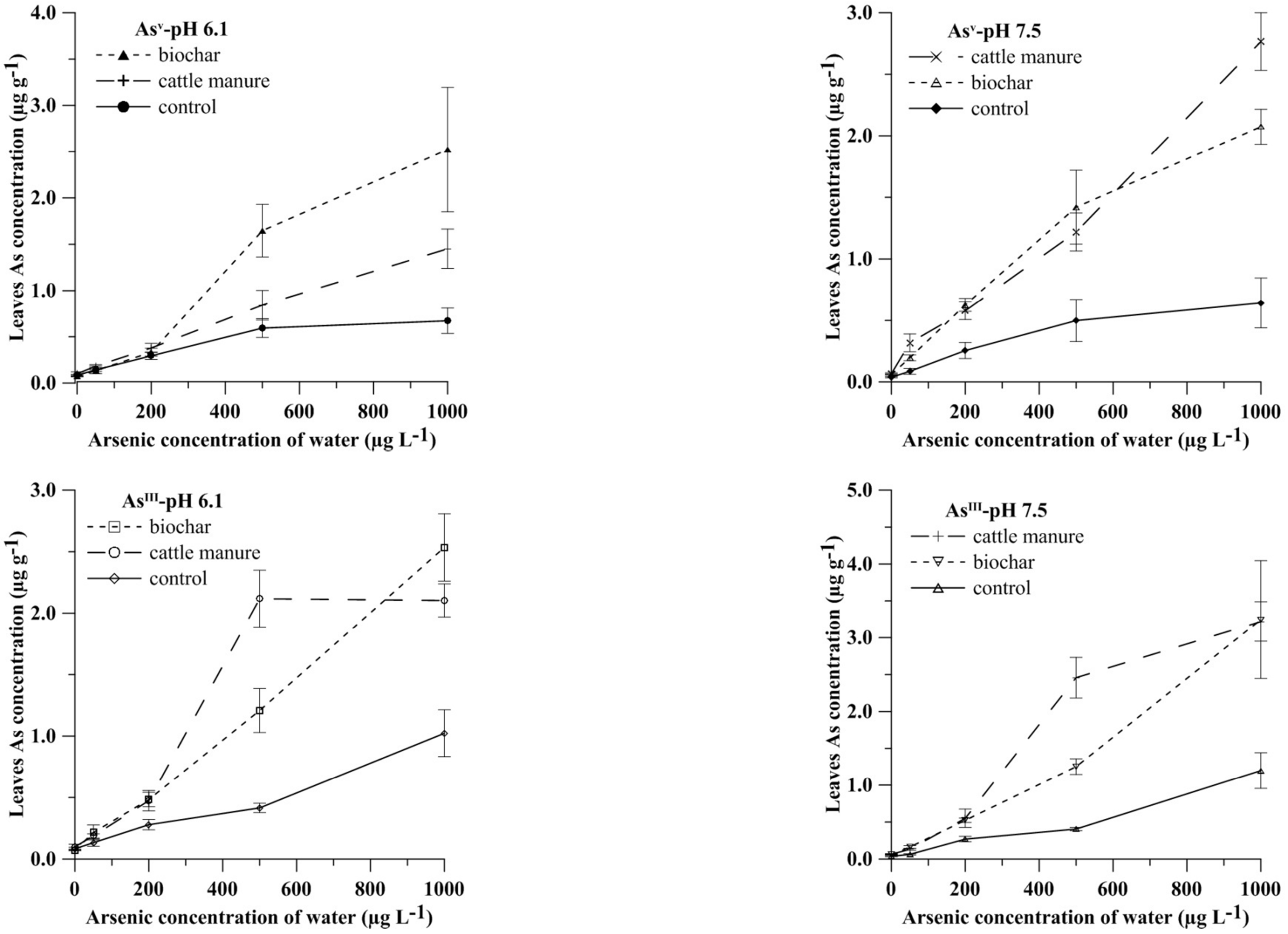

Figure 6.1 Arsenic concentration in spinach leaves (μg g^{-1} dry weight) as influenced by the As concentrations in water and soil amendments for a defined species-pH combination; Data are means ± SE (*n* = 5)

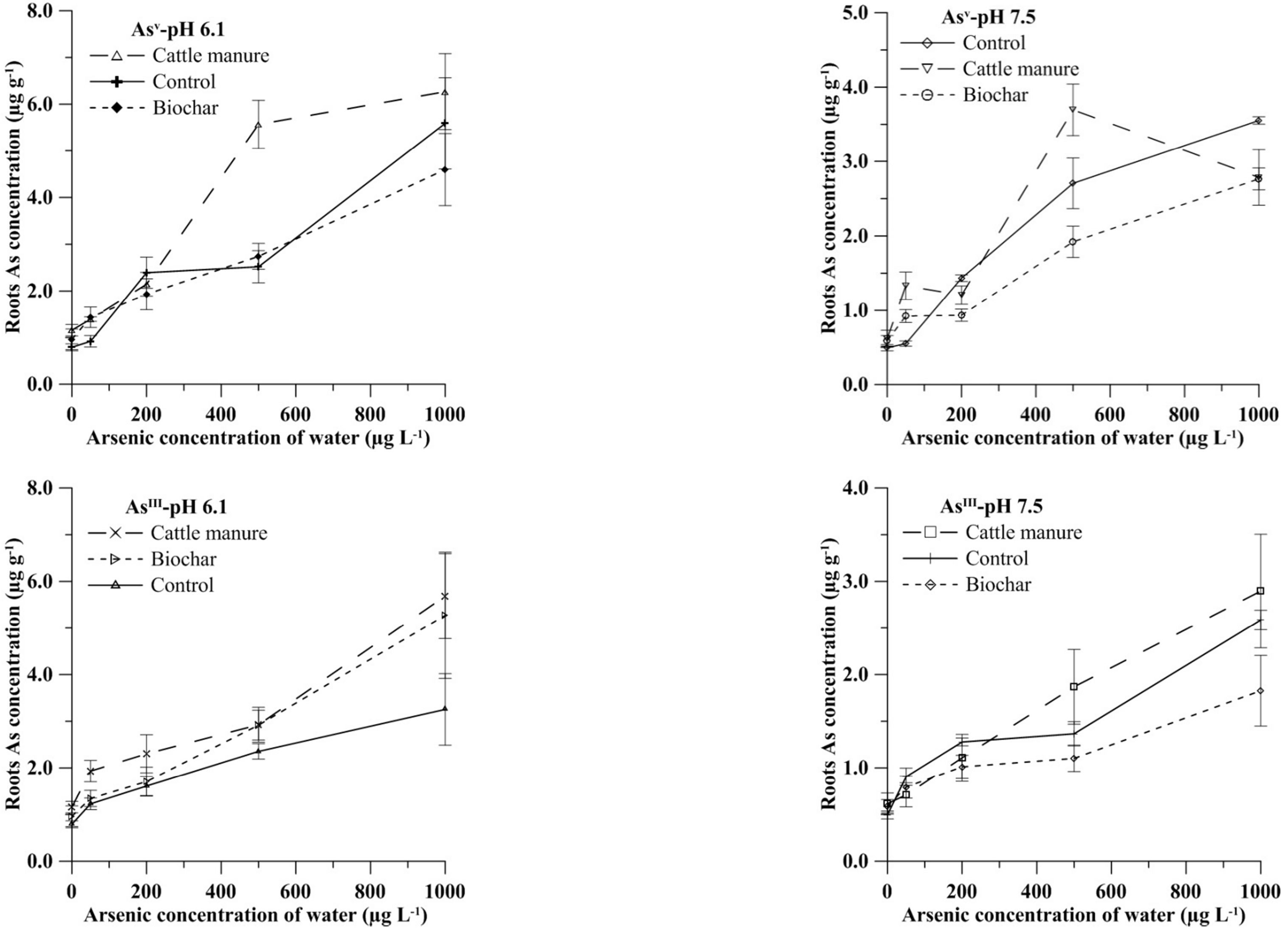

Figure 6.2 Arsenic concentration in spinach roots (µg g^{-1} dry weight) as influenced by the As concentrations in water and soil amendments for a defined species-pH combination; Data are means ± SE (*n* = 5)

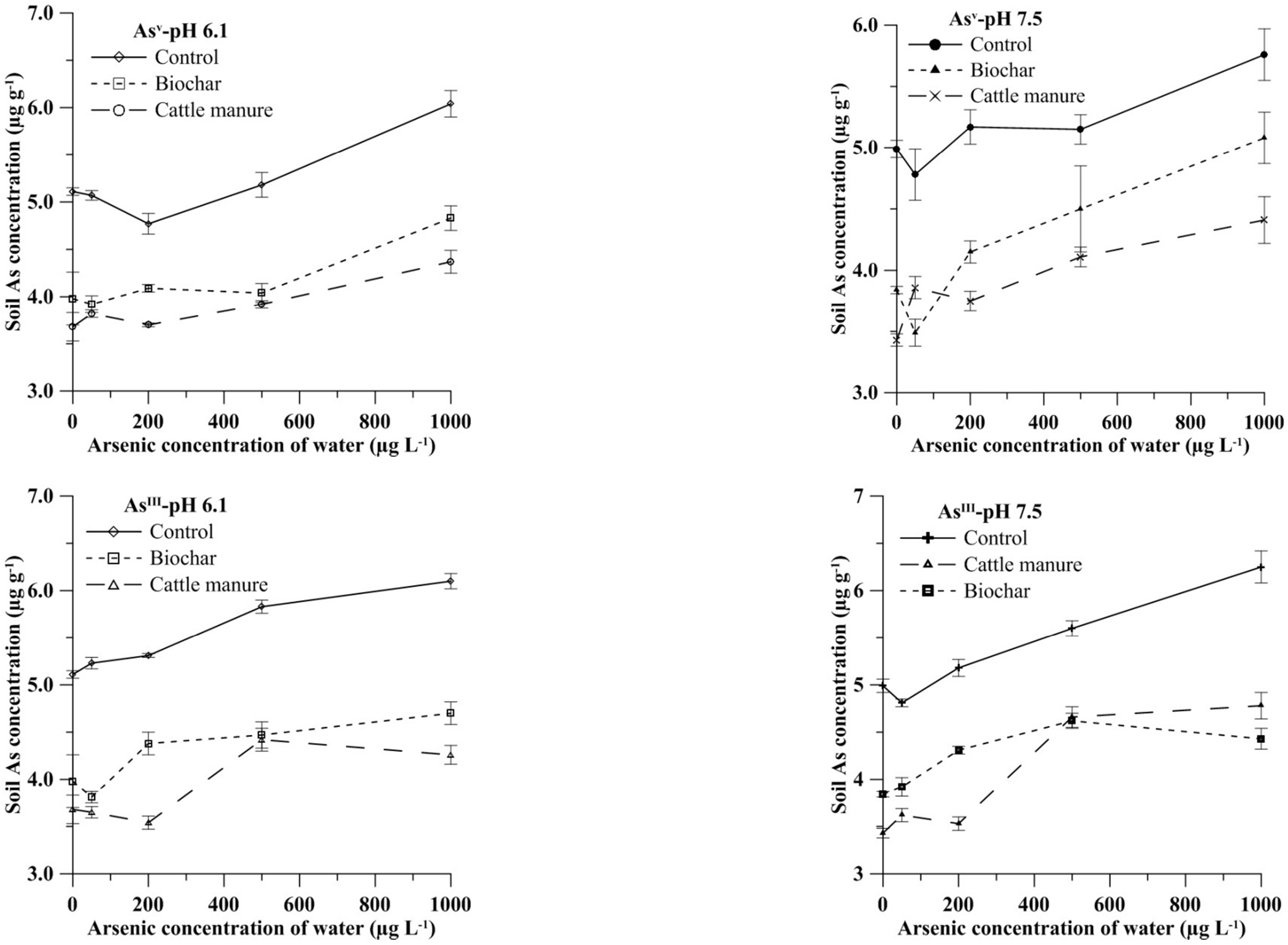

Figure 6.3 Arsenic concentration in soil (µg g^{-1}) after spinach harvest as influenced by the As concentrations in water and soil amendments for a defined species-pH combination; Data are means ± SE (*n* = 5)

6.3.2.2 Effect of As species and soil pH for a specific soil amendment

In this scenario, the data of As concentration in spinach tissues and soil after spinach harvest was statistically analysed as a function of As species and soil pH for a specific soil amendment. This approach was adopted to determine the effect of individual factors (species or pH) and their interaction (species x pH) on As accumulation in spinach tissues and soil for a specific soil amendment.

