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The Effect of the Addition of Fulvic Acid and Straw Water on the Efficiency of Arsenic Uptake from Groundwater by *Vetiveria zizanioides*

by

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Statement of Originality

The entire work created in this master's thesis report is a sole work of the author. He has not used any fragment of text from other sources without providing the proper acknowledgement. The theories, results and designs of original work have been appropriately referenced and all sources of assistance have been fully acknowledged.

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Abstract

The aim of this research project was to investigate the efficiency of fulvic acid or straw water as an amendment to enhance the uptake of arsenic from groundwater by *Vetiveria*. Fulvic acids and straw water were applied to arsenic-contaminated groundwater at different concentrations (0.1% and 0.01%). It was found that when the higher concentration of straw water was added to the groundwater solution, the efficiency of arsenic accumulation by roots was increased 47.8%. Straw water not only enhances the growth of *Vetiveria*, but also improved arsenic accumulation in both shoots and roots. In contrast, the addition of fulvic acids (at high or low concentrations) resulted in the reduction of *Vetiveria* growth. Specifically, a high concentration of fulvic acid reduced arsenic accumulation in roots whilst a low concentration of fulvic acid decreased arsenic accumulation in shoots.

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Table of Contents

List of figures

List of tables

Abbreviations

ADD	Average Daily Dose
As	Arsenic
BF	Bio-concentration Factor
CDTA	Leneditrilotetraacetic Acid
DTPA	Diethylenetriaminepentaacetic Acid
EC	Electrical Conductivity
EDTA	Ethylendiaminetriacetic Acid
EESI	Environmental Earth Science International
EGTA	Ethylene Glycol Tetraacetic Acid
EPA	Environmental Protection Agencies
FH	High concentration of Fulvic Acid 0.1%
FL	Low Level of Fulvic Acid 0.01%
FT	Frist Trial
GW	Groundwater
HEDTA	N-Hydroxyethyl-Ethylenediamine-Triacetic Acid
HI	Hazard Index
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
Maize	*Zea mays*
NOM	Natural Organic Matters
NTA	Nitrilotriacetic Acid
OA	Oleic Acid
PAH's	Polycyclicaromatichydrocarbons
PCB's	Polychlorinatedbiphenyls
PCPs	Pentachlorophenols
RfD	Reference Dose
SF	The Slope Factor
SH	High concentration of Straw Water 0.1%
SL	Low Level of Straw Water 0.01%
SOM	Soil Organic Matter
ST	Second Trial
TF	Translocation Factor
TT	Third Trial
W	Tap Water
WHO	World Health Organization

Chapter 1. Aims and objectives

1.1 Introduction

Arsenic (As) had accidently been released into the ground water and soil in an industrial complex close to Sydney CBD (see Figure 1-1 and Figure 1-2), following pile construction for foundations, which had pierced a buried tank. The arsenic plume had the potential to move into a large water body near the site. From an extensive literature review it was found that *Vetiveria* has the ability to flourish, tolerate and take up arsenic from a moderately contaminated soil and groundwater environment. However, it appears from the literature reviewed that there has been minimal research on the addition of fulvic acid and no research on straw water to *Vetiveria* to enhance the uptake capacity of arsenic from groundwater. The other aspect of the project was to lower the water table to prevent the movement of the arsenic contaminated water from flowing into a nearby water body. It is know that *Vetiveria* is able to achieve this. The company has used other plants (willows and Chinese brake fern) to lower the water table at other sites. The best response has always been associated with *Vetiveria*.

The remedial strategy for the site focused on managing the soil and groundwater contaminated with As resulting from its former use for glass manufacturing. The site also included a batching plant. The site is to be redeveloped for commercial use and requires clean-up to acceptable levels for human health and the environment to the standard set by the NSW Environment Protection Authorities (EPA). The recommended concentration of As in water is <10ppm.

EESI investigations identified a number of contamination 'hotspots' and potential areas of environmental concern (PAECs). These areas required appropriate management to render the site suitable for the proposed commercial/industrial use. No contamination associated with the batching plant was encountered. Historically the glass plant used various products containing metals during the manufacturing process. An As 'hotspot' was recorded in the groundwater plume. Table 1-1 shows the conceptual site model and risk screening for arsenic. EESI suggested a remedial option for arsenic removal on site

using phytoremediation technology. Phytoremediation is the application of plants to remove, transform or stabilize contaminants (Moreal, 2006).

This research project investigated the As remedial option using phytoremediation technology.

Table 1-1 Conceptual site model and risk screening about arsenic

Source	Pathway	Receptor	Risk	Proposed control by EESI
Unknown source onsite(arsenic impacted groundwater)	Direct contact and indirect contact	Site workers and future site users	Low	No action considered necessary for future site users as the redevelopment of the site is proposed to have limited and/or no access to groundwater.
Unknown source onsite(arsenic impacted groundwater)	Lateral and vertical migration of contaminants in groundwater	Soil, shallow/deep groundwater; and a creek close to the site(concrete lined stormwater channel offsite)	Moderate	The investigations to date have not identified a primary source causing the arsenic impact to groundwater onsite.The groundwater monitoring program indicates that a primary source is likely to be located in the vicinity of the furnace building. The arsenic groundwater impacts are located centrally onsite and whilst there is potential for contaminant migration offsite, the risk is noticeably reduced towards the creek.

Figure 1-1 The site under construction environment, taken by February 2012 (provided by EESI)

Figure 1-2 Contaminated groundwater with arsenic in the site, exposed and polluted to the soil (provided by EESI)

1.2 Research aim

One of the plants commonly used in phytoremediation is the Asian grass species *Vetiveria zizanioides* (Vetiver grass). The root system is the major organ for plant uptake of water and absorption of metals from media (USEPA, 2000). This plant is easy to monitor and is suitable for planting in a wide variety of soil types ((Srisatit et al., 2003) ,(Dudai et al., 2006).

The aim of using *V.zizanioides* on the site was also to reduce As concentration and lower the level of the polluted groundwater table. Lowering the ground-water table, would reduce the risk of migration of the As contaminated water to the nearby creek.

This research project undertaken under glasshouse conditions aimed at determining whether the phytoremediation efficiency of removal of As contamination could be enhanced by the addition of fulvic acid or straw water.

The three objectives in the glasshouse experiments were to determine:

- the growth rate of *V.zizanioides* under controlled glasshouse conditions using tap water without adding nutrient;
- water consumption by *V.zizanioides* in each pot; and
- using the groundwater from the site determine the As uptake by *V.zizanioides* after the addition of fulvic acid or straw water at different concentrations.

The findings from the research project could be applied to remediate the As contamination at the site. However, groundwater monitoring of As concentration by EESI would need to continue to ensure concentration does not increase overtime.

Chapter 2. Literature Review

2.1 Background information

Soil is everywhere, and it has always performed and held a wide range of useful functions. The range of these impacts and functions on the environment has changed considerably as expected in the urban environment (Hazelton, 2011).

The soil environment has a complicated biological community containing many types of microorganisms. Soil diversity relies on the microbe activities and the interaction among soil, bacteria and plants is an important process for ecosystem health (Whitbeck and Cardon, 2007). It contains many types of microorganism and the soil diversity relies on the microbe activities. The soil microorganism system might indirectly or directly affect the land productivity (Barrios, 2007). The activities of invertebrates such as earthworms within the soil are essential for the soil functions. Due to endogenic species' mutualistic interactions with micro flora, selective ingestion of soil particles, high rates of ingestion of production of casts, galleries, burrows and chambers can affect nutrient and organic matter dynamics and other pedological processes. Thus, endogenic species play an important role in intensifying agroecosystems as well as enhancing the soil function (Fragoso et al., 1997).

The environment of the rhizosphere includes physical, chemical and biological conditions. The physical structure of the soil is impacted strongly by plants through the rhizosphere biota and bacteria activities around the root system. The rhizospheres in the soil would support microorganism growth and propagation. Also the microorganism activity could stimulate the plants growth and would enrich the soil around them (Chaudhry et al., 2005).

These researches indicate the soil is a part of the environment. There are many interactions between soil and other factors such as bacteria and plants. Due to urbanization, thus soil degradation has become a global issue.

2.1.1 Soil contamination and soil degradation

Soil degradation can occur from physical loss such as soil erosion and reduction of soil quality with nutrient loss such as soil inactivation (Mupenzi et al., 2011). Due to human

activities, the use of xenobiotic chemical compounds is widespread. This leads to many types of pollution within the environment, for example from compounds such as pesticides or herbicides that permeate soil, move to groundwater or directly flow into rivers. Lead from petroleum pollutes ecosystem leading to human health risks through the food chain or drinking water. Toxic elements could evaporate into the atmosphere, adversely affecting the air quality and human health (Mupenzi et al., 2011).

Soil contamination has become a major limitation for sustainable development in the modern world. Global development uses natural or synthetic chemicals compounds with a risk of pollution of the environment (Mupenzi et al., 2011).

2.1.2 Sources of soil contamination

Soil contamination can result from a variety of sources, including the mining industries, agricultural, household, industrial and urban land uses.

The mining industries have been recognized as the major contributors to environmentall pollution, including heavy metals (Liu et al., 2003). Waste heavy metals from other industries such as coal storage and secondary smelting processing also contributes to potential soil pollution (Liu et al., 2003).

The use of chemical compounds for agriculture also generates soil contamination (Bech et al., 1997), namely:

- degradation due to land use conversion from native vegetation to agriculture;
- increased erosion and soil loss due to agricultural practices;
- chemical pollution by fertilizers, pesticides and herbicides; and
- pollution from animal husbandry.

Household chemicals such as shampoo, furniture polish, detergents, disinfectants, and even cosmetics flow into sewage. These household chemicals can infiltrate into the soil resulting in environmental pollution concerns (Eriksson et al., 2003).

Possible contaminants in urban situations include (Li et al., 2001):

- Chemical industries such as dry cleaners, dental laboratories, foundries, all have the potential to contaminate the sites that they use.

- Major urban utilities: public service utilities, for example, power station (burning coal) or gas station, incinerators, liquid waste disposal sites, all may come out contamination.
- Food and animal-related activities: such as meat production, feedlots, poultry processing, cheese production, bakeries, soda and soft beverage factories etc. they are all not 'friendly' to urban soil.
- Construction industry: waste materials may impact on the underground water and water infiltration; it may impact on the soil moisture content, resulting in soil erosion.

2.1.3 The different types of soil pollutant

There are two main classes of pollutants: organic and inorganic. The organic pollution generally comes from utilizing of organic compounds such as polychlorinatedbiphenyls (PCB's), nitroaromatic (explosives), polycyclicaromatichydrocarbons (PAH's), halogenated hydrocarbons, chlorinated solvents. Compared with organic compounds, inorganic compounds are less toxic because they are less active and reactive. Some of the organic compounds are not only toxic and teratogenic but also carcinogenic for humans. The inorganic pollutants are metallic compounds and non-metallic compounds. The metallic pollutant sources includes heavy metals such lead, cadmium, mercury. For most living beings, heavy metals are essential for their growth and important component for their health; however, if the concentration exceeds the required level, it will lead to harm and toxicity for the body and especially be harmful for cells (Chang et al., 1995).

There are many sources of heavy metals come in urbanized areas, including vehicle emissions, industries discharges, and other activities (Li et al., 2001). Heavy metals persist in the soil over a long period time (Alloway and Jackson, 1991). Radioactive soil pollution results from the leakage of the radioactive elements. The radionuclides include uranium, cesium, strontium, tritium, etc. (Beresford, 2005). Moreover, there are non-metallic pollutants such as arsenic; this pollutant represents a widespread and serious threat to human health (Masotti et al., 2009).

2.1.4 Arsenic

2.1.4.1 Background information

'As' is the symbol for the element of arsenic, its atomic number is 33 and its atomic weight is 75. Arsenic occurs in different natural forms (organic or inorganic) and is ubiquitous in our environment. Arsenic is a metalloid. It has various allotropes (yellow, black, grey forms). The most stable arsenic form is a silver-grey, brittle crystalline solid. It accounts of 0.0005% of the earth's crust and it can be found in soil and minerals, it could enter air and underground-water through wind-blown dust and water run-off (Hutton and Symon, 1986).

2.1.4.2 Sources of arsenic

In rocks, arsenic mainly occurs as arsenopyrite (FeAsS). Arsenic is liberated from volcanic activities and weathering of rocks.

Most modern pesticides consist of inorganic arsenic and are widely used for pest control. Inorganic arsenic based defoliants have been used for fruit production. The use of inorganic arsenic chemical compounds in agricultural areas has led to substantial arsenic soil and water pollution. Arsenic is an indispensable material for semiconductor products, hence abandoned semiconductors and other electronic products are of concern for their sustainable use (Elliot, 2006). Marine petroleum exploration may also result in arsenic leakage (Martínez et al., 2007).