Spinach tissues (leaves and roots)

The As concentration in spinach leaves was independent of the As species, soil pH and the interaction of these factors for each of the soil amendments ($P > 0.05$; Figure 6.4). In roots, the As concentration was independent of As species for each of the soil amendments ($P > 0.05$, Figure 6.5). However, the effect of pH was significant for all amendments, and led to a higher As concentration in spinach roots for pH 6.1 compared to pH 7.5 ($P < 0.05$). The interaction of the species with pH was also observed significant for each of the soil amendments ($P < 0.05$). However, there was variability among the soil amendments in determining which species-pH combination could increase the concentration of As in spinach roots. For the control soil, the combination As^{V}-pH 6.1 led to a significantly higher As concentration in spinach roots than As^{III}-pH 7.5. For the biochar amended soil, As^{V} and As^{III} irrigated to plants at soil pH 6.1 resulted in a higher root As concentration than at pH 7.5. For the cattle manure amended soil, again As^{V} and As^{III} irrigated to plants at pH 6.1 resulted in a higher root As concentration than at pH 7.5, although this result was only significant for As^{III}. In summary, for all amendments, the plants irrigated with As^{III} and/or As^{V} water at pH 6.1 had more As in their roots than those irrigated with As^{III} at pH 7.5.

Soil after spinach harvest

The individual effect of soil pH or As species in the irrigation water was not significant for As retention in soils amended with biochar or cattle manure ($P > 0.05$; Figure 6.6). A significant effect of As species on As retention was observed in the control soil ($P < 0.05$), where irrigation with As^{III} led to a significant increase in soil As concentration than where irrigation was achieved with As^{V} species. There was a significant interactive effect of species and pH for the control soil where irrigation with As^{III} at pH 6.1 led to a significant increase in soil As relative to irrigation with As^{V} at pH 7.5.

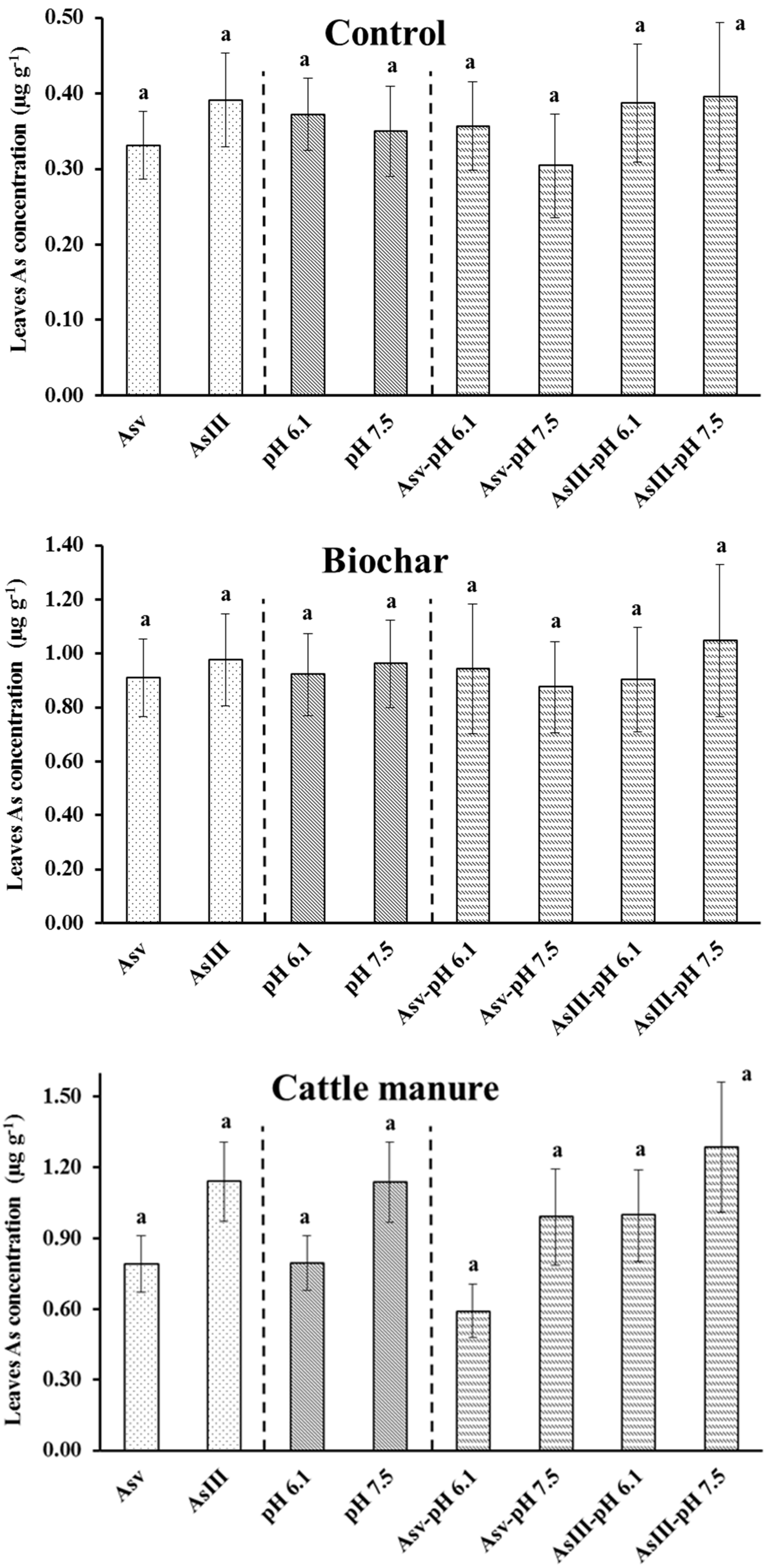

Figure 6.4 Arsenic concentration in spinach leaves (µg g^{-1} dry weight) as influenced by the As species in water and soil pH for a defined soil amendment; Bars with the same letter are not significantly different ($P > 0.05$) within the section they are present in (each section is separated with a dotted line); Each bar represents mean ± SE ($n = 50$ for species and pH whilst $n = 25$ for species-pH combination)

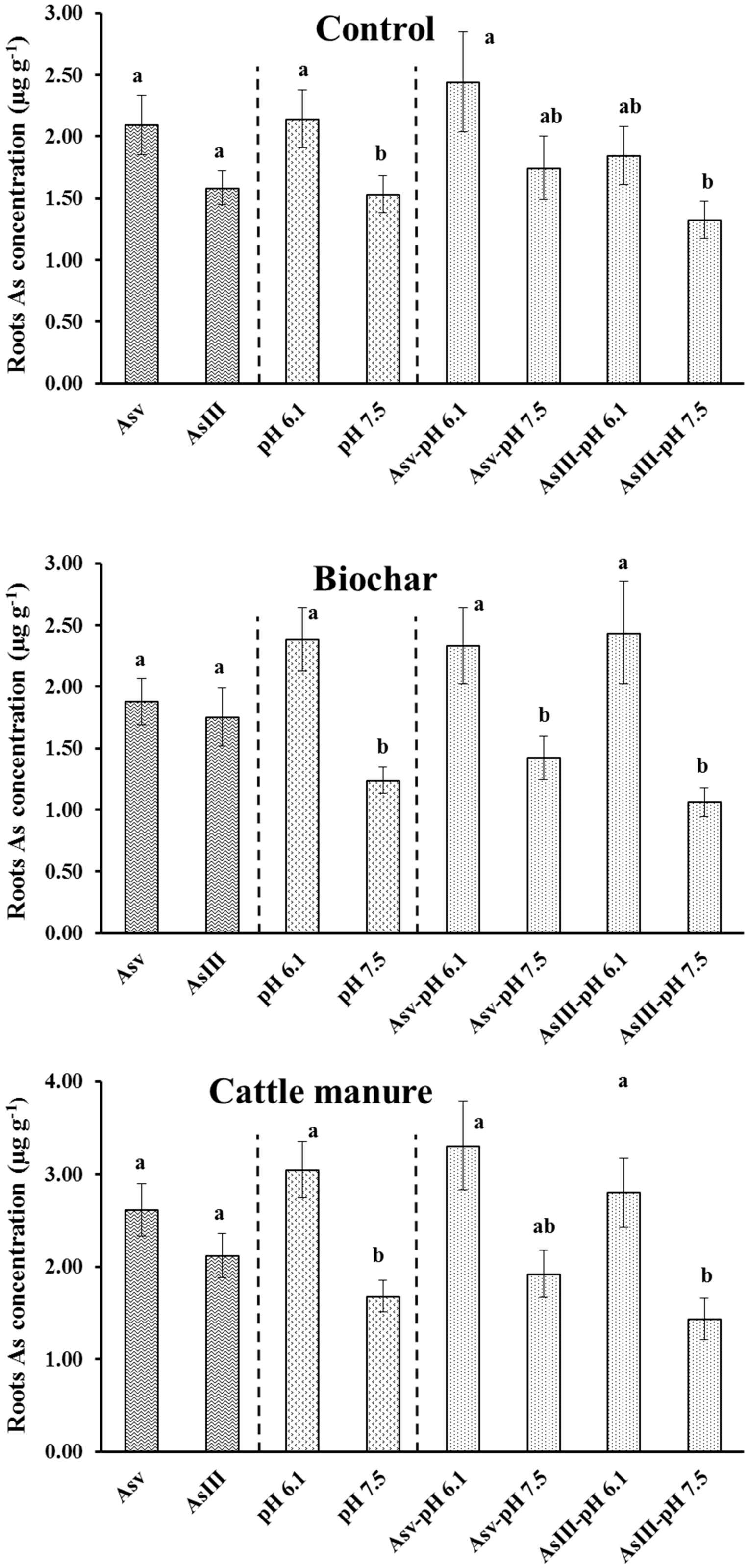

Figure 6.5 Arsenic concentration in spinach roots (µg g^{-1} dry weight) as influenced by the As species in water and soil pH for a defined soil amendment; Bars with the same letter are not significantly different ($P > 0.05$) within the section they are present in (each section is separated with a dotted line); Each bar represents mean ± SE ($n = 50$ for species and pH whilst $n = 25$ for species-pH combination)

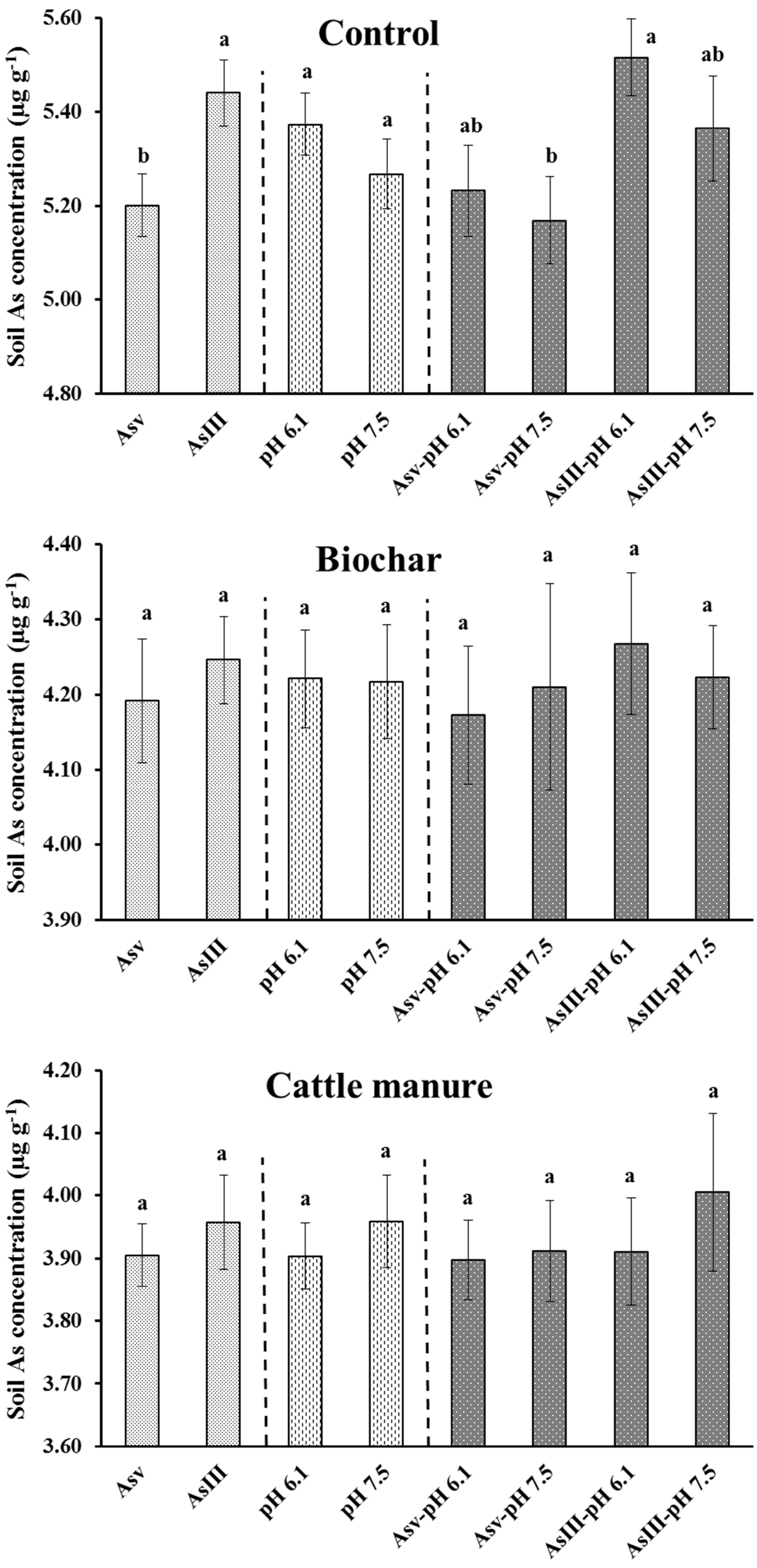

Figure 6.6 Arsenic concentration in soil (µg g^{-1}) after spinach harvest as influenced by the As species in water and soil pH for a defined soil amendment; Bars with the same letter are not significantly different ($P > 0.05$) within the section they are present in (each section is separated with a dotted line); Each bar represents mean ± SE ($n = 50$ for species and pH whilst $n = 25$ for species-pH combination)

6.4 DISCUSSION

There was an increase in the As concentration in spinach tissues (leaves and roots) as a function of the As concentrations in irrigation water irrespective of As speciation, soil pH and/or soil amendment. Similarly, the As concentration in soil at the end of the experiment also increased as a function of the As concentrations of the irrigation water. The high As concentration in spinach tissues and soil for the irrigation treatment of 500 and 1000 µg L^{-1} is proportionate to the increased input of As into the soil-plant system at these treatment levels. An increase in the As concentration of plant tissues as a function of the As concentration of growth media (soil or hydroponic solution) has been extensively reported for many vegetable crops including spinach (Burlo *et al.*, 1999; Carbonell-Barrachina *et al.*, 1999a; Carbonell-Barrachina *et al.*, 1999b; Pavlik *et al.*, 2010) and is in agreement with the findings of Chapter 4. Also, an elevated concentration of As in agricultural soils that have been irrigated with As-contaminated water has been reported (Bhattacharya *et al.*, 2010b; Dahal *et al.*, 2008; Moyano *et al.*, 2009).

The overall picture described in section 6.3.1 shows that plants irrigated with As^{III} had a significantly higher As concentration in their leaves and significantly lower As concentration in their roots relative to plants irrigated with As^{V} (Table 6.2 and 6.3). However, more detailed consideration of this data (section 6.3.2) infers that there was no significant difference between As species when the individual soil amendments are considered (Figure 6.4 and 6.5). It is difficult to speculate on the reason for this difference of results in overall picture (section 6.3.1) and detailed assessment of the experimental factors (section 6.3.2). However, a non-significant effect of As species may be expected under the flood irrigation technique adopted in this work. Flood irrigation will develop an anaerobic condition in soil after each irrigation event which may convert As^{V} species to As^{III} species. Once aerobic conditions develop in soil as a consequence of drainage, As^{V} becomes the dominant As species. Xu *et al.* (2008) reported that As^{III} and As^{V} were dominant under flooded and aerobic conditions respectively regardless of the original speciation of As added to soil (As^{III} or As^{V}). However, the kinetic for the speciation change is less defined in literature.

In soil, the effect of As species was significant and the soil irrigated with As^{III} retained more As than when the soil was irrigated with As^{V} (Table 6.4). However, detailed consideration of the data shows that this difference between species was only observed in the soil without any amendment (control soil, Figure 6.6). The described capacity for the soil to retain As^{III} is in contrast to the findings of the laboratory adsorption study described in Chapter 5 and to most adsorption studies that have been conducted (Lin & Puls, 2000; Smith *et al.*, 1999). The general observation from these studies is that As^{V} is adsorbed by soil to a greater degree than As^{III}. As a possible explanation for this discrepancy, several differences between laboratory adsorption study (Chapter 5) and the current glasshouse study can be cited which could have affected As^{V} adsorption in soil. Two major differences apparent for this glasshouse experiment include (i) the addition of phosphate fertilizer to soil for optimum plant growth, and (ii) the presence of crop species. Phosphate will compete with As^{V} for adsorption sites in soil and can subsequently increase the mobility of this species (Livesey & Huang, 1981; Qafoku *et al.*, 1999; Roy *et al.*, 1986). Plants may increase As mobility in soil by excretion of protons and/or the release of reducing and chelating compounds which could result in either dissolution of As-metal oxides/hydroxides and/or desorption of adsorbed As (Fitz & Wenzel, 2002; Moreno-Jiménez *et al.*, 2012). It appears that As^{III} mobility is less affected by these plant exudates. Moreover, As^{III} retention to soil was not affected by soil pH (6.1 and 7.5). This phenomenon provides evidence for the specific adsorption of As^{III} into the experimental soil (Pierce & Moore, 1982; Sadiq, 1997) which appears to be less affected by change in soil redox conditions.

The As concentration in spinach leaves was unaffected by soil pH (Table 6.2; Figure 6.4). However, in roots, there was a significantly higher As concentration in the plants growing at pH 6.1 relative to those growing at pH 7.5 (Table 6.3). The reported higher As concentration in spinach roots at pH 6.1 was apparent for the control, biochar and cattle manure soil amendments (Figure 6.5). There could be two possible reasons for a higher As concentration in spinach roots at pH 6.1. Firstly, a higher As concentration in roots would be expected if there was higher As availability in soil at this pH level. However, the soil retention data does not support this possibility; there was no significant difference in As retention to soil between the two soil pH levels (Table 6.4; Figure 6.6). Because retention is inversely related to mobility or bioavailability, no difference of retention between the two pH levels suggests no difference of As availability in soil. A second possible factor could be related to the activity of proton-coupled solute transporters within plants. White (2012) suggested that a decrease in pH can increase the activity of proton-coupled solute transporters and enhance anion uptake.

These transporters may have been enhanced at the acidic pH leading to a relatively higher As influx into root cells in current study.

The effect of soil amendments on the As concentration in spinach tissues (leaves and roots) was significant (Table 6.2 and 6.3; Figure 6.1 and 6.2). The plants grown in biochar and cattle manure amended soil had a higher As concentration in spinach tissues relative to the plants with no amendment. The opposite trend was observed for the concentration of As in soil at the end of the experiment (As retention), where the control treatment had a higher concentration of As than the biochar and cattle manure amended soil (Table 6.4; Figure 6.3). These findings indicate that biochar and cattle manure amendments increase As mobility in soil, and plants consequently accumulate this mobile As. This is a significant finding. Soil amendment with cattle manure is a common agronomic practice in South Asia. Flood irrigation and As contamination of irrigation water are also common scenarios. Soil amendment with cattle manure therefore may be increasing the risk of As exposure to the population through increase of As concentration in food crops. A possible reason for this increase is explored in the next section.

Possible mechanism of arsenic concentration in spinach tissues (leaves and roots)

The discussion in the preceding section shows that the As concentration in spinach tissues (leaves and roots) was primarily influenced by the As concentration in irrigation water and the soil amendments. Cattle manure and biochar addition to the soil increased the As concentration in spinach tissues and a possible mechanism for this increase can be proposed.