2.1.4.3 Effects of arsenic

A high concentration of arsenic leads to a variety of impacts for ecosystems. Arsenic can be introduced in to marine environment through anthropogenic or natural sources therefore ingestion of a food source containing arsenic by crab and fish may lead to arsenic poisoning. Arsenobetaine is the major form within in marine organisms with arsenic concentration within crabs up to 100 ppm As (Amlund and Sloth, 2011). It not only impacts on the marine organism growth and breeding, but also once arsenic contained seafood is sold from the market it can affect human health. Amlund and Sloth (2011) also assert that a high concentration of arsenic can be harmful to ecosystems. Considering the impact of high arsenic concentration in the environment is a reason for the development of this research project.

Arsenic decreases enzymatic activity in cell, and causes tissue damage. Ingestion of excessive arsenic will lead to death. The arsenic poisonous dose in humans is 5 to 50 ppm, and the fatal dose is 60 to 200 ppm (Saha et al., 1999).

2.1.5 Vetiver grass

The plant selected by EESI for the phytoremediation of the site was *Vetiveria* (synonym of *Chrysopogon zizanioides*), which is commonly known as *Vetiveria*. *Vetiveria* is a perennial grass of the Poaceae family, native to India. EESI had successfully used this plant for remediation at other sites. The main characteristic is strong ecological adaptability and large biomass. The above ground part can grow up to approximately 1.5 meters high. The root system is the major organ for plant uptake water and absorbs metals from media (USEPA, 2000).

Compared with other grasses, *Vetiveria* has an advantage root system, unlike most grasses. Most grasses form horizontally spreading, mat-like root systems. The roots of *Vetiveria* can extend downward and reach 2 to 4 metres in depth (USEPA, 2000).

Vetiveria is easy to monitoring and is suitable for planting in different soil types (Srisatit et al., 2003). *Vetiveria* could grow well in a wide range of soil environment, such as loamy sand, sandy soil, clay soil, crushed limestone, sandy clay loam, and peat/tuff (Dudai et al., 2006). Although *Vetiveria* is likely to thrive in the sandy soil of the study site (Dudai et al., 2006), there is very little research on the effectiveness of this plant in remediation when grown in sandy soil compared with research by Srisatit et al. (2003) who explored *Vetiveria* growing in silt loam soil.

2.1.6 Fulvic acid

EESI had used fulvic acid as an amendment and was therefore chosen to be used in this research. Fulvic acid is a type of humic acid but with lower molecular weight and higher oxygen content than other humic acids. Fulvic acid is not a single acid; it encompasses many different acids including carboxyl and phenolic OH functional groups (Hofrichter and Steinbüchel, 2001). This amendment could also improve metals bioavailability and solubility, thus increase metals translocation in plants (Lagier et al., 2000).

From the molecule aspect, fulvic acid contains a large portion of the ubiquitous organic matter reservoir in natural water or soil environments (Thurman, 1985), and these

organic matters could be complexed with heavy metals (Alberts et al., 1986). Consequently, complexation by humic substances can improve or retard uptake metals from groundwater or soil by plants organisms (McCarthy, 1989).

Plant growth relies on uptake of nutrients by roots from water or soil. Fulvic acid could free up nutrients from the soil resulting in increased bioavailability of metals for plants assimilation. Chen et al. (2006) found that humic substances (including humic acid and fulvic acid) can be substantially complexed with arsenic in groundwater. Chen and Zhu (2006) determine that there is a distinct positive correlation between humic substances and arsenic. Ghosh et al. (2012) explored the compounds between humic or fulvic acid and arsenic. He found that arsenic in aqueous phase distribution could impact on three processes such as covalent bonds, ionic associations and cationic bridging complexes. All of these processes could be impacted by humic substances. Therefore, fulvic acid as a type of humic substance has a potential benefit to enhance phytoremediation efficiency, but may potentially inhibit phytoremediation efficiency. These researchers also indicated interaction between utilize of fulvic acid and arsenic in phytoremediation. As an alternative amendment, it has achieved positive result in some previous reports such as the research by Rauthan and Schnitzer (1981).

2.1.7 Straw water

The straw water was the other amendment which was suggested to be used in this research. It also contains phenolic acid. Phenolic is a natural organic matter (NOM) which may substantially impact on arsenic geochemistry and speciation. In a neutral pH situation, it is negatively changed (Atanasova et al., 2002). The phenolic group is also the major component for humic acid (Chris Conoley of EESI, personal communication, March 2012). Therefore, the impact of straw water on removal of arsenic is likely to have a similar mechanism as fulvic acid.

Wuana and Okieimen (2011) in their resaerch, straw is consiedered as one of organic amendments for immobilisation of heavy metals, such as cadmium, chromium and lead. This was cited in a paper by (Guo et al., 2006) which investigated the availability and assessment of fixing additives for the in situ remediation of heavy metal contaminated soils.

Straw was considered to be suitable for the immobilisation of cadmium, chromium and lead tin soil. There was no research on other metals such as arsenic using straw as an amendment. In the research project straw water not straw was used to amend into groundwater rather than the soil. (For the production of straw water refer to Chapter 3)

2.2 Method for soil remediation

Soil contamination is a social and sanitary problem, leads to economic concern worldwide; nearly 12% of land is affected by soil degradation, totalling two billion hectares (Bini, 2009). Sites with polluted soil or groundwater are identified by agencies such as the EPA as contaminated land. An investigation report contains the pollution sources and contaminant level. If a site is polluted, the site cannot be used for any purposes until the contaminants reached an acceptable level. Moreover, contaminated soil may mix with underground water and will directly affect residents' health. Risk assessment is a tool used by governments or local councils to clearly understand the risks and be a guide for decision making and management. Choosing a method of remediation for the polluted site considers the clean-up duration and cost. There are two main treatment methods, one is in-situ treatment and the other is ex-situ treatment.

2.2.1 In-situ soil treatment

There are three major technologies for in-situ soil treatment (see Table 2-1):

- Physical and chemical treatment;
- Thermal treatment; and
- Biological treatment.

The selected methods of remediation depend on the type of pollutants and specific site situations. In some cases, two or more methods should be combined for one site treatment due to multi-polluted sources (Mahajan, 1985).

The physical and chemical treatment includes soil vapor extraction, soil flushing, chemical oxidation, solidification/stabilization, and electro kinetic separation. Physical/chemical treatment has the most diverse of treatments and has the longest historical background.

Thermal treatment heats the soil substantially increasing the soil volatilization rate therefore leading to pollutant vaporising into the atmosphere. There are five thermal treatments, namely electrical resistivity heating, stream injection and extraction, conductive heating, radio-frequency heating, and vitrification. Vitrification is a high-temperature approach designed to immobilize pollutants by incorporating them into the vitrified end product, which is chemically durable and leach resistant. This technology is used to treat soil and sediments contamination in situ and ex situ

Biological treatments involve microorganism or vegetation for contamination degradation, removal, or immobilization. Bioventing, phytoremediation and monitored natural attenuation are used.

When choosing one technology for soil remediation, the treatment duration and cost has to be considered before the final treatment. The potential risks also should be considered, for example, if soil flushing is chosen for a site then during the process it will generate noise pollution; and if thermal treatment is chosen, then toxic elements will come out from the soil layer through volatilization which may resulting in air pollution. The subsequent or secondary pollution will affect the local residents and directly impact on people who are involved in this treatment. Therefore, protection should be used under the treatment situations, such as wearing earplugs and respirators (Evanko and Dzombak, 1997).

2.3 Land risk assessment

The environmental risk assessment identifies and classifies different sites depending upon the basis of intervention priority. It is used to select the most appropriate method, to establish the goals before treatment and expected endpoints (Bini, 2009).

Table 2-1 is a feasibility guideline for the best treatment for different source of pollutants. For example, bioremediation has a better treatment effect for non-halogenated VOCs (volatile organic compounds), halogenated VOCs, non-halogenated SVOCs, fuels and explosives. However, it shows that average treatment effect for non-halogenated VOCs, halogenated VOCs and non-halogenated SVOCs by phytoremediation from the time (U.S.EPA, 1996).

Table 2-1 The feasibility of each treatment for different sources of pollutants (U.S.EPA, 1996)

Key: ☆Better ★Worse ◇Average S=specific to chemical type	Non-halogenated VOCs	Halogenated VOCs	Non-halogenated SVOCs	Halogenated SVOCs	Fuels	Inorganics	Radionuclides	Explosives
Physical and Chemical Treatment								
Soil Vapor Extraction	☆	☆	★	★	☆	★	★	★
Solidification/stabilization	★	★	◇	◇	★	☆	☆	★
Chemical OXidation	◇	◇	★	◇	★	S	★	◇
Soil Flushing	☆	☆	◇	◇	◇	☆	★	★
Electrokinetic Separation	◇	◇	◇	◇	★	☆	◇	★
Biological Treatment								
Bioremediation	☆	☆	☆	S	☆	S	S	☆
BIovending	☆	☆	☆	★	☆	★	★	★
Phytoremediation	◇	◇	◇	S	◇	◇	★	★
In-situ Thermal								
Thermal treatment including the five methods	☆	☆	☆	☆	☆	★	★	★

2.4 Biological treatment

Biological treatment is the process using microorganisms or vegetation to degrade or transform pollutants (Skipper and Turco, 1995). This approach is widely used for wastewater treatment, and also been used for contaminated soil and solid waste treatment. The biological treatment method aims to accelerate natural biodegradation processes (Calvo et al., 2009). It can involve application of phosphorous and nitrogenous fertilizers, changing the pH and soil or water content, even adding chemical compounds or addition of bacteria. The basic techniques of in-situ biological treatment are shown in Table 2-2 (Skipper and Turco, 1995):

Table 2-2 Techniques of in-situ bioremediation

Method	Process
Stimulation	Using addition of nutrients, regulation of redox condition, pH change, or removal of other limiting conditions in order to increase indigenous microorganism activities.
Inoculation	Inoculation of microorganisms of specific bio transforming abilities into contaminated environments.
Enzyme Treatment	Utilization of active enzymes to degrade or transform specific pollutants
Phytoremediation	Application of plants to transform or stabilize contaminants.

2.4.1 Phytoremediation

Phytoremediation is the application of plants to transform or stabilize contaminants. Plants play a very important role in the ecosystem; they act as the earth's 'lung'. Plants can accumulate potential toxic elements from the terrestrial environment. Therefore, using plants to remediate soil pollution has become a feasible option (Thompson, 1995). This research provides important information for readers to understand the general process of 'Phytoremediation'.

The uptake capacity is influenced by the species of plant and the genotype and therefore, the impact on the cleanup efficiency. Depending on species and genotypes, plants have different sensitivity or tolerance to metals, resulting in dividing plants into three different groups (Adriano, 2001; Moreal, 2006):

- Indicators: there is a linear relationship between plants' content of elements and the soil content of elements, this group plants mostly belong to crops, such as wheat, maize and oats.
- Excluders: these plants are insensitive for uptake or accumulation of potentially toxic elements. Mainly monocotyledon grasses are belonging to this group. The plants have the restricted uptake of metals into roots and their limited translocation to shoots even under high contamination in the growth medium (Baker and Walker, 1990).

- Accumulators: these are plants which can accumulate high concentration of elements. Plants which can accumulate extremely high amounts elements are called hyper-accumulators (Baker, 1987).

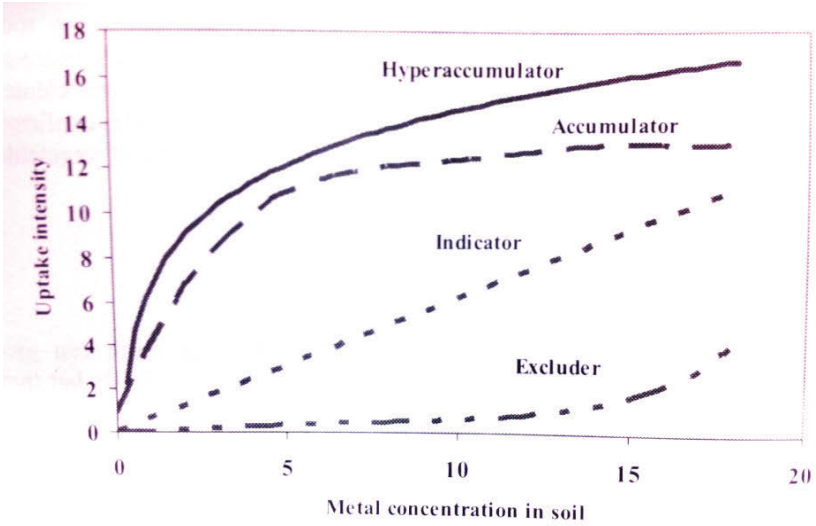

Figure 2-1 Relative uptake and bioaccumulation potential among plant species (Adriano, 2001)

For example, Adriano (2001) showed that the three different plant groups (refer to Figure 2-1). In that figure, the x-axis shows the metal concentration in soil, and y-axis indicates the plant uptake intensity.

The phytoremediation approach is a cost-efficient method for long-term treatment, and it has an expected social acceptance. This method is an emerging technology that holds great potential for land remediation where:

- Treatment is close to the surface.
- Treatment is able to cover a large area, suitable for big scale remediation.
- There is little immediate risk to environment and human health.
- Contaminants are relatively non-leachable, no secondary pollution during treatment.