Once the cattle manure was applied to the experimental soil, decomposition may have been initiated by soil microbes because of the chemical composition of the manure (C:N ratio, 16:1) and the favourable environmental conditions. The favourable conditions that could be expected to exist in the soil include near to neutral pH, sufficient soil moisture, good aeration once the soil had drained, and warm temperature (Brady & Weil, 2008). The decomposition of organic matter releases many organic and inorganic compounds (for example humic and fulvic acids, nitrogen, phosphorus and sulphur) in the soil (Brady & Weil, 2008; McLaren & Cameron, 1996). Alternate periods of drying and rewetting (flood irrigation) can also accelerate organic matter decomposition and increase the concentration of dissolved organic compounds in soil (Miller *et al.*, 2005).

The ability of organic compounds and phosphate to compete with As^{V} and/or As^{III} and thereby prevent As species retention onto soil particles is well documented (Creger & Peryea, 1994; Dobran & Zagury, 2006; Qafoku *et al.*, 1999; Redman *et al.*, 2002). There is evidence for this phenomenon in the current study as less As retention (3 to 31%) was observed in soil amended with cattle manure relative to the soil with no amendment (Table 6.4).

Increasing the As concentration in the irrigation water leads to an increase in the concentration of As in soil solution. The consequence of this increase in soluble As is the recorded increase in the As concentration in spinach tissues across all the treatment levels (0 to 1000 µg L^{-1}). However, at low As concentrations in water (≤ 200 µg L^{-1}), the effect of concentration was limited. There was an abrupt increase in the As concentration in spinach tissues for the 500 and 1000 µg As L^{-1} irrigation treatments when applied to the cattle manure amended soil. An increased rate of As uptake into plant tissues at these concentration levels may be linked to soil adsorption capacity. It appears that most adsorption sites in soil may have been saturated at some point between 200 and 500 µg L^{-1} and the As introduced into the system above this concentration remains in soil solution and is available for uptake by plants. The previous laboratory adsorption study (Chapter 5) showed that the Rangitikei soil had some capacity to adsorb As, however this adsorption varied with the As species applied to the soil. At the highest As concentration (1000 µg L^{-1}), 98% of As^{V} and 55% of As^{III} was adsorbed by the Rangitikei soil during the reaction time of 36 hours. However, this glasshouse study differs from the adsorption study described in Chapter 5. The laboratory adsorption study was only conducted on the control soil (no amendment) where the added effect of organic molecules and phosphate ions may have been limited in comparison to the cattle manure amended soil. As stated earlier, these molecules may compete with As for adsorption sites thereby limiting adsorption capacity of soil. Flood irrigation further nullifies the effect of As species by inducing species inter-conversion, thereby mitigating the effect of species on As retention in soil (Figure 6.6).

Biochar amendment increased the concentration of As in spinach leaves to a similar level as the cattle manure amendment. In roots and soil, biochar had an intermediate effect on As retention in comparison to other amendments. As described for the cattle manure amendment, an increased As concentration in spinach tissues in the biochar amended soil may also be related to release of dissolved organic molecules (Beesley & Dickinson, 2011; Beesley *et al.*, 2010). Joseph *et al.* (2010) suggested that when biochar is added to the soil, the release of organic compounds present in biochar will be among the first reactions, especially when the soils are moist (a condition prevalent in the current experiment because of flood irrigation). The release of soluble organics may be more common in biochar produced at low pyrolysis temperature (< 500 ^{0}C) because of the predominance of amorphous C structure with a low aromaticity (Joseph *et al.*, 2010; McBeath & Smernik, 2009). In the current study, the biochar was pyrolyzed at 350 to 450 ^{0}C, therefore an increased organic compounds influx can be expected. An increased As concentration in soil pore water after biochar application has also been reported recently (Beesley *et al.*, 2013; Hartley *et al.*, 2009). This effect has been attributed to (i) competition between P and As for binding sites, and (ii) increased soil pH as a function of biochar application (Hartley *et al.*, 2009). Increase in soil pH of the Rangitikei soil may be expected from addition of biochar and cattle manure amendment which had a pH of 7.4 and 7.6 respectively (Table 6.1). However, an increase in soil pH does not enhance As accumulation in spinach tissues, as evidenced by the lack of significant effect of pH on the As concentration in spinach leaves (Table 6.2) and the reduced concentration of As in spinach roots at pH 7.5 (Table 6.3).

Organic matter can also increase As solubility and mobility in soil by acting as an electron donor for microbes which are involved in the reductive dissolution of As-rich metal oxides/hydroxides minerals (Anawar *et al.*, 2006; Anawar *et al.*, 2013; Islam *et al.*, 2004). Islam *et al.* (2004) observed a marked increase in the rate of Fe (III) reduction followed by arsenic release when sodium acetate (a proxy for organic matter) was added to sediments under anaerobic conditions. The released As^V may subsequently be reduced to As^{III} by soil microbes (Islam *et al.*, 2004; Postma *et al.*, 2007) and the species As^{III} is generally more soluble and mobile in soil than As^V species. The increased As concentration in amended soils (cattle manure and biochar) of the current study may also be related to an increased influx of As by reductive dissolution of metal oxides/hydroxides minerals that may have been promoted due to presence of these carbon sources. McArthur *et al.* (2001) suggested that As will not be released from Fe oxides/hydroxides unless organic matter is present. This is an area of future research.

A general model for enhanced As concentration in spinach tissues as affected by cattle manure and biochar amendments is graphically presented in Figure 6.7. This model proposes that the application of cattle manure and biochar to the Rangitikei soil increased microbial activity. These microbes initiated weathering of these carbon amendments and released a substantial amount of organic compounds and inorganic molecules (for example phosphate) into the soil solution which compete with As for retention onto soil particles. As a result, a large proportion of As remained in soil solution which was taken up by spinach plants.

6.5 RISK ASSESSMENT

To investigate the potential health risk to humans that could be associated with ingestion of spinach grown in this study, the As concentration in spinach leaves was recalculated and re-expressed in fresh weight as a function of the As concentration in irrigation water and soil amendments (Table 6.5). There is a discrepancy between the As concentration in the control treatment for this experiment when compared to the earlier experiment described in Chapter 4. The As concentration in spinach leaves for the current experiment was lower than the concentration reported, for the same As irrigation levels, in Chapter 4. This may be due to a greater number of days under submerged conditions for the plants grown in the previous study (three days in glasshouse experiment 1 but two days for this experiment). Longer submerged conditions may increase As solubility and thereby increase uptake by plants (Li *et al.*, 2009; Masscheleyn *et al.*, 1991; Xu *et al.*, 2008).

The As concentration in spinach leaves grown in the biochar and cattle manure amended soil was generally equivalent. However, the As concentration in spinach leaves of these amended soils was two-to-three fold higher than the As concentration of spinach leaves grown in unamended soil for all As treatment water levels (50 to 1000 µg L^{-1}).

The As concentration for the plants grown in unamended soil was above the Chinese maximum permissible concentration (MPC) value (0.05 µg g^{-1} fresh weight) when irrigated with 500 and 1000 µg As L^{-1}. For amended soils, the As concentration in spinach leaves exceeded the MPC level where they were irrigated with 200 µg As L^{-1} or greater. For carbon treatments, the As concentration in spinach leaves was 1.6 to 8.3 times higher than the MPC level, and therefore present an unacceptable risk to consumers.

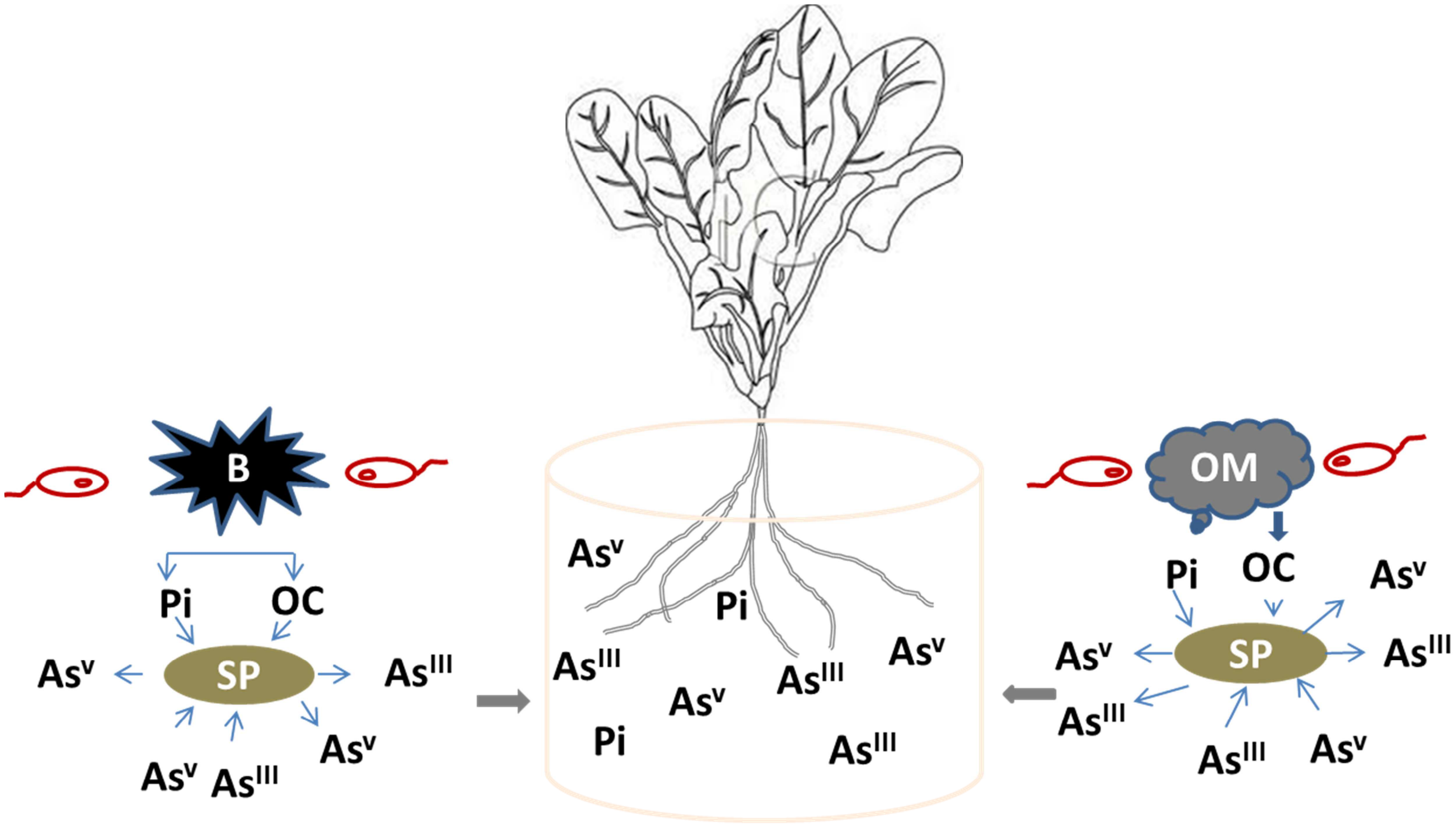

Figure 6.7 Possible mechanism of As concentration in spinach tissues (leaves and roots) when irrigated with As treatment waters and amended with biochar and cattle manure. B stands for biochar; OM for organic matter; Pi for $H_2PO_4^-$ or HPO_4^{-2} ions; OC for organic compounds; SP for soil particles involved in adsorption;  represents soil microbes

Table 6.5 Total As concentration in spinach leaves (µg g^{-1}) as a function of the As concentration in irrigation water and soil amendments

Arsenic in water (µg L^{-1})	Control		Biochar		Cattle manure	
	Dry weight [a]	**Fresh weight** [b]	**Dry weight**	**Fresh weight**	**Dry weight**	**Fresh weight**
0	0.061 ± 0.007	0.010	0.066 ± 0.002	0.011	0.083 ± 0.008	0.013
50	0.106 ± 0.015	0.017	0.178 ± 0.017	0.028	0.205 ± 0.024	0.033
200	0.275 ± 0.022	0.044	0.492 ± 0.033	0.079	0.496 ± 0.044	0.079
500	0.479 ± 0.050	0.077	1.382 ± 0.113	0.221	1.659 ± 0.178	0.265
1000	0.885 ± 0.105	0.142	2.594 ± 0.268	0.415	2.386 ± 0.183	0.382

[a] each value is mean ± SE (*n* = 20); [b] Mean fresh weight As concentration was calculated as a product of mean As concentration (µg g^{-1} dry weight) and mean water content of spinach (84% in this case) according to formula: fresh weight µg g^{-1} = (dry weight µg g^{-1}) x (1- % moisture/100)

The risk was further explored using the USEPA hazard quotient (HQ) and cancer risk (CR) parameters. Only the calculations for spinach leaves grown in cattle manure amended soil are presented for further discussion because of the prevalence of cattle manure as common agronomic practice in South Asian countries. The results for the biochar amended soil will be similar to cattle manure soil because of the more or less similar As concentrations in spinach leaves (Table 6.5). Additionally, two vegetable consumption scenarios were considered. In scenario one, West Bengal India data was used where the reported vegetable consumption per capita is 450 to 500 grams per day (Roychowdhury *et al.*, 2003; Samal *et al.*, 2011). In scenario two, Bangladesh vegetable consumption data, ranging from 130 to 205 grams per day, was used (Alam *et al.*, 2003; Khan *et al.*, 2009; Rahman *et al.*, 2012).

Calculation and comparison of As intake from spinach leaves with USEPA parameters

Considering the HQ values, the people of West Bengal India appear to be exposed to greater non-carcinogenic risk than the people of Bangladesh, because of their higher vegetable consumption per day (Table 6.6 and Appendix VI and VII). The HQ values for Indian people exceeded the critical value of 1 for both adults and adolescents, where the spinach is irrigated with 500 or 1000 µg As L^{-1}. Interestingly, the CR values for both scenarios was in excess of 1 in 10,000 where the irrigation water with an As concentration $\geq$ 500 µg L^{-1} was applied. The CR value also exceeded the critical value (1×10^{-4}) for scenario one, where 200 µg As L^{-1} was used for irrigation. These calculations indicate that both an unacceptable non-carcinogenic and carcinogenic risk may develop if an individual continues to eat As-contaminated spinach during his/her lifetime period. However, these calculations do not describe the type of effect (acute or chronic) and associated symptoms and targeted body organs that may be affected by this As poisoning.

The specific effects to an individual through consumption of As-enriched spinach leaves were assessed using following models.

Calculation and comparison of As intake from spinach leaves with ATSDR notified As intake values

The daily As intake (mg kg^{-1} body weight) from consumption of As-contaminated spinach leaves is summarized in Table 6.7.

Table 6.6 Hazard quotient (HQ) and cancer risk (CR) for the ingestion of spinach leaves grown in cattle manure amended soil

As in water (µg L^{-1})	Leaves As (µg g^{-1} fresh weight)[a]	**Scenario 1 (Example West Bengal India): where vegetables are consumed 500 grams per day**				**Scenario 2 (Example Bangladesh): where vegetables are consumed 205 grams per day**			
		Hazard Quotient (HQ)		Cancer risk (CR)		Hazard Quotient (HQ)		Cancer risk (CR)	
		Adults[c]	Adolescent[d]	Adults	Adolescent	Adults	Adolescent	Adults	Adolescent
200	0.079[b]	0.31	0.38	1.4×10^{-4}	1.7×10^{-4}	0.13	0.15	5.8×10^{-5}	6.9×10^{-5}
500	0.265	1.05	1.26	4.7×10^{-4}	5.7×10^{-4}	0.43	0.52	1.9×10^{-4}	2.3×10^{-4}
1000	0.382	1.51	1.81	6.8×10^{-4}	8.2×10^{-4}	0.62	0.74	2.8×10^{-4}	3.3×10^{-4}

[a] refer to Table 6.5; [b] parameters have been calculated where the As concentration in spinach leaves exceeds the Chinese MPC value (0.05 µg g^{-1} fresh weight); [c] adults (> 18 years); [d] Adolescent (12-18 years old); An HQ value greater than 1 defines an unacceptable non-carcinogenic risk to humans while a CR value greater than 1 in 10,000 propose an unacceptable cancer risk in humans.

The As intake per day of Indian people (adults and adolescents) is higher than the As intake of Bangladesh people (adults and adolescent) due to their higher vegetable intake per day (500 grams; Table 6.7). However, within each vegetable intake scenario (500 or 205 grams per day), the As intake values of adults and adolescent are similar. Therefore, the As intake values for adults attributable to the ingestion of As-enriched spinach leaves was compared with ATSDR notified As intake values.

Table 6.7 Arsenic daily intake (mg kg^{-1} body weight) for the ingestion of spinach leaves grown in cattle manure amended soil

As in water (µg L^{-1})	Leaves As (µg g^{-1} fresh weight) [a]	**Scenario 1 (Example West Bengal India): where vegetables are consumed 500 grams per day**		**Scenario 2 (Example Bangladesh): where vegetables are consumed 205 grams per day**	
		Adults [b]	Adolescent [c]	Adults	Adolescent
200	0.079 [d]	0.0007 [e]	0.0008	0.0003	0.0003
500	0.265	0.0022	0.0027	0.0009	0.0011
1000	0.382	0.0032	0.0038	0.0013	0.0016

[a] refer to Table 6.5; [b] adults (> 18 years and 60 kg body weight); [c] Adolescent (12-18 years old and 50 kg body weight); [d] parameters have been calculated where the As concentration in spinach leaves exceeds the Chinese MPC value (0.05 µg g^{-1} fresh weight); [e] calculated as, As concentration in spinach leaves (mg kg^{-1}) x mass of spinach eaten each day (kg) / body weight of an individual.

The As intake for an adult due to the consumption of As-enriched spinach leaves ranged from 0.0007 to 0.0032 mg kg^{-1} body weight day^{-1} where 500 grams vegetables are eaten per day (Table 6.7). At these intake values, an individual may develop bladder and lung cancers, and skin lesions (Table 6.8) through continuous ingestion of As-enriched spinach during his/her lifetime.

For the areas where vegetables are consumed at a rate of 205 grams per day, the As intake associated with the ingestion of As-enriched spinach leaves ranged from 0.0003 to 0.0013 mg kg^{-1} body weight day^{-1}. At these As intake values, As will affect the lung and bladder and therefore the probability of developing bladder and lung cancers increases. An increase in incidence of skin lesions may also develop at these As intake values (Table 6.8).

Table 6.8 Summary of the health effects of arsenic

Dose (mg kg^{-1} day^{-1})	**Type of poisoning**	**Effects**
> 2	Acute	Vomiting, diarrhea, and abdominal pain, headache, lethargy, mental confusion, hallucination, seizures, and coma
> 0.065 to 0.14	Chronic	Cardiovascular diseases such as "blackfoot disease", which is endemic in an area of Taiwan where average arsenic concentrations in drinking water range from 0.17 to 80 µg L^{-1}
0.03 to 0.1	Chronic	Peripheral neuropathy, characterized initially by numbness of the hands and feet and a "pins and needles" sensation and progressing to muscle weakness, wrist-drop and/or ankle-drop, diminished sensitivity, and altered reflex action. Reports of neurological effects at lower arsenic levels (0.004 to 0.006 mg kg^{-1} day^{-1}) have been inconsistent Minor respiratory symptoms such as cough, sputum, rhinorrhea, and sore throat
0.01	Chronic	Vomiting, diarrhea, and abdominal pain; symptoms diminish after cessation of exposure
> 0.002	Chronic	Skin lesions (hyperkeratinisation and hyperpigmentation)
0.0012	Chronic	Lowest reported dosage associated with increased incidence of skin lesions
0.0000086	Chronic	Bladder and lung cancers- negligible risk based on consumption of 0.3 µg L^{-1} in drinking water
0.000001 to 0.000002	Chronic	Bladder and lung cancer

Source: Adopted from Ministry for the Environment (2011) and the references cited therein

Calculation and comparison of As intake from spinach leaves with drinking water As intakes

As stated earlier, the As intake for an adult ranges from 0.0007 to 0.0032 mg kg^{-1} body weight per day^{-1} where 500 grams vegetables are eaten in a single day (Table 6.7). These intake values are equivalent to drinking 2 litres of water each day that has an As concentrations above 50 µg L^{-1} (but less than 115 µg L^{-1}; Table 6.9). At these intake values, As may lead to the development of cancer (bladder, lung and skin) in an individual during his/her lifetime. Children could be more susceptible at these intake values and may have intellectual impairment.

Table 6.9 Effects and symptoms linked to various concentrations of As in drinking water [a]

Arsenic conc [†] (μg L^{-1})	**Type of poisoning**	**Identified symptoms and effects so far linked to arsenic in drinking water**	**Daily Intake / Dose (mg kg^{-1} bw [††] day^{-1}) [b]**
Above 1200	Acute	Abdominal pain, vomiting, diarrhea, muscular weakness and cramping, pain to the extremities, erythematous skin eruptions (erythema is redness of the skin caused by dilatation and congestion of the capillaries), swelling of eyelids, feet and hands. Possible progressive deterioration in motor and sensory responses (depending on exposure and vulnerability), finally leading to shock and death	> 0.04
Above 115	Chronic	**Non-cancerous effects:** skin lesions, diabetes mellitus (also known as type 1 diabetes and insulin-dependent diabetes), adverse effects on the digestive, respiratory, cardiovascular, and nervous systems, bronchiectasis (chronic permanent widening of the bronchial tubes), reproductive and developmental effects. A number of effects depend also on nutritional and other factors: variation between countries seen in degree of skin pigmentation, hyperkeratosis (an excessive thickening of the outer layer of the skin-appearance of thickened, horny, verruca-like scales over the entire body), respiratory stress, polyneuropathy (inflammation of multiple nerves causing loss of sensation or movement), and peripheral vascular disease. **Caners:** skin, bladder, kidney, liver, lung, colon, uterus, prostate, and stomach. Lung cancer a leading cause of death among those exposed	> 0.004
Above 50	Chronic	Intellectual impairment in children, cancer risk significant	> 0.002
20	Chronic	Lifetime risk of bladder or lung cancer from the exposure, estimated to be about 1 in 140 people	0.0007
10	Chronic	Lifetime excess bladder or lung cancer risk, estimated at about 1 in 300. Excess skin cancer risk estimated at 1 in 1700 people	0.0003
5	Chronic	Lifetime excess risk of bladder or lung cancer, estimated at about 1 in 500 people	0.0002
3	Chronic	Lifetime excess risk of bladder or lung cancer, estimated at about 1 in 900 people	0.0001

[a] adopted and modified from Piper and Kim (2006) and references cited therein; [b] calculated by the author using the formula: As concentration in drinking water (mg L^{-1}) x Amount of water used per day (2 L) divided by body weight of an adult (60 kg); [†] concentration, and [††] body weight

For a daily vegetable consumption of 205 grams, As intake ranges from 0.0003 to 0.0013 mg kg^{-1} body weight day^{-1} (Table 6.7). These values correspond to an As concentration in drinking water above 20 µg L^{-1} (but less than 50 µg L^{-1}; Table 6.9). At these intakes, there is an increased risk of developing bladder, lung and skin cancer by an individual if he/she eats spinach that has been enriched with As through irrigation of As-contaminated water (≥ 200 µg L^{-1}).