2.4.2 Phytoremediation Strategies

The root system is the major component of plant for contaminant uptake from the soil and water. Once the contaminant is absorbed by the roots, next, the contaminant will translocate into other organs such as stem and shoot. During this process, the xylem plays the role of the transportation. According to Saleh et al. (2004) different purpose

and mechanisms, phytoremediation includes some different sub treatments depending on site specificity.

- Phytoextraction: this process involves root system uptake of contaminants and translocation into other parts. Normally, the pollutants are translocated by harvesting the plants. This approach is widely used to concentrate metal remediation and is feasible for large scale land remediation.
- Phytostabilization: mobile pollutants such as heavy metals within soil can be immobilized by this method depending on root system' accumulation and absorption, and root adsorption mechanism. Moreover, the pollutants also can be precipitated within root zone. This method can prevent mobile metals to leach into other environments which are close to the contaminated area. Nevertheless, this process is generally used for inorganic (metal) pollution within soil, sludge and sediments.
- Phytovolatilization: through the plant metabolism and plant transpiration, the contaminant or a modified form of the contaminant which is absorbed by the root system then can be released into the atmosphere. This approach mainly focuses on aquatic plants to clean up groundwater; however, it can also be used for soil remediation. Moreover, this method is more suitable for chlorinated solvents and metals treatment.
- Phyto(rhizo)filtration: toxic substances and excess nutrients can be removed by a mass of roots. The contaminant is contained in the roots or precipitated onto the roots. This method is selected to remediate underground water pollution. Wastewater, surface water or groundwater with metals and radionuclides pollution is primarily cleaned up using this technology.
- Rhizodegradation: it is a process used to degrade contaminants in the rhizosphere by the roots releasing compounds which enhance microbial activity. Organic pollutants such as PAHs, BTEX (stands for benzene, toluene, ethylbenzene and xylenes, these chemicals are some of the volatile organic compounds which are notorious for soil and groundwater contamination), pesticides, PCPs (pentachlorophenols) and PCBs (polychlorinated biphenyls) in soil or sediment are normally remediated using this approach (Saleh et al., 2004). There are some advantages and limitations of each sub-treatment shown in Table 2-3 (McIntyre, 2003).

Table 2-3 Advantages and limitations of sub-processes under phytoremediation (McIntyre, 2003)

Advantage	Limitations
Phyto-extraction	
Plant provides abundant biomass in a short period time.	Hyper-accumulator plants are generally slow growing and bio-productivity is rather small; phytomass after treatment has to be disposed of.
Phyto-stabilization	
Low-cost, less disruptive and may enhance soil restoration/re vegetation.	Extensive fertilization is required and it is necessary to use amendments for soil modification; long term treatment may result in problems such as leaching.
Phyto-volatilization	
Toxic contaminants can be transferred into a less toxic form; for instance, As^{3+} can be transfered into As^{5+}. The latter is less toxic.	After the treatment, low concentration of metabolites still can be found in plant tissue.
Phyto(rhizo)-filtration	
In-situ or ex-situ treatments are both suitable for this approach; terrestrial or aquatic	Need to understand the interaction between chemical speciation and all species; medium pH value has to be monitored during the whole process in order to insure metal uptake is continuous.
Rhizo-degradation	
This approach is normally combined with phytoremediation and bio- augmentation. Beneficial to cultivation of new microorganisms in the soil.	In some researches, microbial inoculation inhibited rhizodegradation relative to the non-inoculated control.

2.4.3 Evaluation of Phytoremediation

Low cost is the major factor for phytoremediation; compared to the other methods such as physical treatment, phytoremediation generally has a lower operating cost and entails less capital (Raskin and Ensley, 2000). Moreover, it is easily monitored. The whole treatment includes the following steps:

1) soil preparation;
2) planting the seeds;
3) pest and weed control;
4) harvesting;
5) disposal of the plants and biomass.

In addition, this approach can be turned into a permanent treatment solution. The harvested metals can be recycled and reused. This approach is also suitable to remediate radionuclides. Furthermore, its greater social acceptance is another benefit for phytoremediation (Tucker and Shaw, 2000).

In contrast, if cost is the greatest potential advantage of phytoremediation, the duration (time) is the biggest drawback (Raskin and Ensley, 2000). Utilizing this treatment might require a long of period time. The purification period is primarily dependent on the plants' life cycle. Therefore, the treatment is limited by the plant growth and harvesting (Raskin and Ensley, 2000).

Phytoremediation does not result in a total cleanup of the contaminated site. There is an interaction between metal reduction in soil and accumulation of metals within the plants. Therefore, when the concentration of metals decreased this might lead to reaction rate decreasing. High concentration pollutants might be harmful for plants growth and slow the uptake of the contaminants (Salt et al., 1998).

The treatment mainly relies on the root system, and the root cannot extend into deeper soil layers. Therefore, phytoremediation is generally limited to the root zone. According to this research, phytoremediation have many advantages.

2.4.4 Plant Selection for Phytoremediation

The choice of plant is the most important factor during the whole treatment process, it impacts on the project duration and the operating cost. It influences the remediation efficiency and rate. The rules for selection of suitable plants for soil remediation (Chaney et al., 1997) are:

- Plants are able to have high tolerance for high concentrations of the element within root and shoot cells;

- The ability to translocate an element from underground part to aboveground part is required; and
- The plant has to have a rapid uptake capacity in order to maximize the remediation efficiency.

Apart from the basic information of phytoremediation, these researchers (Chaney et al., 1997) found a way to select adaptable species for phytoremediation. And provide a guide for choosing plants for this project.

2.4.5 Mechanism of phytoremediation

There are many pathways for plant to absorb dissolved external elements. Root tissue structure allows the plant some degree of control over the uptake. Cell membranes and hydrophobic barriers control the quantity of solutes entering the vascular tissue. Organic substances primarily are taken into the tissues by diffusion and stored or degraded in cells. Inorganic substances such as charged metal ions have a tendency to persist to a greater extent in root tissue (Steinberg, 2009).

Some plants have shown the capacity to withstand relatively high concentration of organic pollutants without toxic impact, additionally they can uptake and convert these organic compounds to less toxic metabolites.

a) Mechanism of phytoremediation for organic contaminants

Plants can stimulate biodegradation of organic compounds in the rhizophere by the release of root enzymes, exudates and build-up of organic carbon in the soil.

b) Mechanism of phytoremediation for inorganic contaminants

Major source comes from heavy metals pollution. There are many different mechanisms to remediate heavy metals. Plants can uptake and translocate heavy metals into the above-ground biomass; filtering metals from water onto root system; or stabilizing waste sites by erosion control and evapotranspiration of large quantities of water.

Selection of a specific plant for phytoremediation depends on the type of pollutants on site. Different plants are chosen for different purposes of treatment. Therefore, the mechanisms for particular treatments are different. Sometimes two or more sub-approaches have to be used to achieve a goal for remediation.

2.5 Case studies

There has been a lot of research looking at phytoremediation from different perspectives. For example, some research has explored the mechanisms of how the plant uptakes elements from soil and water environment; some researchers have focused on using different organic or inorganic amendments to enhance plant uptake capacity under contaminated environment (Datta et al., 2011; Datta and Sarkar, 2004; Srisatit et al., 2003; Tu and Ma, 2002)They have drawn attention to the fact that there are some approaches which enhance the efficiency of phytoremediation. Thus, regarding to this research project, selected should improve arsenic uptake in order to improve the phytoremediation efficiency of the plant .Additionally, transgenic plants are generally used for phytoremediation, and researchers contribute by analysing the relationship between relative gene and plant physiology, in order to overexpress the gene and improve plant tolerance and absorption for a particular requirement (Ryan et al., 2001). Ingham et al. (1985) studied the importance of rhizosphere for plant growth. They pointed out that the microfloral grazers such as protozoa, nematodes and microarthropods increased plant growth as well as increased N uptake by plants. Some papers discuss the element's transpiration from molecular level, and they use molecular chemistry to explain how the elements react and their mobility in the plant. Some researchers claim the plant absorption mechanism depends on cell wall protection (Fry, 1998).

The case studies are relevant to current research that explores phytoremediation from different biological levels to determine plant uptake and detoxication mechanisms.

(Tu and Ma, 2002) used *Pteris vittata* (Ladder brake) to explore different arsenic forms and concentrations impacting on plant uptake capacity. They argued different arsenic forms will impact on plant uptake capacity which is relative to this project. *Pteris vittata* is a hyper-accumulator. The experiments were conducted under greenhouse conditions with As concentrations between 50 ppm to 1000 ppm and various the arsenic forms (inorganic and organic, arsenate and arsenite) were studied. The soil in their experiment was mainly sandy soil. Arsenic chemicals were synthesized in the laboratory in order to prepare for different concentrations. The chemical components included $AlAsO.2H_2O$, $Ca_3(AsO_4)_{2\sim}14H_2O$ and $FeAsO_4.2H_2O$.

The experiment had two parts. In the first part, the soil was mixed with arsenic solution at six different concentrations (0, 50, 100, 200, 500 or 1000 ppm as K_2HAsO_4) to determine the effect of different arsenic concentrations on *Pteris vittata*; the second part of their experiment was to mix different arsenic forms solution at the same concentration (50 ppm) to determine the form of arsenic is best for ladder brake uptake. The main contribution of this research was to show that the biomass around the root system is an important factor for metals uptake. The amount of biomass was measured after the two different treatments. Moreover, plants and soil samples were digested using USEPA Method 3051 and the arsenic concentrations determined at the beginning and end of the experiments. The arsenic concentration in different parts of plant such as fronds and roots were measured. For the fronds, it was observed that growth rate and measured arsenic concentrations at different life stages (young fronds, mature fronds and old fronds) (Tu and Ma, 2002). Table 2-4 and Table 2-5 show some results in their experiment.

Table 2-4 As concentration in ladder brake and soil as affected by different soil arsenic concentrations (Tu and Ma, 2002)

	Water-soluble As			Fronds		
Total soil As	Initial	Final	Roots	Young	Mature	Old
	mg As kg^{-1}					
0.69 (control)	0.02	0.01	1.0 ± 0.2†	7.3 ± 0.6	4.4 ± 0.8	1.6 ± 0.1
50	5.8	4.0	131 ± 2.8	2 642 ± 59	3 357 ± 27	3 662 ± 207
100	13.5	9.2	379 ± 55	4 178 ± 237	6 674 ± 446	7 021 ± 381
200	29.8	22.7	990 ± 79	4 999 ± 358	7 516 ± 51	7 624 ± 374
500	89.2	78.1	2 318 ± 86	11 203 ± 1 163	8 069 ± 35	9 779 ± 841

† Mean ± standard error.

Table 2-5 As concentration in ladder brake and soil as affected by different arsenic forms (Tu and Ma, 2002)

Arsenic forms	Water-soluble As		Roots	Fronds		
	Initial	Final		Young	Mature	Old
	mg As kg^{-1}					
Control	0.01	0.05	3.4 ± 0.1†	5.3 ± 0.3	4.8 ± 0.3	1.3 ± 0.02
$FeAsO_4$	0.2	0.2	58 ± 3.6	255 ± 22	149 ± 11	59 ± 1.5
$AlAsO_4$	0.5	0.4	178 ± 11	411 ± 13	297 ± 16	242 ± 3
$NaAsO_2$	5.0	3.5	390 ± 4.4	1532 ± 89	1862 ± 106	2390 ± 9.5
Na_2HAsO_4	5.5	4.3	211 ± 3.3	1088 ± 119	1551 ± 15	2796 ± 50
NaMMA‡	12.6	5.2	213 ± 5.1	969 ± 23	1592 ± 67	2819 ± 11
K_2HAsO_4	6.0	4.7	373 ± 37	1195 ± 49	1653 ± 4	2899 ± 24
CaMMA‡	15.8	5.6	180 ± 29	1518 ± 53	2089 ± 46	3628 ± 102
$Ca_3(AsO_4)_2$	8.4	5.5	190 ± 16	1467 ± 15.5	2205 ± 26	4559 ± 77

† Mean ± standard error.
‡ NaMMA, sodium methylarsonic acid; CaMMA, calcium acid methanearsenate.

The arsenic contents in different plant's parts have been clearly examined. Table 2-4 and Table 2-5 visually indicate the decrease of arsenic by phytoremediation in the fronds. Therefore, the arsenic has been translocated and accumulates in the fronds as the plant matures (Tu and Ma, 2002). The tables (Table 2-4 and 2-5) showed the arsenic accumulation by fronds was greater than the roots, and implied different arsenic compounds are a factor which may impact on the arsenic uptake and translocation rate within plant organs.

Tu and Ma (2002) explained two new concepts: BF (bio-concentration factor) and TF (translocation factor) to illustrate the uptake efficiency between plant and biomass or fronds and roots.

BF is defined as the ratio of arsenic concentration in plant tissue to those in soil. This factor can be used for comparing the effectiveness of the plant in concentrating arsenic from soil into its biomass.

TF is the ratio of arsenic concentration in fronds to those in roots, indicates the effectiveness of a plant in this translocation (Tu and Ma, 2002).

Subsequently, the BF and TF values under two time periods, 12 weeks and 18 weeks have been examined. Table 2-6 and Table 2-7 separately show the BF and TF values after 12 weeks and 18 weeks under different treatments

Table 2-6 BF and TF values for Ladder brake as impacted by arsenic concentrations after 12 weeks (Tu and Ma, 2002)

	Bioconcentration factor†		
Total soil As	Fronds	Roots	Translocation factor‡
mg As kg^{-1}			
0.69 (control)	6.15a§	1.48a	4.2a
50	63.3de	2.58b	24.6d
100	59.8d	3.77c	15.9c
200	36.8c	4.94de	7.5b
500	21.0b	4.63d	4.5a

† Ratio of As concentration in plant tissue to that in soil.
‡ Ratio of As concentration in frond to that in root.
§ All numbers are the averages of four replicates. Values in a column followed by the same letter are not significantly different ($p < 0.05$).

Table 2-7 BF and TF values for Ladder brake as impacted by different arsenic forms after 18 weeks treatment (Tu and Ma, 2002)

	Bioconcentration factor†		
Arsenic forms	Fronds	Roots	Translocation factor‡
Control	6.36b§	4.86bc	1.3a
$FeAsO_4$	2.68a	1.14a	2.4a
$AlAsO_4$	6.21b	3.51b	1.8a
Na_2HAsO_4	32.8c	4.15bc	7.9c
K_2HAsO_4	34.2cd	7.36d	4.6b
NaMMA¶	34.9cd	4.20bc	8.3c
$NaAsO_2$	36.8de	7.68d	4.8b
CaMMA¶	43.6f	3.54b	12.3d
$Ca_3(AsO_4)_2$	51.6g	3.75b	13.7d

† Ratio of As concentration in plant tissue to that in soil.
‡ Ratio of As concentration in frond to that in root.
§ All numbers are the averages of four replicates. Values followed by the same letter in a column are not significantly different ($p < 0.05$).
¶ NaMMA, sodium methylarsonic acid; CaMMA, calcium acid methanearsenate.

Tu and Ma (2002) measured the phytoextraction capacity of *Pteris vittata*. They found that application of a plant for phytoremediation should be considered in terms of its ability to absorb and uptake contaminants directly or indirectly from other media. For the first part of their experiments, they examined four different arsenic concentrations (50, 100, 200 and 500 ppm As) in soil samples and found that the greatest arsenic accumulation rate by *Pteris vittata* is at 100ppm As. A 500 ppm As in the soil led to a reduction in plant biomass (Table 2-8).

For the second part of their experiments, they used different inorganic and organic arsenic forms to determine the difference of arsenic uptake capacity by *Pteris vittata*. It is apparent that the arsenic removal by the hyperaccumulator plants was substantially affected in the arsenic forms in the soil (Table 2-9).

Table 2-8 Arsenic phytorextraction capacity by ladder brake after 12 weeks treatment of amended different arsenic concentrations (Tu and Ma, 2002)

Total soil As	Fronds	Roots	Total	Percent of soil As
mg As kg^{-1}		mg $plant^{-1}$		%
0.69 (control)	0.006 ± 0.001†	0.001 ± 0.0001	0.007 ± 0.001	0.67
50	9.30 ± 1.2	0.13 ± 0.042	9.43 ± 1.17	12.4
100	13.8 ± 4.1	0.46 ± 0.12	14.3 ± 4.2	9.5
200	8.86 ± 2.0	0.79 ± 0.27	9.65 ± 2.2	3.2
500	5.27 ± 0.8	0.70 ± 0.011	5.96 ± 1.04	0.79

† Mean ± standard error.

Table 2-9 Arsenic phytorextraction capacity by ladder brake after 12 weeks treatment of amended different arsenic concentrations (Tu and Ma, 2002)

Arsenic forms	Fronds	Roots	Total	Percent of soil As
	g $plant^{-1}$			%
Control	0.04	0.01	0.05	4.74
$FeAsO_4$	0.82	0.18	1.00	1.31
$AlAsO_4$	2.02	0.68	2.70	3.55
$NaAsO_2$	13.6	0.30	13.9	18.2
Na_2HAsO_4	13.5	0.61	14.1	18.6
NaMMA†	14.4	0.69	15.1	19.8
CaMMA†	15.1	0.65	15.8	20.7
K_2HAsO_4	18.4	1.20	19.6	25.8
$Ca_3(AsO_4)_2$	19.1	0.72	19.8	26.0

† NaMMA, sodium methylarsonic acid; CaMMA, calcium acid methanearsenate.

Srisatit et al. (2003) examined and observed *Vetiveria* (synonym of *Chrysopogon zizanioides*) and *Vetiveria nemoralis (synonym of Chrysopogon zizanioides)* (two ecotypes) for 90 days of arsenic treatment (0-150 ppm As). They directly explored *Vetiveria* for arsenic uptake rate in two different ecotypes. They analysed the plant arsenic content every 15 days in different plant parts (roots, stems and leaves). It was found that there was a clear arsenic accumulation trend during the treatment.

The aim of this research was to study the accumulation of arsenic in different parts of the plants and then to compare the arsenic uptake efficiency between two ecotypes of *Vetiveria*. All of the experimental pots were under outdoor environment. Therefore, it was difficult to control temperature and the final results would have been affected by

rainfall, insects or other external elements. The most important contribution of this research is the equation for arsenic removal rate. This equation can be used in further research for other elements removal calculation (Srisatit et al., 2003).

$$Efficiency\ of\ arsenic\ removal\ (\%) = \frac{[As\ in\ shoots + As\ in\ roots]\ (mg) \times 100}{Total\ As\ in\ the\ pot\ (mg)}\ (Eq.1)$$

This study shows that the arsenic removal efficiency of the *Vetiveria* grasses increased as the soil arsenic concentration increased. The two different ecotypes of *Vetiveria* have different removal efficiency. For *Vetiveria nemoralis*, the best efficiency was 0.0398% at the 90th day under 125 mg As/kg soil treatment. The maximum efficiency for *Vetiveria* was 0.0488% under 75 mg As/kg soil treatment. Based on the results (Table 2-10) the average accumulation efficiency of *Vetiveria* was slightly higher than that of *Vetiveria nemoralis*. It was explained that the different efficiency was due to the *Vetiveria* ecotype having more developed root systems than *Vetiveria nemoralis*, therefore the more root hairs providing more surfaces area for arsenic absorption, resulting in higher accumulation efficiency.

Srisatit et al. (2003) focused on different ecotypes of *Vetiveria* grass. A comparison of arsenic uptake capacity or arsenic tolerance between *Vetiveria* grass and other species was not addressed.

Datta et al. (2011) investigated the effect of soil properties on phytoremediation efficiency, and found out the soil properties will impact on phytoremediation efficiency. They conducted a greenhouse study to assess the *Vetiveria* grass for clean-up pesticide-contaminated soils. Five different soil samples were collected from five different places. The soils have different properties such as pH, EC (electrical conductivity), SOM (soil organic matter) and metal contents. The five soil samples are shown in Table 2-11.

Table 2-10 Arsenic removal for both *Vetiveria* (Surat Thani ecotype) and *Vetiveria* (Prachuabkirikhan ecotype) under 90 days treatment (Srisatit et al., 2003)

Vetiver grass	$Na_2HAsO_4.7H_2O$ concentration (mg As/kg soil)	Time (days)					
		15	30	45	60	75	90
Prachuab-kirikhan ecotype	50	$^{a}0.0139^{a}$	$^{a}0.0209^{a}$	$^{c}0.0116^{a}$	$^{d}0.0384^{a}$	$^{d}0.0249^{a}$	$^{e}0.0288^{a}$
	75	$^{a}0.0124^{a}$	$^{a}0.0150^{b}$	$^{c}0.0081^{b}$	$^{d}0.0198^{b}$	$^{d}0.0332^{b}$	$^{e}0.0275^{b}$
	100	$^{a}0.0123^{c}$	$^{a}0.0159^{c}$	$^{c}0.0157^{c}$	$^{d}0.0238^{c}$	$^{d}0.0271^{c}$	$^{e}0.0390^{c}$
	125	$^{a}0.0118^{d}$	$^{a}0.0123^{d}$	$^{c}0.0195^{d}$	$^{d}0.0251^{d}$	$^{d}0.0201^{d}$	$^{e}0.0398^{d}$
	150	$^{a}0.0109^{e}$	$^{a}0.0147^{e}$	$^{c}0.0150^{e}$	$^{d}0.0200^{e}$	$^{d}0.0228^{e}$	$^{e}0.0357^{e}$
Surat Thani ecotype	50	$^{a}0.0157^{a}$	$^{b}0.0194^{a}$	$^{c}0.0138^{a}$	$^{d}0.0233^{a}$	$^{d}0.0365^{a}$	$^{f}0.0344^{a}$
	75	$^{a}0.0154^{b}$	$^{b}0.0193^{b}$	$^{c}0.0201^{b}$	$^{d}0.0312^{b}$	$^{d}0.0391^{b}$	$^{f}0.0488^{b}$
	100	$^{a}0.0122^{a}$	$^{b}0.0264^{a}$	$^{c}0.0170^{a}$	$^{d}0.0180^{a}$	$^{d}0.0281^{a}$	$^{f}0.0421^{a}$
	125	$^{a}0.0132^{c}$	$^{b}0.0228^{c}$	$^{c}0.0148^{c}$	$^{d}0.0217^{c}$	$^{d}0.0326^{c}$	$^{f}0.0285^{c}$
	150	$^{a}0.0100^{d}$	$^{b}0.0192^{d}$	$^{c}0.0167^{d}$	$^{d}0.0177^{d}$	$^{d}0.0322^{d}$	$^{f}0.0228^{d}$

Note: - The same letters on the same corner mean there is no significant difference at 95% confidence level.

- The letters on the right corner is the different of concentrations.
- The letters on the left corner is the different of period of times

Table 2-11 Soil properties from five different places (Datta et al., 2011)

Soil properties	Eufaula	Millhopper	Orelia	Orla	Pahokee Muck
pH	6.1	6.4	8.2	8.2	5.9
EC (ds/cm)	0.02	145	203	205	558
SOM (%)	2.0	4.38	2.39	2.29	85.4
P (mg/kg)	67	4,875	1,688	1,688	6,812
Ca–Mg (mg/kg)	64.6	3,155	13,125	59,200	40,800
Fe–Al (mg/kg)	847	4,745	6,060	17,550	6,010

After measuring the physical and chemical properties for each soil sample, then sodium arsenite ($Na_2HAsO_4 \cdot 7H_2O$) was added to the soils at three concentrations: 45, 225 and 450 mg As/kg soil. Then the soil was transferred into PVC columns and *Vetiveria* grass was planted into each column. Sixty columns were used for their experiments [5 groups' soil× 4 rates (0, 45, 225, and 450 mg As/kg soil (0, 45, 225, 450 ppm As) × 3

(triplicates)]. Arsenic concentration within soil was measured at the beginning of the experiment (initial) and after four month harvested (final). After 4 months harvest, the As concentration of plants was measured and divided into two parts (roots and shoots). The arsenic removal rate calculation also followed Equation of (Srisatit et al., 2003). Plant biomass, root and shoot length were also measured. It was found that no external symptoms of toxicity were observed in plants at the concentration of 45 mg/kg soil As concentration. At 225 mg/kg As concentration, there is a slight decline in biomass accompanied with yellowing of leaves. Soil properties such as clay content and pH could vary the arsenic accumulation rates in plant tissue. The increased soil arsenic concentration resulted in increased plant accumulation rate. From literature review, apparently, root tissue always has a higher arsenic accumulation rate than the shoot tissue. This result confirms that the root part is the major organ for metal uptake. Secondly, in their discussion, they found that arsenic was strongly adsorbed to Fe/Al oxides, especially in acidic soil, resulting in a decrease in the plant uptake efficiency of arsenic (Datta and Sarkar, 2004). Linked to this project, these researches provided evidences to support using *Vetiveria* the current research study.

2.6 Efficiency of phytoremediation for contaminated sites

Although phytoremediation is a natural approach for contamination treatment, the major limitation of this technology is lower efficiency and long-time operating period than physical treatments. A body of research has been published on how to improve phytoremediation efficiency. These researchers focus on different aspects, some of them explore the use of organic amendments such as chelating agents to enhance plant accumulation (Chen et al., 2004; Chiu et al., 2005; Lou et al., 2007) and other organic amendment as dairy waste , mycorrhizae and Azotobacter strains (Singh et al., 2007). In contrast, Römkens et al. (2002) argued about the drawbacks of adding organic amendment for phytoremediation which may lead to secondary pollution or be harmful for plant growth. There is other recent research which addresses inorganic amendment such as humic compounds and fulvic acid (Ghosh et al., 2012; Winarso et al., 2011). These papers implied that some inorganic compounds can activate the metal solubility in order to improve the phytoremediation efficiency.