Summary of quantified risk assessment (USEPA parameters, ATSDR and drinking water intake reference values)

Each of the three risk assessment models show that there is an unacceptable risk to individuals associated with the ingestion of As-enriched spinach, regardless of the amount of vegetable eaten each day (500 or 205 grams). An individual may develop cancer of the bladder, lung and skin as a consequence of continuous ingestion of As-enriched spinach during his/her lifetime. There is also an increased possibility of developing skin lesions with these As intakes from spinach. Children may be subjected to increased severity of these effects and may experience intellectual impairment. The daily As intake (mg kg^{-1} body weight) that can be attributed to the ingestion of spinach leaves corresponds to an As concentration in drinking water of 10 µg L^{-1} or greater.

Implications of this risk assessment approach

The findings of this study have major implications for areas where drinking water (surface or groundwater) has an acceptable As concentration (≤ 10 µg L^{-1}) but As-contaminated water (groundwater and/or wastewater) is used for vegetables cultivation (typical example of Karachi, Pakistan). While the risk of As in drinking water is well described, the equivalent risk of As in vegetables in poorly discussed in scientific literature. The WHO has proposed a maximum permissible As concentration in drinking water of 10 µg L^{-1} and efforts are being made to keep the As concentration in drinking water below this level. In the scenarios where vegetables are irrigated with As-contaminated water and accumulate this metalloid, then the intake of As through the vector of As-enriched vegetables (spinach in this case) will offset the efforts of countries to safeguard their people against As in drinking water. As per the findings of this study, the continuous ingestion of As-enriched spinach will have carcinogenic (cancer of bladder, lung and skin) and non-carcinogenic (skin lesions and intellectual impairment of children) effects on people who eat this As-enriched spinach during their lifetime.

This study also has profound implications for areas where As-contaminated drinking water (> 10 µg L^{-1}) is ingested in conjunction with As-contaminated foods (rice, vegetables). In these areas, the risk would possibly be many orders of magnitude higher due to the double exposure pathway (food and water). As a result, the people of these areas will be at greater risk of more serious As-induced poisoning compared to the people of areas where there is only a single exposure pathway (drinking water or food) to As poisoning.

6.6 CONCLUSIONS

The As concentration in spinach leaves was significantly higher where plants were irrigated with 500 and 1000 µg As L^{-1} treatment water relative to other treatment water levels (0 to 200 µg L^{-1}). The effect of As^{V} or As^{III} was found to be insignificant, possibly because of the use of flood irrigation which may have inter-converted these As species with a change in soil redox conditions. The As concentration in leaves was independent of soil pH. However, pH had a significant effect on the root As concentration. Plants grown at pH 6.1 contained a higher concentration of As than the plants grown at pH 7.5. Amendment of soil with biochar or cattle manure led to an increase in the As concentration in leaves (nearly two to three fold) and reduced the level of As retention in soil relative to the unamended soil. The concentration of As in the spinach leaves was above the Chinese MPC value (0.05 µg g^{-1} fresh weight) for unamended soil when irrigated with water that had an As concentration $\geq$ 500 µg L^{-1}. For amended soils, the concentration of As in leaves exceeded the MPC value when the concentration of As in irrigation water was 200 µg L^{-1} or greater. The risk was further explored for spinach leaves grown in cattle manure amended soil by considering two vegetable consumption scenarios for India and Bangladesh, USEPA proposed reference values, and the comparison of As intake with ATSDR and drinking water intakes. The USEPA cancer risk (CR) was greater than the critical value (1×10^{-4}) for people of both countries. The USEPA non-carcinogenic risk parameter (HQ) was above the critical value of 1 only for Indian people because of their higher vegetables consumption (500 grams per day). Arsenic intake (mg kg^{-1} body weight day^{-1}) associated with the ingestion of spinach leaves corresponds to proposed ATSDR oral reference As intakes values that may lead to development of bladder and lung cancer, and skin lesions. When compared to daily drinking water intakes, the As intake values associated with the ingestion of spinach correlate to an As concentration in drinking water that is 10 µg L^{-1} or greater. At these As concentrations in drinking water, As may lead to bladder, lung and skin cancer in an individual during his/her lifetime. Intellectual impairment in children may also be expected at these exposure levels. This work indicates that cultivation of spinach using flood irrigation with an irrigation water concentration greater than 50 µg L^{-1} and where cattle manure or biochar is used as soil amendment, will lead to an unacceptable risk to human health during his/her lifetime.

6.7 REFERENCES

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CHAPTER 7

KEY FINDINGS FROM THIS RESEARCH: SCALE OF RISK AND RECOMMENDATIONS FOR FOOD SAFETY

7.1 INTRODUCTION

This chapter reviews the defined objectives of this study (Chapter 1 and 2) in relation to research conducted, to state what the results have revealed about the objectives. The role of stakeholders in proposed As irrigation management strategy and future research needs in the subject area are also discussed here.

7.2 OBJECTIVES OF THE STUDY

7.2.1 A better understanding of the risk associated with irrigating vegetables with As-contaminated water in soil environment

Arsenic contaminated water and rice are currently considered the main sources of As poisoning in humans in South Asia. Vegetables are given less attention and believed to contribute little to overall As ingestion because of their low consumption rate per day.

My research shows that vegetable cultivation with As-contaminated water can pose an unacceptable risk to food safety and thus to human health. As shown in this study, vegetable species can alone be a major source of As poisoning in humans and should not be overlooked. Even at a low vegetable consumption rate per day (205 grams, Chapter 6), an individual vegetable species (spinach in this case) can contain enough As to pose an unacceptable carcinogenic risk (defined by USEPA) to humans, when irrigated with 500 and 1000 $\mu g\ L^{-1}$ As. When compared with ATSDR and drinking water reference daily intake values, the As intake through consumption of spinach may cause skin, bladder and lung cancer, skin lesions and intellectual impairment in children (Chapter 6). The As intake values associated with the ingestion of spinach correspond to an As concentration in drinking water that is 10 $\mu g\ L^{-1}$ or greater.

In the above discussion, the USEPA defined carcinogenic risk was calculated for the scenario where spinach consumption frequency (Ef) is 52 days in a year (once or twice in a week). Consider a scenario where various vegetables are irrigated with As-contaminated water, and are eaten every day during a year (Ef 365 days). Risk for this scenario can be again calculated for two vegetable consumption rates, 205 and 500 grams (fresh weight) per day (Table 7.1). In this scenario, the risk of As poisoning is unacceptable even at low As concentrations in vegetables tissues. An arsenic concentration of 0.040 µg g^{-1} fresh weight can present an unacceptable non-carcinogenic and carcinogenic risk to consumers when vegetables are consumed at a rate of 500 grams per day. When vegetables are consumed at a rate of 205 grams a day, unacceptable carcinogenic and non-carcinogenic risks are apparent from an As concentration of 0.097 µg g^{-1} fresh weight. Thus, a continuous ingestion of As by humans from As-contaminated vegetables, even at low concentrations in the vegetable's edible tissues, is a potential health risk. In the scenario of eating vegetable every day, the As intake (mg kg^{-1} body weight) will be many orders of magnitude higher than the scenario where vegetables are eaten once or twice in a week. Individuals who eat As-enriched vegetables each day may be exposed to heightened risk of experiencing chronic and acute effects of As poisoning. These may include both acute and chronic non-carcinogenic (abdominal pain, vomiting, diarrhea, skin lesions, diabetes) and carcinogenic (bladder, lung and skin cancer) effects. A detailed description of these acute and chronic effects is summarized in Chapter 6 (Table 6.8 and 6.9).

Table 7.1 Hazard quotient (HQ) and cancer risk (CR) for the ingestion of vegetable species eaten every day of the year

As concentration (µg g^{-1} fresh weight)	**Hazard Quotient (HQ)**		**Cancer risk (CR)**	
	Adults [a]	**Adolescent** [b]	**Adults**	**Adolescent**
Scenario 1: where vegetables are consumed 500 grams per day (Example West Bengal India)				
0.040	1.11	1.33	5.0×10^{-4}	6.0×10^{-4}
Scenario 2: where vegetables are consumed 205 grams per day (Example Bangladesh)				
0.097	1.10	1.33	5.0×10^{-4}	6.0×10^{-4}

[a] adults (> 18 years); [b] Adolescent (12-18 years old)

7.2.2 The screening of vegetable species that may accumulate a higher concentration of As in their edible parts and therefore pose a greater risk to humans through their consumption

Chapter 4 shows that vegetable species vary in their abilities to accumulate As in their edible tissues under similar growth conditions. Spinach had the highest As concentration in its edible tissues relative to other vegetable species. The As concentration in spinach leaves was above the Chinese food safety standard for inorganic As (0.05 µg g^{-1} fresh weight) by a factor of 1.6 to 6.4 times. This increase was observed for As treatment water concentrations 100, 200, and 1000 µg L^{-1} in flood irrigation and for 1000 µg L^{-1} in non-flood irrigation. The As concentration in spinach leaves shows an unacceptable carcinogenic and non-carcinogenic risk to humans upon their consumption (CR value greater than the critical value of 10^{-4} and HQ value greater than the critical value of 1; Chapter 4).

7.2.3 Determination of an acceptable level of As in irrigation water that can be used for vegetable cultivation

An acceptable level of As in irrigation water may be defined as, *the As concentration in irrigation water that can be used for food crops cultivation without a risk of promoting As concentration in their edible tissues to a level that can be risky for humans.*

Chapter 4 shows that an acceptable level of As in irrigation water depends on the vegetable species and technique adopted to irrigate them. The As concentration in irrigation water which may be acceptable for a specific vegetable species can be proposed from this study. An As concentration of 50 µg L^{-1} appears acceptable for spinach cultivation when subjected to flood irrigation. Under non-flood irrigation, an As concentration of 200 µg L^{-1} appears acceptable for spinach. For carrot, As concentration up to 1000 µg L^{-1} appears acceptable under both flood and non-flood irrigation. The As concentration in tomato fruit and radish edible parts (taproot without skin) was below the Chinese food safety standards when irrigated with water concentrations up to 1000 µg L^{-1} under non-flood irrigation, therefore, this concentration level (1000 µg L^{-1}) appears acceptable for their cultivation.