Chen et al. (2004) explored the use of *Vetiveria* for different heavy metals treatment (Table 2-12). The soil samples contained heavy metals (Pb) and with the addition of EDTA and EDTA-heavy metal complexes (such as NTA, CDTA, EDTA, EGTA and DTPA) for soil amendment. It was observed that the organic amendment increased the heavy metals bioavailability resulting in enhancing the plants uptake efficiency. Chen et al. (2004) found that the root is the major part for metal accumulation. The 14 days experiment demonstrated that by adding EDTA into soil there was a substantial increase in the accumulation of Pb in the roots and shoots. Adding EDTA into soil facilitated Pb translocation from roots to shoots. They acknowledged that amending EDTA would lead to secondary pollution for soil or groundwater.

Table 2-12 Lead concentration in the roots and shoots of *Vetiveria* and the translocation ratios (TR*) of Pb from underground part to aboveground part after 14 days treatment by applying different EDTA concentrations for the pot experiment (Chen et al., 2004)

Pb amendment soils (mg kg ^{1})	The EDTA application concentration (mmol kg ^{1} soil)			
	0	0.5	2.5	5.0
Shoot				
0	0.81 ± 0.06	–	–	3.59 ± 0.42
500	0.82 ± 0.12 a A	6.06 ± 0.37 b A	25.7 ± 3.23 c B	42.2 ± 4.09 d C
2500	6.52 ± 0.17 a A	21.3 ± 3.63 a A	86.3 ± 13.5 b B	160 ± 28.1 c C
5000	43.0 ± 0.71 a A	68.6 ± 4.76 b B	127 ± 7.7 c C	243 ± 13.5 d D
Root				
0	4.16 ± 0.42	–	–	15.6 ± 4.13
500	60.3 ± 5.08 a A	83.5 ± 7.75 a A	200 ± 22.5 b B	266 ± 43.0 c B
2500	205.8 ± 21.8 a A	242 ± 29.6 a A	464 ± 50.3 b B	951 ± 126 c C
5000	556 ± 28.4 a A	871 ± 82.8 a AB	1440 ± 303 b B	2280 ± 462 c C
*TR**				
0	19.7 ± 3.0	–	–	23.4 ± 2.5
500	1.35 ± 0.06 a A	7.37 ± 1.30 ab AB	13.9 ± 5.3 bc B	17.2 ± 2.0 c B
2500	3.18 ± 0.29 a A	8.76 ± 0.91 b B	18.9 ± 0.2 c C	18.0 ± 1.0 c C
5000	7.73 ± 0.37 a A	7.88 ± 0.14 a A	9.48 ± 2.98 a A	11.4 ± 3.8 a A

Note. Mean and standard deviation (n = 3–4); TR*: defined as the % of shoot Pb concentration versus root Pb concentration; The different capital and small letters stand for significance at 0.01 and 0.05 levels, respectively.

Chen et al. (2004) did not study *Vetiveria* grass for arsenic uptake but Pb uptake. It was found that EDTA will improve the metals bioavailability in soil and therefore can enhance phytoremediation efficiency for Pb.

In two papers, different plant species were studied for phytoremediation using chelating agents. Chiu et al. (2005) compared *Zea mays* (Maize) and *Vetiveria* for three metals (arsenic, zinc and copper) accumulation rate by adding different chelating agents, such as NTA (nitrilotriacetic acid), CDTA (leneditrilotetraacetic acid), EDTA

(ethylendiaminetriacetic acid), EGTA (ethylenebis tetraacetic acid), DTPA (diethylenetriaminepentaacetic acid) and so on. (Chiu et al., 2005) showed that compared with other chelating agents, at 20mmol NTA could maximally improve the arsenic and zinc bioavailability by plants in sandy loam soil. In the control group (soil contained arsenic at 100 mg/kg) without added chelating agents, the results show that *Vetiveria* is more suitable and more effective in terms of accumulation of arsenic than *Zea mays*.

Chiu et al. (2005) added chelating agents (EDTA, HEDTA and OA (oxalic acid)). It was found that the addition of chelating agent decreased the dry-weights (both aboveground and underground part) for *Pteris vittata* and *Sesbania rostrata*. In contrast, for *Vetiveria*, EDTA and HEDTA increase its dry-weight for aboveground, whereas OA can slightly decrease its dry-weight for both parts (Table 2-13 and Table 2-14).

Table 2-13 Water-soluble arsenic in contaminated soil after three species harvested (mean ±SD, n=4) (Chiu et al., 2005)

	Control	EDTA	HEDTA	OA
As				
P. vittata	0.038 ± 0.008c	0.14 ± 0.016a	0.12 ± 0.011b	0.026 ± 0.003c
V. zizanioides	0.032 ± 0.002c	0.16 ± 0.016a	0.14 ± 0.014b	0.032 ± 0.003c
S. rostrata	0.040 ± 0.013b	0.16 ± 0.011a	0.16 ± 0.009a	0.035 ± 0.004b

Table 2-14 Amendment of 2.5g/kg chelating agents on dry weights of three species (pteria vittata, *Vetiveria*ia zizanioides and Sesbania rostrata(g/pot) grown in metal-contaminated soil(mean ± SD, n=4) after four weeks treatment (Chiu et al., 2005)

		Dry Weights (g)	
Plants	Treatments	Aboveground part	Underground part
P. vittata	Control	2.56 ± 0.65a	2.59 ± 0.21a
	EDTA	1.70 ± 0.60ab	1.63 ± 0.47b
	HEDTA	1.51 ± 0.26b	1.16 ± 0.20b
	OA	1.27 ± 0.44b	1.16 ± 0.35b
V. zizanioides	Control	4.74 ± 0.98b	2.64 ± 0.26a
	EDTA	5.67 ± 0.98ab	2.20 ± 0.28ab
	HEDTA	6.88 ± 1.05a	2.80 ± 0.44a
	OA	4.61 ± 0.40b	1.85 ± 0.46b
S. rostrata	Control	4.29 ± 0.52a	1.47 ± 0.31a
	EDTA	3.67 ± 0.53ab	1.41 ± 0.06a
	HEDTA	3.02 ± 1.44ab	0.86 ± 0.39b
	OA	2.35 ± 0.72b	0.75 ± 0.04b

OA: Oxalic acid.
*Different letters in the same plant and same part (aboveground or underground) indicate a significant difference at $p < 0.05$ according to the Duncan test.

The total dry weight of *Vetiveria* is higher than of *Zea mays* after the treatment. Despite adding chelating agents, both plants demonstrated decreased dry weight in soil contained arsenic than the BL (control) group. For the physiological observations, for all of *Zea mays* plants grown, including the control groups (without adding chelating agents), were stunted and exhibited various symptoms such as the striping of leaves and shoots and reddening, leaf curling, necrosis on leaf edges and stems. In contrast, there were no substantial symptoms for *Vetiveria* (Chiu et al., 2005). Moreover, amended NTA for both plants in soil can increase arsenic accumulation in plant tissue, especially for the shoots part.

Although adding chelating agents can enhance the arsenic concentration in soil extraction, plant accumulation of arsenic is not merely related to arsenic concentration in the soil solution. Figure 2-2 and Figure 2-3 show the impact of adding different concentrations of chelating agents on metals solubility. It clearly indicates at the concentration of 2.5 g/kg, all the chelating agents can substantially enhance arsenic solubility in soil. Figure 2-4 and Figure 2-5 show no substantial difference for arsenic accumulation between control group and adding chelating agents groups for *Pteris vittata* and *Sesbania rostrata*. Addition of OA and HEDTA can insubstantially increase arsenic accumulation for *Vetiveria* but this is not observed by EDTA.

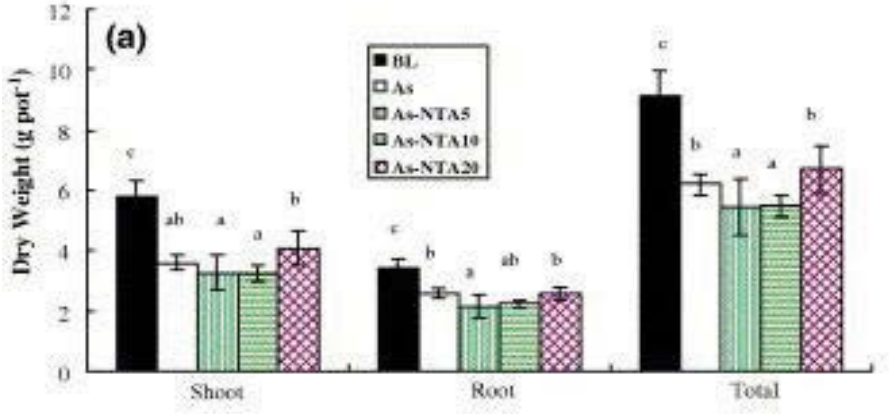

Figure 2-2 Root, shoot and the total dry weights of *Vetiveria* under soil contained 100 ppm arsenic condition, treatment including BL(soil only, no metals addition), only arsenic, arsenic plus 5mmol NTA, arsenic plus 10 mmol NTA and arsenic plus 20 mmol NTA (Chiu et al., 2005)

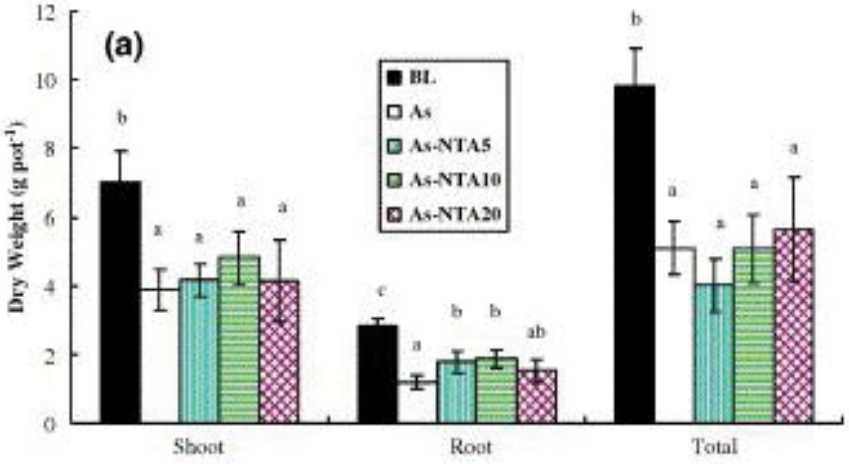

Figure 2-3 Root, shoot and the total dry weights of maize under soil contained 100 ppm arsenic condition, treatment including BL(soil only, no metals addition), only arsenic, arsenic plus 5mmol NTA, arsenic plus 10 mmol NTA and arsenic plus 20 mmol NTA (Chiu et al., 2005)

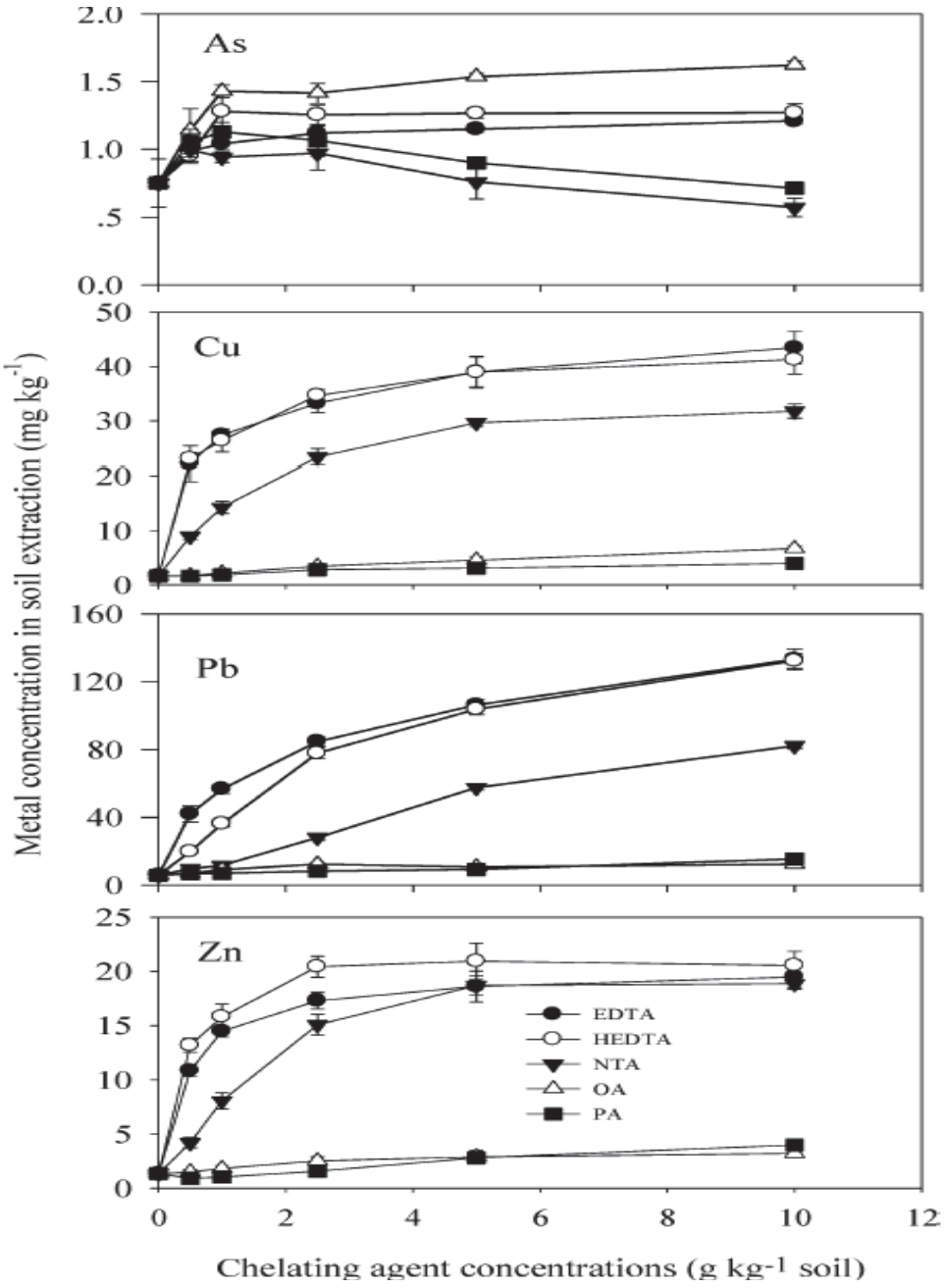

Figure 2-4 Effects of different chelating agents and their application rates ranging from 0 to 10 g/kg on soil AS, Cu, Pb and Zn extraction. Error bars represent ± SD (n=4) (Chiu et al., 2005)

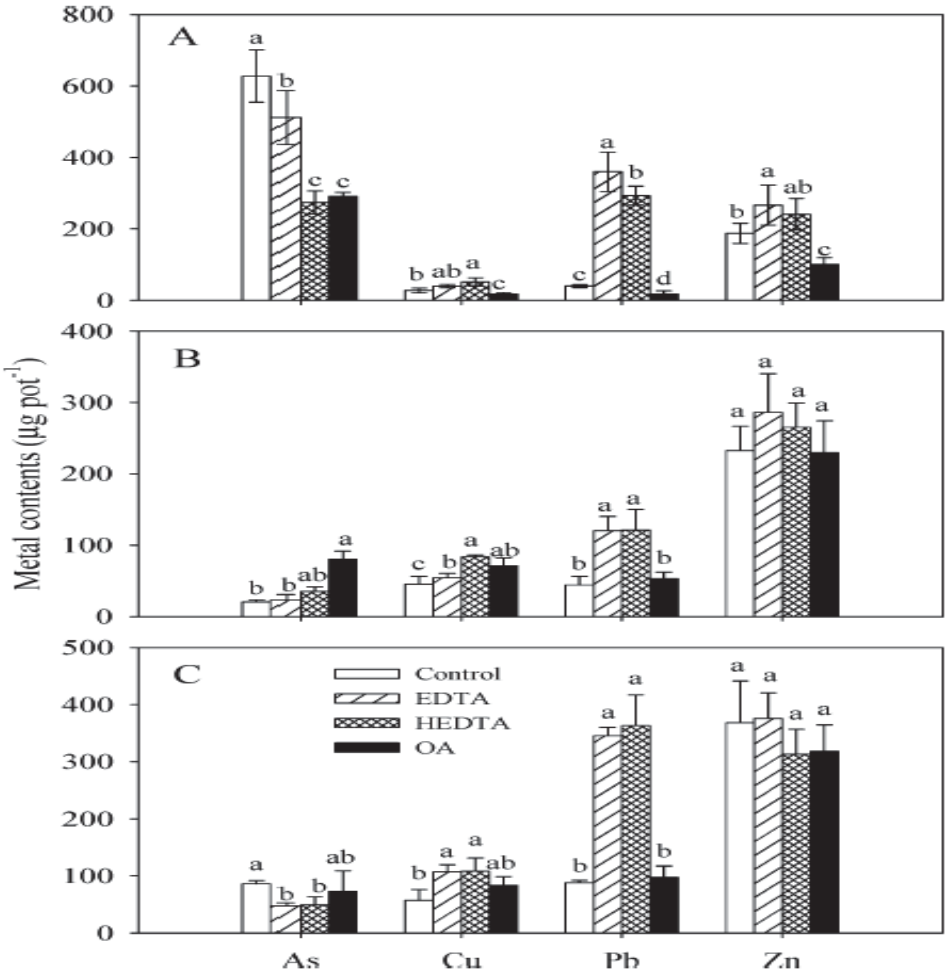

Figure 2-5 Different Chelating agents impact on As, Cu, Pb and Zn accumulation in the aboveground parts of three plants (A=*P.vittata*; B=*V.zizanioides*; C=*S.rostrata*) treated in metal-contaminated soil when they were added to the soil for four weeks in concentration of 2.5 g/kg. Error bars represent ± SD (n=4). Different letters in the same plant and same metal indicate a substantial difference at $p<0.05$ according to the Duncan test (Chiu et al., 2005)

2.7 Conclusion

From the literature review, it has been shown that *Vetiveria* is able to tolerate and uptake arsenic from moderately contaminated soil environment. It appears that there has been very little research on the addition of fulvic acid and no research on straw water to *Vetiveria* plants to demonstrate whether these amendements enhance the uptake capacity of arsenic in groundwater. It was decided by EESI to use straw water as an amendment because of the low cost of production. The availability of arsenic uptake by straw water

was then compared with fulvic acid, to determine which amendment would be more successful.

Chapter 3. Methodology

3.1 Introduction

After the review of literature, *Vetiveria zizanioides* was the selected plant to undertake the task to remediate arsenic. *V.zizanioides* has been widely used for water and soil conservation in Asia (USEPA, 2000). In this report, *Vetiveria* was used under glasshouse conditions and arsenic concentrations in plants were measured by ICP-MS. The soil and groundwater taken directly from the site were used as materials for the plant's growth. Fulvic acid and straw water were mixed at different concentrations with groundwater in order to explore those amendments' functions for enhancing arsenic uptake. This chapter describes and explains the experimental and analytical procedures used (soil and glasshouse).

The research began on 10 May 2012 and was mainly conducted in the glasshouse laboratory. The analytical techniques operations were undertaken from 3 October to 20 December 2012. As this research involved a toxic element (arsenic), it was necessary to obtain Biosafety Approval (Appendix 1) from the Biosafety Committee prior to introducing groundwater (containing arsenic) into the glasshouse.

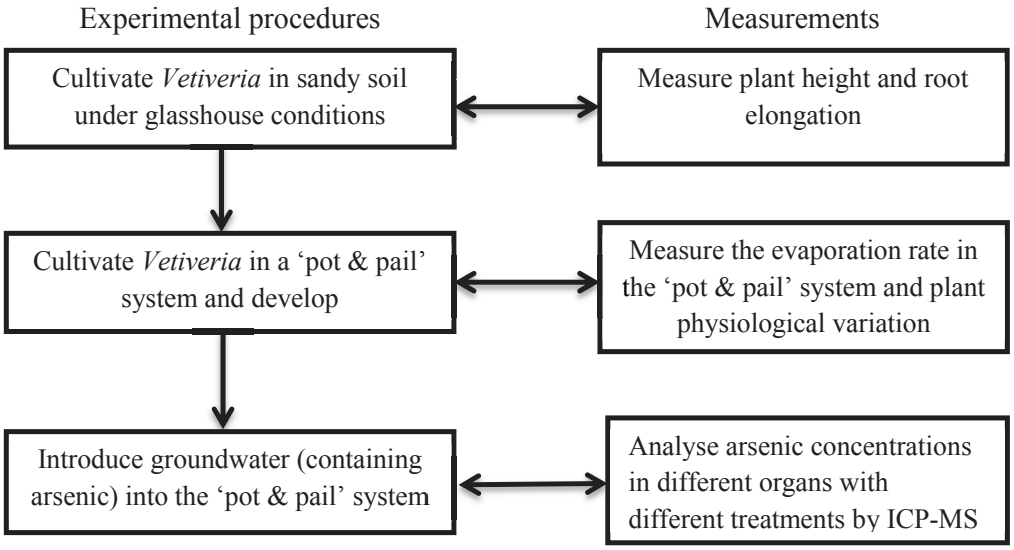

Figure 3-1 Experiment timetable

There were three glasshouse trials. To simulate the site conditions, the sandy soil samples and groundwater used in these three trials were all collected from the site. The experimental design is presented systematically in Table 3-1. Figure 3-1 outlines the procedures employed in the glasshouse experiment. Further information will be described in this chapter.

Table 3-1 The procedures used in glasshouse experiments

Experiment	Time(Day)
Frist trial- cultivate *Vetiveria* in the glasshouse	140
Second trial- cultivate hydroponic roots in 'pot & pail' system. Measure the evaporation rate	79
Third trial- introduce groundwater into pail; *Vetiveria* starts to accumulate and take up arsenic from treatment solutions. Arsenic concentration analysed by ICP-MS	78

3.2 Materials

3.2.1 Vetiveria

26 *Vetiveria* plants were provided by EESI. Special care was taken to wash any soil which had adhered to the roots. Then the height of each plant was recorded for reference in future experiments. 20 *Vetiveria* plants were used in the FT and 6 *Vetiveria* plants were kept as storage in case of failure of the control groups.

3.2.2 Soil collection and characterization

Arsenic-contaminated sandy soil from the site was used for this study. This soil provided by EESI, had not undergone any treatment such as sieving or spiked amendment. Selected chemical properties of the soil are shown in Table 3-4

Table 3-2 Data for soil moisture

Sample	Weight of container/tin (g)	Wet mass soil + tin (g)	Dried mass soil + tin(g)	Moisture content (%)
1	25.0	95.2	85.1	16.8
2	24.8	77.9	69.3	19.3
3	27.5	77.3	68.7	20.8

A soil moisture content test was conducted in soil laboratory. The formula and data follow:

$$MC = \frac{w_{wt} - w_{dt}}{w_{dt} - w_t} \times 100\%$$

Where:

MC = moisture content

W_{wt} = weight of the moisture specimen with tare

W_{dt} = weight of the dried specimen with tare

W_t = weight of the container

The average soil moisture is $MC_a = \frac{MC_1 + MC_2 + MC_3}{3} = 19.0\%$ and the field capacity is half of soil moisture, thus the field capacity is 9.5%. In order to give an indication of wilting point and is part of soil physical properties.

3.2.3 Groundwater properties and amendment preparation

There are two stages for watering plants. In the first and second trials, tap water (no arsenic detected) was used to water plants twice per week. However, in the third trial, groundwater containing 5.5 mg/L of arsenic was introduced in the glasshouse laboratory. *Vetiveria* began to depend solely on roots to take up water from the groundwater samples. The groundwater was kept in five plastic containers with different mixtures. Each container with labelled according to the solution constituents. The coding of the label on each container is shown in Table 3-3. The pH in each container is shown in Table 3-5.

The straw water was prepared by EESI. A 10L container was filled with straw supplied by Pet barn (12kg bail used as bedding material). The straw was loosely packed in the container and then the container was filled with tap water and sealed. After 45 days, the water contained maximum amount of phenolic acid (220 mg/L refer to Appendix 8).

Table 3-3 Coding of the solutions in differently marked plastic containers

Label	Solution
SL	10 Litres groundwater mixed with 1 ml straw water; amendment concentration=0.01%
SH	10 Litres groundwater mixed with 10 ml straw water; amendment concentration=0.1%
FL	10 Litres groundwater mixed with 1 ml straw water; amendment concentration=0.01%
FH	10 Litres groundwater mixed with 10 ml straw water; amendment concentration=0.1%
GW	10 Litres groundwater mixed with 1 ml straw water

Table 3-4 Soil chemical properties at the research site (Sydney Analytical Laboratories)

Soil sample	TOC	TKN	Available .N (mg/kg)	Bray.P (mg/kg)	CEC MEQ%
	0.29	63	1.5	12	6.5
Soluble	Na (%)	K (mg/kg)	Ca (mg/kg)	Al (mg/kg)	As (mg/kg)
	0.12	0.06	0.61	<0.01	2
	Cr (mg/kg)	Hg (mg/kg)	Zn (mg/kg)	Ni (mg/kg)	Cd (mg/kg)
	18	0.035	33	4.0	<0.5

Table 3-5 pH in each container

No.	pH	Total amount(L)	Arsenic concentration(mg/L)	Amendment concentration
SL	7.84	10	5.5	Straw water 0.01%
SH	7.78	10	5.5	Straw water 0.1%
FL	7.83	10	5.5	Fulvic acid 0.01%
FH	7.76	10	5.5	Fulvic acid 0.1%
GW	8.11	10	5.5	None

3.3 Methods

3.3.1 First trial (FT)

3.3.1.1 Objectives

The first trial was for 140 days. The main purpose was to grow *Vetiveria* under glasshouse conditions in sandy soil, because there are few researchers who have explored *Vetiveria* growth rate in sandy soil. In addition, this trial was a pilot for the next two trials. If plants grew well in this situation, it would demonstrate that the plant would grow successfully on site. Moreover, the soil used in this trial already contained 2 mg/kg total arsenic (mainly arsenate). Although the arsenic concentration was not high for *Vetiveria* growth, as per the information from the literature review, plant could uptake arsenic, and could accumulate arsenic both in shoots and roots.