7.2.4 Improved understanding of the various As and soil factors, and soil management practices, that affect the As concentration in edible parts of vegetables

Chapter 6 shows that the As concentration in spinach leaves was mainly dependent on both the As concentration in irrigation water and soil amendments used (biochar or cattle manure). Increased influx of As into spinach leaves in biochar-and-cattle manure amended soils may be attributed to release of organic compounds and phosphate ions from these amendments by soil microbes. These results are significant, considering the common use of cattle manure as a soil amendment in South Asia and biochar as an emerging soil amendment. The effect of As species and pH was insignificant, possibly because of change in As speciation and soil chemistry due to a change in soil redox conditions under flood irrigation.

7.2.5 Improved understanding of soil adsorption parameters and models, and elucidation of key soil properties which control As adsorption in soils of variable physical and chemical characteristics

Results of Chapter 5 show that As^{V} and As^{III} vary in their adsorption kinetics. Most of the As^{V} was adsorbed in the first 3 hours while most of As^{III} was adsorbed in the first 6 hours of reaction time. Similarly, considering the adsorption parameters, As^{V} was adsorbed to a greater degree than As^{III} in each soil, as defined by high adsorption maxima, bonding energy and As partition coefficient values. This difference in As adsorption between As species is a significant finding and highlights the risk of irrigating crops with As^{III}-laden water, as this species will remain bioavailable and leachable in these soils.

The Freundlich isotherm better modeled the adsorption behavior of selected soils than the Langmuir model, indicating a heterogeneous nature of adsorbing sites in the studied soils. Adsorption of As was mainly controlled by amorphous Al, total C and Olsen P content of selected soils. This understanding is important from an As management perspective. Future experiments using these soil properties (amorphous Al, total C and Olsen P) with different application rates may help to refine As management in selected soils.

7.2.6 Propose management strategies for South Asian horticulture where As-contaminated irrigation water is often used for vegetable cultivation

Arsenic is ubiquitous and unavoidable in South Asia because it occurs naturally in ground water that is ultimately used for drinking and irrigation. A decision to ban As-contaminated irrigation water will have several impacts including (i) farmers, who are already experiencing a poor quality life in South Asian countries, will have their livelihood and income reduced, (ii) will cause a decrease in the food supply of the area where the vegetables are grown, and ultimately the food supply of the country, and (iii) a need for the provision of alternative water resources for irrigation of crops. Therefore, management of As-contaminated irrigation water will be helpful for sustainable agriculture.

Based on the results of this study, I propose some management strategies which can be used where As-enriched irrigation water is used for cultivation of vegetables and other food crops.

Adoption of proper irrigation techniques

The flood irrigation technique should be discouraged, since this technique increases As mobility in soil and thereby its uptake by crops. Instead, alternative irrigation techniques should be adopted, such as non-flood irrigation, which involves less input of As in soil and development of aerobic conditions, thereby increasing As adsorption by soil minerals. Drip or sprinkler irrigation techniques may be adopted; however, their effect on As cycling is uncertain because of lack of research data.

Arsenic concentration in irrigation water

An acceptable level of As in irrigation water should be adopted which does not promote As in vegetables edible tissues to a risk point. An acceptable As concentration level in irrigation water can only be determined by scientific research, since it depends on many factors including crop species, soil types, and agronomic practices. Based on the results of my study, I propose that an As concentration greater than 50 $\mu g\ L^{-1}$ should not be used for spinach cultivation with existing flood irrigation technique in South Asia. Irrigation water which contains unacceptable concentrations of As should be used for non-food crops, for instance cotton, cut flowers etc.

Selection of crop species

Crops should be selected with caution. The crops which actively accumulate As in their edible tissues (for example spinach) should be replaced with the crops which accumulate less As in their edible parts. Such knowledge can be obtained by future studies where various vegetables are tested for their uptake potential under different As concentrations in irrigation water. From the results of the current study, I propose that radish and tomato could be grown in a silt loam soil with As concentration levels up to 1000 µg L^{-1} using non-flood irrigation. Similarly, carrot may be grown using both flood and non-flood irrigation where As concentration in irrigation water is up to 1000 µg L^{-1}. However, the skin of root vegetables (e.g. carrot, radish) should be removed before ingestion as a significant portion of the As burden of vegetables is found in the skin. The validity of these propositions should be tested in a range of soil types and environmental conditions.

Adoption of proper agronomic practices

Addition of cattle manure to soils should be made with caution as it may increase As concentration in crop tissues. The results of Chapter 6 show that the As concentration in spinach leaves of cattle manure-amended soil was 2 to 3 times higher than the As concentration of spinach leaves grown in unamended soil for all experimental As concentrations (50 to 1000 µg L^{-1}).

South Asian soils are generally deficient in organic matter (≤ 1.0 %) and addition of cattle manure is an important practice to improve soil fertility status. Future research is therefore urgently required to test our glasshouse results over a wider range of soil types and cattle manure application rates to find an optimum application rate where irrigation with As-contaminated water occurs.

7.3 ROLE OF STAKEHOLDERS

A number of stakeholders would be required to contribute in this As irrigation management strategy.

Governments

Governments of South Asian countries should consider the severity of As poisoning to humans from vegetables which are being irrigated with As-enriched water. Funds should be allocated for food safety and sustainable agriculture research. Various relevant sectors such as agriculture, water, and environmental agencies should be involved. These agencies should conduct research in relevant fields and develop appropriate management strategies for the affected areas. Government should also enforce robust environmental regulations and proposed management strategies.

The areas where As-enriched untreated industrial and sewage effluents are used for irrigation of food crops should be identified. In Pakistan, the majority of industries (95% of the industries) dispose of their untreated industrial effluent to sewers, which is used by farmers for cultivation of food crops (Husaini *et al.*, 2011; WWF, 2007). Industrial effluent with an As concentration near to 100 mg L^{-1} is used in Faisalabad, Pakistan to grow vegetables and other food crops (Husaini *et al.*, 2011). In general, one fourth of the vegetables grown in Pakistan are irrigated with untreated wastewater (Zia & Aziz, 2007). Government should impose and enforce appropriate industrial water quality standards for effluent discharge. For contaminated sewage waters, treatment plants should be established and equipped with latest technologies to control As concentrations in sewage waters.

Drip and sprinkler irrigation techniques are unaffordable for most farmers. Therefore, government should expand provision of financial assistance to farmers to adopt these modern technologies. Such financial assistance has been underway in Pakistan, where 80% of the cost of drip irrigation was being paid by the government. The remaining 20% of the cost was borne by the farmers.

Non-governmental organizations (NGOs) & environmental organizations

National and international organizations should increase efforts to help governments and people of South Asian countries to promote food safety and sustainable agriculture. The role of FAO, UNICEF and WHO is appreciated in this regard; these organizations are actively working for the supply of acceptable drinking water, mitigating adverse health impacts of arsenic poisoning, and highlighting issues of excessive levels of As in irrigation water (Heikens, 2006). These national and international organizations should continue to educate farmers, general public and governments about health concerns which arise from irrigating the food crops with As-contaminated water. The latest scientific knowledge and management strategies in the subject area should also be promoted.

Farmers

Farmers should be educated to understand the risk of irrigating vegetables with As water in terms of human health and land degradation. They should adopt the management strategies proposed to them by government, national and international agencies for their personal wellbeing and safety of their fellow countrymen.

Scientific community

Scientists from various organizations (such as universities, national and international organizations) should develop projects to understand the As uptake potential of various food crops. Field trials should be established on farmers’ fields where the practice of As-contaminated irrigation water is executed. Such trials will help to understand the As uptake potential of vegetables in real situation. Food intake and vegetables consumption pattern of affected areas should also be explored. Low cost As treatment technologies should be investigated which keep As concentrations in groundwater and industrial effluent as low as possible. Finally, general and site-specific management strategies should be developed to minimize risk to human health.

7.4 RECOMMENDATIONS FOR FUTURE WORK

- The concentration of As in irrigation water was limited to 1000 µg As L^{-1} in this research. In some instances, the As concentration in irrigation water is many orders of magnitude higher than this concentration, especially where industrial effluent is used for vegetable cultivation. The As concentration in wastewater used for irrigation has been reported to range from 5.9 to nearly 100 mg L^{-1} (Husaini *et al.*, 2011; Tiwari *et al.*, 2011). Therefore, experiments should be conducted with higher As concentration levels to explore the uptake potential of various vegetable species, particularly spinach.

- Plant response experiments were only conducted in one soil, Rangitikei silt loam, (Chapter 4 and Chapter 6) where spinach shows a high risk to human health. Spinach irrigation experiments should be conducted in a range of soils varying in texture (e.g. sandy or clayey soil) to determine if the risk is mitigated or further aggravated by specific soil properties.

- Radish and tomato were grown only under non-flood irrigation in this research. Their response to As-contaminated water should also be investigated under flood irrigation to determine if they will concentrate As in their edible tissues above the critical level.

- Leachate from pots should be collected and analyzed for its total, and particularly inorganic As species concentration. This analysis is important for flood irrigation practices where a non-significant effect of species on As concentration in vegetables tissues was observed. The lack of effect is proposed to be the inter-conversion of As species, and this should be tested with speciation studies.

- Arsenic species in vegetable tissues should be determined to compare the values with the Chinese food safety standard for inorganic As. Currently, the As species in vegetables tissues has been assumed to be inorganic, based on the fact that inorganic species has been used for irrigation (Chapter 4 and Chapter 6).

- To better quantify the USEPA risk calculation used in this research, the vegetable consumption habits of the people of affected areas should be surveyed. The data should be recorded for individual vegetables to quantify the risk that can be imposed by an individual vegetable species.

- In Chapter 6, cattle manure and biochar were applied at the rate of 10 tons per hectare. Various application rates should be included in the future experiments to quantify the effect of variable treatment rates and to propose optimum rates of these agricultural amendments.

- In Chapter 6, the effect of cattle manure and biochar on As concentration in spinach tissues and soil was attributed to the release of organic compounds and phosphate ions from these soil amendments. This generalization should be tested by various ways including:
    1. Analysis of soil samples at the end of the experiment to determine if there is an increase in C and P concentration in soil
    2. Soil solution and leachate analysis to determine if there is high influx of As (As^{V} or As^{III}) concentration in soil solution or leachate
    3. Laboratory adsorption studies for cattle manure-and-biochar amended soils against a range of As^{V} or As^{III} concentrations to determine if there is more or less As retention in amended soil

- The laboratory batch adsorption study was limited to three soils only (Chapter 5). In these soils the amorphous Al, total C and Olsen P content controlled the As adsorption behavior. A greater number of soils, varying in physical and chemical properties, should be included in future experiments to conform the observed results.