3.3.1.2 Soil sample collection and treatment

Topsoil (sandy loam) from site field was excavated to be used in this experiment. A sample (duplicate) was analysed at The Sydney Analytical Laboratories (Table 3-4). The soil was transferred into the UTS soil laboratory, put into pots and taken to the glasshouse.

The moisture content of the soil was determined in the soil laboratory (Table 3-2).

2 kg of soil containing arsenic was then placed in each pot (40cm deep 30cm diameter). The soil depth in each pot was approximately 20cm. The average temperature in the glasshouse ranged from 24 ℃ (night) to 26 ℃ (daytime). The complete glasshouse conditions information from May to December was recorded by glasshouse laboratory computer system (refer Appendix 2).

3.3.1.3 Plant preparation

20 *Vetiveria* plants were used in the First Trial. The roots and stems were cleaned and each plant was planted in a pot. Figure 3-2 shows how the *Vetiveria* is cultivated in large pot conditions.

3.3.1.4 Growth observation and monitoring

Once the plants were put into the glasshouse, the grass growth rate was recorded. The plants' growth period started from 10th May to the end of September 2012. During this

period, the new stem's length and health were observed and recorded. This period continued for 4 months is the First Trial (FT).

Tap water was used to water each plant. Due to the presence of arsenic in the soil, a plastic tray was put under the bottom of the large pot in order to prevent arsenic leaching. Figure 3-2 and Figure 3-3 show how the FT ran.

The root system was measured at the beginning and end of FT (Figure 3-4 and Figure 3-5). Measurement comprised the root growth, elongation of roots and shoots. Arsenic concentrations in both organs were analysed at the end of FT.

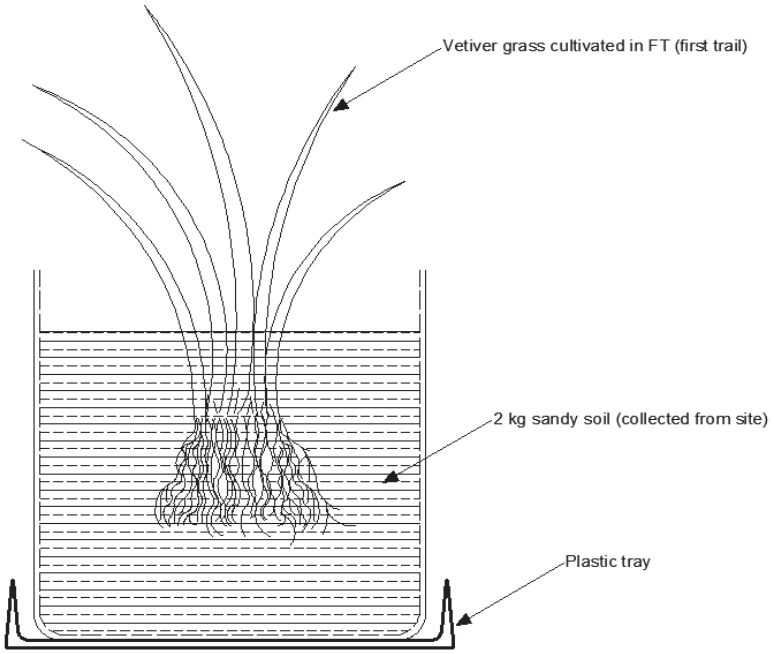

Figure 3-2 The pot trial in FT

Figure 3-3 Initial planting of the *Vetiveria* into the pot under glasshouse conditions. This is the beginning of the FT. The height of each plant was measured and recorded including its health condition.

Figure 3-4 Measurement of length for roots. This measurement was undertaken twice (beginning and end of the FT)

Figure 3-5 The base of a pot after FT, showing the root system

3.3.2 Second trial (ST)

3.3.2.1 Objectives

The aims of this trial (ST) were to determine whether *Vetiveria* could survive in the 'pot & pail' system and to develop hydroponic roots across 'nylon gauze' in order to accumulate arsenic directly from the solution. 'Nylon gauze' (Figure 3-13) was used to prevent sandy soil particles entering the solution. This was a preliminary stage for the final trial. The volume of water taken up by the plant and the evaporation in the 'pot & pail' system was measured.

3.3.2.1 Transfer into the 'pot & pail' system

After FT growth, 20 *Vetiveria* plants were removed. Rhizosphere soil was washed off , the shoots and roots were cut into the same size sections (shoots: 20 cm; roots; 10 cm). The prepared grasses were planted in smaller pots (containing 200g sandy soil), wrapped in a 40cm×40cm nylon gauze. A plastic pail (1 L) was placed outside each pot as a container in order to prevent arsenic leaching out from the pot. Tap water was added directly into the pail, so that the soil within the pot and plant root systems could immediately absorb the water. The water table was established at the 400 ml level. A

plastic wrap cover was placed over the top of pail, to prevent evaporation. Figure 3-6 shows the *Vetiveria* at the beginning of the ST and Figure 3-7 shows *Vetiveria* plants developed roots that having crossed the 'nylon gauze' extending into the pail.

3.3.2.2 Observation of new shoots growth

Observations were based on the growth rate of the above-ground shoots. At the beginning the above-ground part was 20cm long. This period lasted 79 days. The elongation of the shoots was recorded every week. The aim of this timing was to ensure that the cut plant would grow well at high moisture content. This period was from 11 July to 31 September 2012.

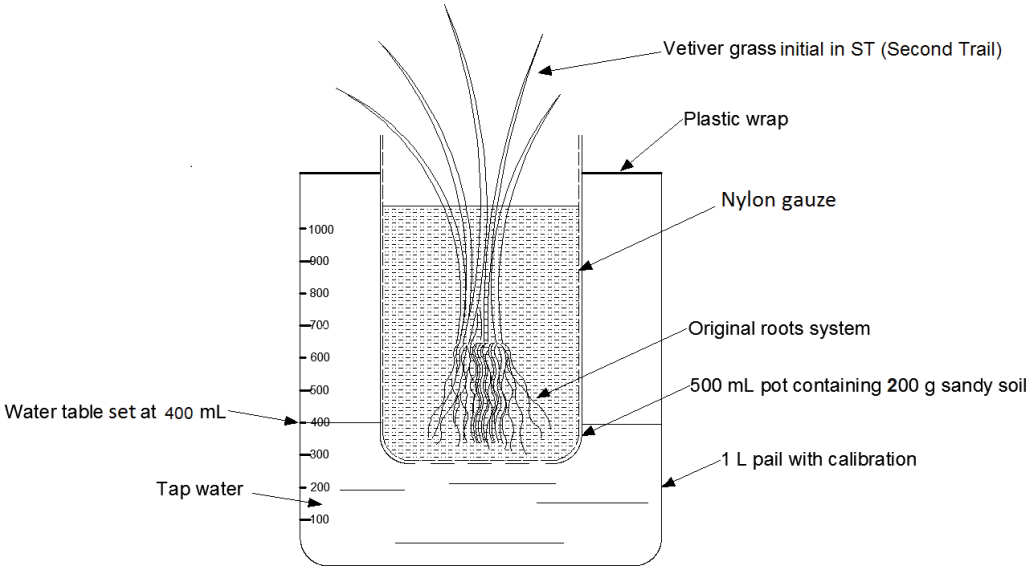

Figure 3-6 A *Vetiveria* plant grows at the initial situation in 'pot & pail' system

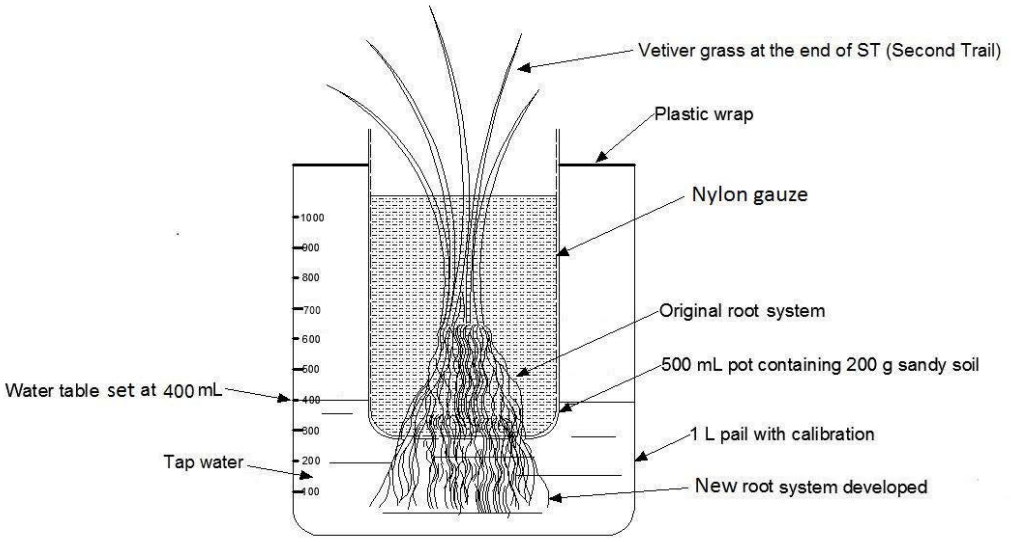

Figure 3-7 A *Vetiveria* plant grows at the end of ST developing roots into pail

3.3.2.3 Evaporation rate measurement

A lysimeter was used to measure plant evaporation rates, using Water Budge technology to determine the soil evaporation rate within a smaller pot. The initial soil moisture content had been determined (Table 3-2). Depending on the evaporation rate of both soil and plant, the total volume of groundwater solution was determined for the next step.

3.3.3 Third trial (TT)

3.3.3.1 Introduction

The aim of this trial is to measure arsenic concentrations in different organs by ICP-MS. The enhancement efficiencies by adding two amendments were determined (fulvic acid and straw water). The Figure 3-8 shows *Vetiveria* growing in this third trial.

3.3.3.2 Experiment technique

When the immersed roots crossed the 'nylon gauze' water and arsenic were taken up directly from the bathing solution. Arsenic could accumulate within roots and translocate into the above-ground parts. The *Vetiveria* root uptake capacity can be affected by the application of different treatments, resulting from different metal bio-availabilities.

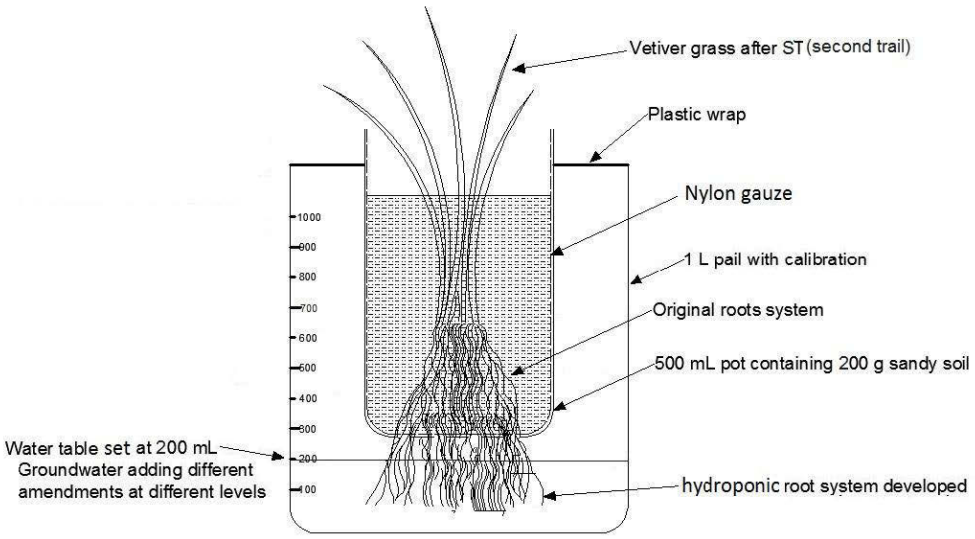

Figure 3-8 The *Vetiveria* plant treated in the third trial

In this trial, 18 'pot & pail' units were used and the tap water in the pail (ST) was replaced by groundwater. The 18 units were divided into 6 groups. Each group had different treatment. The water table was controlled (at 200 ml) below the bottom of smaller pot, thus there was no connection between the soil (in smaller pot) and groundwater. The amendments, fulvic acid and straw water at two different concentrations (0.1% and 0.01%), were used and mixed with the groundwater. Each treatment was conducted in triplicate (Table 3-6).