- Through pot or field trials, the response of other common vegetables (for example cauliflower, cabbage, lettuce, potato, turnip) should be quantified where they are also commonly irrigated with As-contaminated water. The methodology reported in this thesis could be adopted as a standard method to test the As uptake response of vegetables under both flooded and non-flooded irrigation conditions.

7.5 REFERENCES

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APPENDIXES

Appendix I Recommended conditions for As analysis on GFAAS

Wavelength (nm)	193.7
Low Slit (nm)	0.7
Pretreatment/Pyrolysis Temperature (^{0}C)	1200
Atomization Temperature (^{0}C)	2000
Lamp used	EDL (Electrodeless Discharge Lamp)

Source: Perkin Elmer Manual (*The THGA Graphite Furnace: Techniques and Recommended Conditions*)

Appendix II Installation and optimization of HGAAS

Installation of the FIAS (Model FIAS-400) components was followed by the instructions provided in the Perkin Elmer manual(s) (*FIAS-Pump Module Series, Description and Maintenance; FIAS for Atomic Spectroscopy-Setting up and performing Analysis*). After installation, the FIAS was optimized and a reference solution (10 µg As L^{-1}) was analyzed for As concentration as per Perkin Elmer manuals (*FIAS for Atomic Spectroscopy-Setting up and performing Analysis; Flow Injection Mercury/Hydride Analyses-Recommended Analytical Conditions and General Information*). Once the output readings for the reference solution stabilized (analysis of 500 µL of a 10 µg L^{-1} arsenic solution should provide a signal of about 0.45 A), plant samples were prepared and analyzed for their total As concentration.

Appendix III Recommended and adopted analytical parameters for As determination using HGAAS

Spectrometer						
Technique	AA (Atomic Absorption)					
Integration time (s)	15					
Data Processing	Peak height, Smoothing: 19 points					
Lamp	EDL (Electrodeless Discharge Lamp)					
Slit (nm)	0.7 (Low)					
Wavelength	193.7					
FIAS						
Cell Temperature (^{0}C)	900					
Carrier gas	Nitrogen (N_2)					
Optimum gas flow rate (mL/min)	In between 150-200					
Optimum inlet gas pressure	50 psi					
Pump speed	Step	Time (s)	Pump1 (U/min)	Pump2 (U/min)	Valve	Read
	Prefill	15	100	120	Fill	
	1	10	100	120	Fill	
	2	15	0	120	Inject	0
Reagents						
Carrier solution	10% (v/v) HCl					
Reducing agent	0.2% $NaBH_4$ in 0.05% NaOH					
Sample solution	As^{III} in 10% (v/v) HCl					

Source: Manual (*Flow Injection Mercury/Hydride Analyses-Recommended Analytical Conditions and General Information,* Perkin Elmer Inc.)

Appendix IV Hazard Quotient (HQ) and Cancer Risk (CR) calculation

Adults (> 18 years old)										
C	**Fi**	**Ef**	**Ed**	**W**	**Te**	**EDI** (C x Fi x Ef x Ed) / (W x Te)	**RfD**	**HQ** (EDI/RfD)	**CSF**	**CR** (EDI x CSF)
0.080	0.5	52	70	60	25550	0.000095	0.0003	0.32	1.5	0.00014
0.106	0.5	52	70	60	25550	0.000126	0.0003	0.42	1.5	0.00019
0.122	0.5	52	70	60	25550	0.000145	0.0003	0.48	1.5	0.00022
0.319	0.5	52	70	60	25550	0.000379	0.0003	1.26	1.5	0.00057
Adolescents (12-18 years old)										
C	**Fi**	**Ef**	**Ed**	**W**	**Te**	**EDI** (C x Fi x Ef x Ed) / (W x Te)	**RfD**	**HQ** (EDI/RfD)	**CSF**	**CR** (EDI x CSF)
0.080	0.5	52	70	50	25550	0.000114	0.0003	0.38	1.5	0.00017
0.106	0.5	52	70	50	25550	0.000151	0.0003	0.50	1.5	0.00023
0.122	0.5	52	70	50	25550	0.000174	0.0003	0.58	1.5	0.00026
0.319	0.5	52	70	50	25550	0.000454	0.0003	1.51	1.5	0.00068

C: Concentration of As in the edible part of the plant (mg kg^{-1} fresh weight)

Fi: Food intake rate (kg per person per day; assumed 0.5 kg)

Ef: Exposure frequency (days per year; assumed 52 days per year)

Ed: Exposure duration (equivalent to the average lifetime, about 70 years)

W: Average body weight (60 kg for an adult, and 50 kg for adolescents)

Te: Average exposure time (= Ed x 365 days)

EDI: Estimated daily intake (mg kg^{-1} day^{-1}) calculated as EDI = (C x Fi x Ef x Ed) / (W x Te)

RfD: Oral reference dose (0.0003 mg As kg^{-1} body weight day^{-1})

HQ: Hazard Quotient (calculated as HQ = EDI/RfD)

CSF: Cancer slope factor for As (1.5 mg As kg^{-1} day^{-1})

CR: Cancer risk (calculated as: EDI x CSF)

Appendix V Amount of water applied to treatment pots at an irrigation event [a]

As in water (µg L^{-1})	As^{V}						As^{III}					
	pH 6.1			pH 7.5			pH 6.1			pH 7.5		
	control	biochar	cattle manure	control	biochar	cattle manure	control	biochar	cattle manure	control	biochar	cattle manure
0	1.38 ± 0.03 [b]	1.22 ± 0.02	1.28 ± 0.01	1.23 ± 0.02	1.46 ± 0.03	1.40 ± 0.05	1.38 ± 0.03	1.22 ± 0.02	1.28 ± 0.01	1.23 ± 0.02	1.46 ± 0.03	1.40 ± 0.05
50	1.24 ± 0.04	1.22 ± 0.03	1.29 ± 0.01	1.13 ± 0.03	1.32 ± 0.03	1.46 ± 0.03	1.25 ± 0.02	1.36 ± 0.01	1.26 ± 0.01	1.32 ± 0.03	1.42 ± 0.03	1.53 ± 0.03
200	1.20 ± 0.08	1.16 ± 0.07	1.25 ± 0.01	1.23 ± 0.02	1.47 ± 0.05	1.52 ± 0.05	1.29 ± 0.04	1.33 ± 0.02	1.29 ± 0.01	1.33 ± 0.02	1.44 ± 0.02	1.47 ± 0.01
500	1.35 ± 0.06	1.28 ± 0.01	1.26 ± 0.03	1.19 ± 0.06	1.47 ± 0.06	1.49 ± 0.03	1.36 ± 0.06	1.33 ± 0.03	1.25 ± 0.02	1.38 ± 0.03	1.54 ± 0.05	1.53 ± 0.02
1000	1.26 ± 0.04	1.33 ± 0.02	1.31 ± 0.01	1.27 ± 0.03	1.47 ± 0.03	1.45 ± 0.05	1.23 ± 0.06	1.32 ± 0.01	1.36 ± 0.02	1.36 ± 0.02	1.49 ± 0.03	1.57 ± 0.04
Average amount of water applied to a pot in liters between trials: Control soil: 1.28 ± 0.01; biochar soil: 1.37 ± 0.01; cattle manure soil:1.39 ± 0.01												

[a] data is presented only for final irrigation before spinach harvest; [b] unit in litres

Appendix VI Hazard Quotient (HQ) and Cancer Risk (CR) calculation for the ingestion of spinach leaves grown in cattle manure amended soil (Scenario 1 where vegetables are consumed 500 grams per day)

Adults (> 18 years old)										
C	**Fi**	**Ef**	**Ed**	**W**	**Te**	**EDI** (C x Fi x Ef x Ed) / (W x Te)	**RfD**	**HQ** (EDI/RfD)	**CSF**	**CR** (EDI x CSF)
0.079	0.5	52	70	60	25550	0.000094	0.0003	0.31	1.5	0.00014
0.265	0.5	52	70	60	25550	0.000315	0.0003	1.05	1.5	0.00047
0.382	0.5	52	70	60	25550	0.000454	0.0003	1.51	1.5	0.00068
Adolescents (12-18 years old)										
C	**Fi**	**Ef**	**Ed**	**W**	**Te**	**EDI** (C x Fi x Ef x Ed) / (W x Te)	**RfD**	**HQ** (EDI/RfD)	**CSF**	**CR** (EDI x CSF)
0.079	0.5	52	70	50	25550	0.000113	0.0003	0.38	1.5	0.00017
0.265	0.5	52	70	50	25550	0.000378	0.0003	1.26	1.5	0.00057
0.382	0.5	52	70	50	25550	0.000544	0.0003	1.81	1.5	0.00082

Appendix VII Hazard Quotient (HQ) and Cancer Risk (CR) calculation for the ingestion of spinach leaves grown in cattle manure amended soil (Scenario 2 where vegetables are consumed 205 grams per day)

Adults (> 18 years old)										
C	**Fi**	**Ef**	**Ed**	**W**	**Te**	**EDI** (C x Fi x Ef x Ed) / (W x Te)	**RfD**	**HQ** (EDI/RfD)	**CSF**	**CR** (EDI x CSF)
0.079	0.205	52	70	60	25550	0.000038	0.0003	0.13	1.5	0.000058
0.265	0.205	52	70	60	25550	0.000129	0.0003	0.43	1.5	0.00019
0.382	0.205	52	70	60	25550	0.000186	0.0003	0.62	1.5	0.00028
Adolescents (12-18 years old)										
C	**Fi**	**Ef**	**Ed**	**W**	**Te**	**EDI** (C x Fi x Ef x Ed) / (W x Te)	**RfD**	**HQ** (EDI/RfD)	**CSF**	**CR** (EDI x CSF)
0.079	0.205	52	70	50	25550	0.000046	0.0003	0.15	1.5	0.000069
0.265	0.205	52	70	50	25550	0.000155	0.0003	0.52	1.5	0.00023
0.382	0.205	52	70	50	25550	0.000223	0.0003	0.74	1.5	0.00033

C: Concentration of As in the edible part of the plant (mg kg^{-1} fresh weight)

Fi: Food intake rate (kg per person per day; assumed 0.5 kg or 0.205 kg)

Ef: Exposure frequency (days per year; assumed 52 days per year)

Ed: Exposure duration (equivalent to the average lifetime, about 70 years)

W: Average body weight (60 kg for an adult, and 50 kg for adolescents)

Te: Average exposure time (= Ed x 365 days)

EDI: Estimated daily intake (mg kg^{-1} day^{-1}) calculated as EDI = (C x Fi x Ef x Ed) / (W x Te)

RfD: Oral reference dose (0.0003 mg As kg^{-1} body weight day^{-1})

HQ: Hazard Quotient (calculated as HQ = EDI / RfD)

CSF: Cancer slope factor for As (1.5 mg As kg^{-1} day^{-1})

CR: Cancer risk (calculated as: EDI x CSF)