Table 3-6 The different treatments for each group

Number	Content	Abbreviation
1	Tap water with plant (control 2)	W
2	Ground water +plant (control 1)	GW
3	Ground water + fulvic acid 0.1%+plant	FH
4	Ground water + fulvic acid 0.01%+plant	FL
5	Ground water + straw water 0.1%+plant	SH
6	Ground water + straw water 0.01%+plant	SL

Vetiveria can accumulate arsenic in the root system. The arsenic concentration in the root system was measured at the beginning and at the end of the third trial. The variation on efficiency of the roots to accumulate arsenic can be calculated from Eq. 2.

$$\Delta E_r = \frac{C_1 - C_c}{C_c} \times 100\% \text{ (Eq. 2)}$$

Where:

ΔE_r= change in root uptake efficiency

C_1= arsenic concentration increment in roots in different treatment (μg/kg)

C_c= the arsenic concentration increment in the control group (groundwater with no amendment)

For the above-ground parts, it is more feasible to measure arsenic in the shoots every two weeks. Approximately 0.1~0.2 g fresh shoots were collected and the arsenic concentration variation was analysed during this period.

3.3.3.2 Analytical techniques

Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry which is capable of detecting metals and several non-metals at concentrations as low as one part in 10^{12} (parts per trillion). In this experiment, the arsenic concentration was very low in the soil and in the groundwater. Compared to atomic absorption techniques, ICP-MS has greater speed, precision, and sensitivity. Therefore, ICP-MS was selected to measure arsenic concentrations. However, analysis by ICP-MS is also more susceptible to trace contamination from glassware and reagents. In addition, the presence of some ions can interfere with the detection of other ions. Figure 3-9 shows the ICP-MS in Science Faculty at the University of Technology, Sydney.

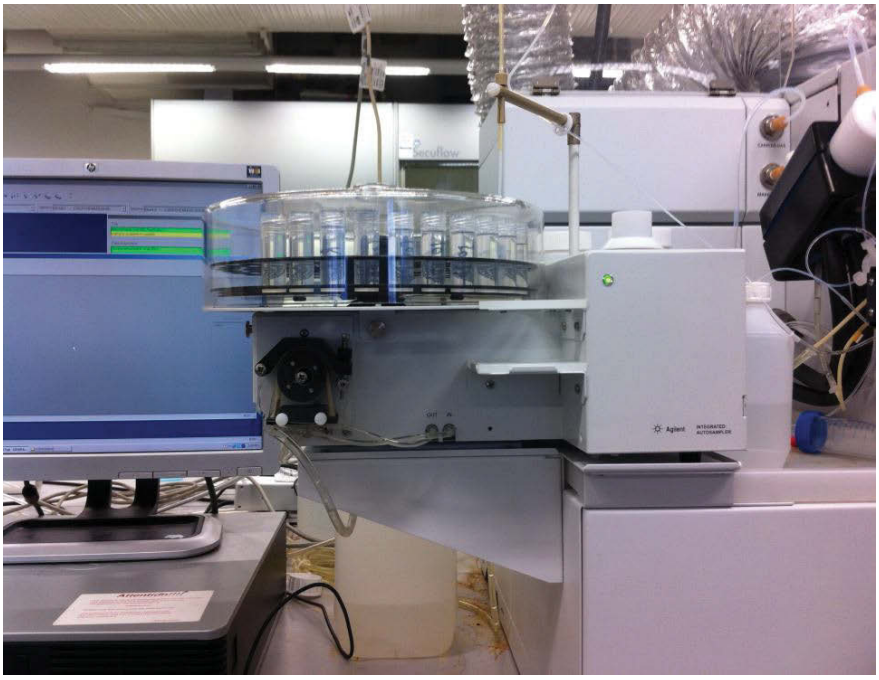

Figure 3-9 The ICP-MS operating to test plant tissue samples

3.3.3 Plant sample preparation

In order to prepare plant samples for ICP-MS, they need to be digested. Method 3050B (USEPA, 1986) was used to digest plants. The procedures and instruments are summarised in Figure 3-10, Figure 3-11 and Appendix 3.

3.3.4 'Pot & pail' monitoring and Vetiveria treatment

Each pail was periodically checked to ensure the water table remained at the 200 ml mark. The grass height was also recorded. The groundwater solution was carefully transferred from the container to the pail (Figure 3-12).

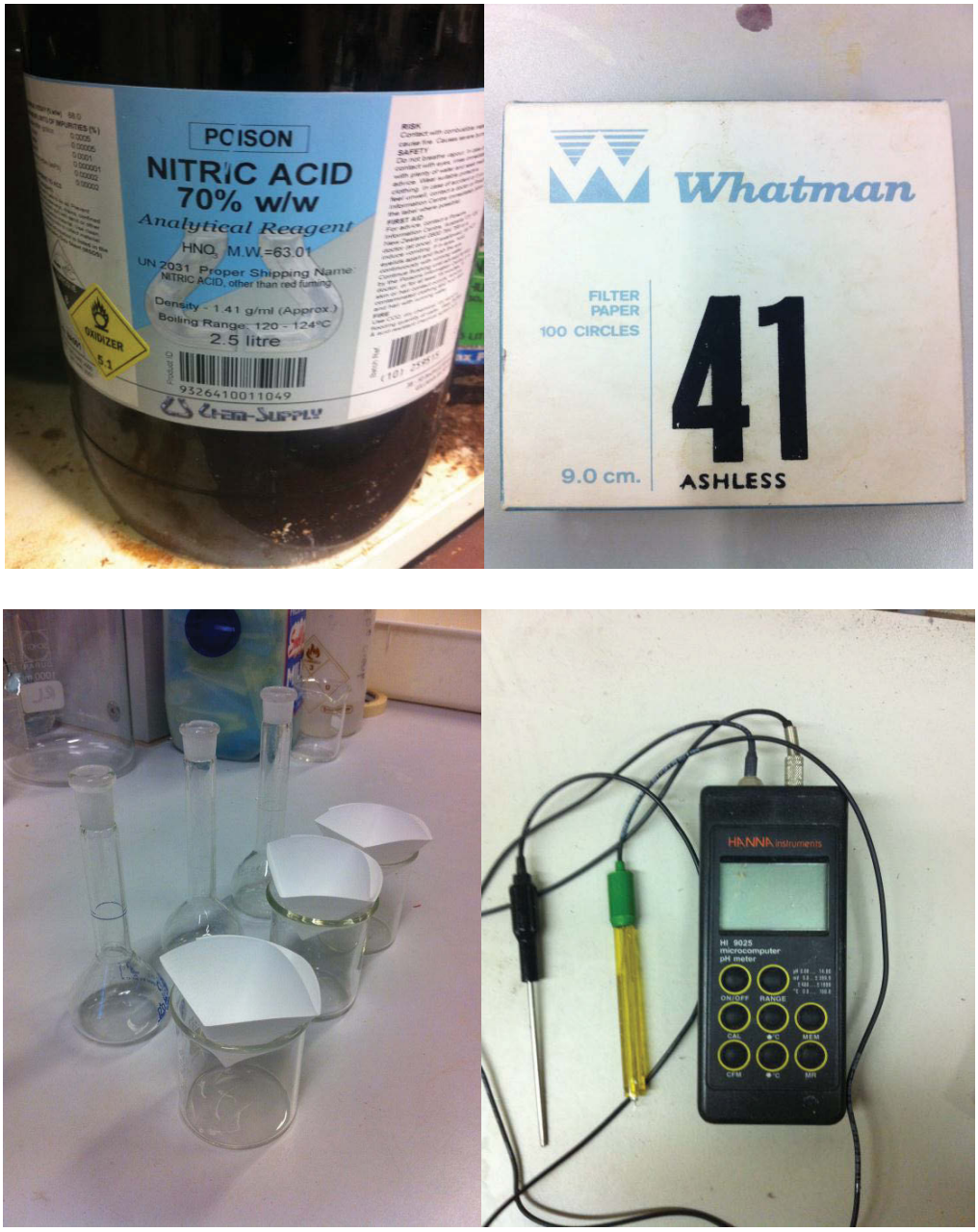

Figure 3-10 Equipment used in the soil and plant tissue experiments

Figure 3-11 electronic balance

Figure 3-12 All the pots within the last treatment, already have introduced groundwater and organic amendments

Figure 3-13 The regenerated roots, grow and cross the ‘nylon gauze’ into the water environment. The ‘green’ parts are root exudates. Once the newly regenerated roots come into the groundwater, the plant will actively take up metals.

Chapter 4. Results

This chapter shows the experimental results from May to December 2012. The raw data from the three glasshouse trial are presented in Appendices 4, 5, 6 and 7.

The 26 plants of *Vetiveria* plants used in this research were grown from rooted tillers obtained by splitting "mother plants" supplied by EESI.

The glasshouse conditions during the trials (May to December 2012) varied, with the maximum temperature in May being 31.9 ^{o}C and in October 35.3 ^{o}C (details in Appendix 2).

In the third trials, the data included average height values of the rooted tillers (details in Appendix 6). In the third trial, the plants were grown in fulvic acid (low and high concentration) and straw water (low and high concentration). The experiments were performed in triplicate. The two control groups were conducted in triplicate.

4.1 Plant growth in the first trial

The height of 20 plants used in the first trial (May to October 2012) were record weekly (Figure 4-1, Appendix 4). The average height increased from about 20 cm to 130 cm over the six month period.

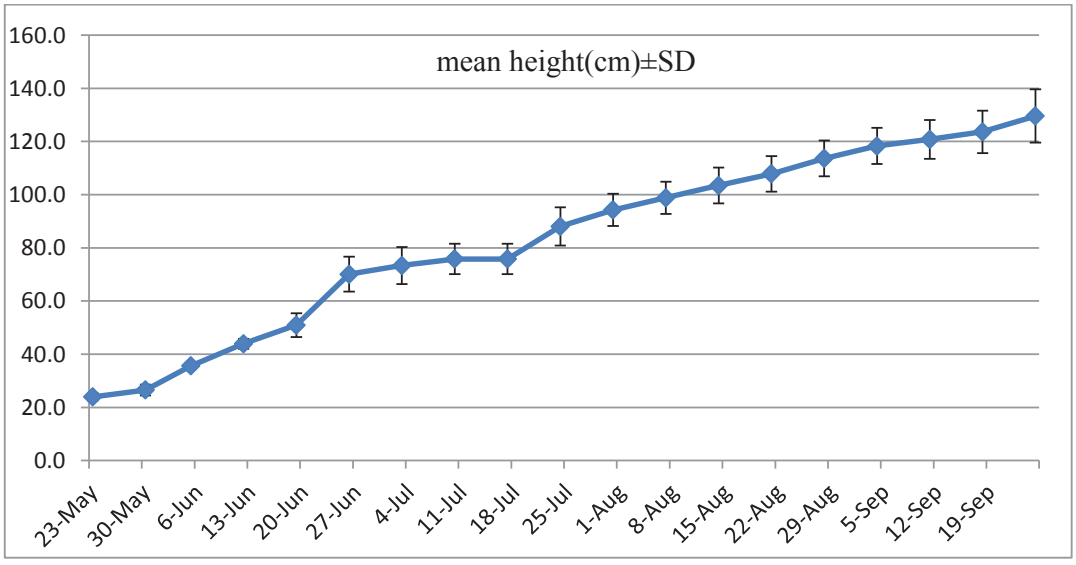

Figure 4-1 increase in plant height (mean) over six months period

4.2 Evaporation rate in the second trial (Split root and shoots)

At the beginning of the second trial (in June), the 18 plants (6 treatment with 3 replicates) were transferred into the 'pot & pail' system and labelled 1 to 18. These 18 plants were split from the 2 plants clumps, used in Trial 1. The number of days for plant roots to cross the 'nylon gauze' and reach the aquatic environment was on average 13 days with a range of 9 to 16 days (Table 4-1).

The total volume of water recorded in each 'pot & pail', removed by evaporation or by plant uptake or transpiration in the first week, was on average 202.4 g per pot & pail with a range of 165 to 288 g per pot & pail (Table 4-2).

Table 4-1 The time for *Vetiveria* roots crossing 'nylon gauze' and reaching the aquatic environment

No.	Days	No.	Days	No.	Days	No.	Days
1	13	6	16	11	14	16	13
2	14	7	11	12	13	17	14
3	12	8	12	13	12	18	16
4	13	9	10	14	14	Average	13
5	15	10	9	15	12		

Table 4-2 The water transpired by *Vetiveria in* 'pot & pail' system during the second trial

No.	Weight (gram)	No.	Weight (gram)	No.	Weight (gram)	No.	Weight (gram)
1	227	6	168	11	235	16	205
2	166	7	189	12	156	17	288
3	187	8	168	13	184	18	255
4	165	9	231	14	179	Average	202.4
5	197	10	263	15	181		

4.3 Results in the third trial

4.3.1 pH variation

The pH value in each pail was measured with a HANNA HI 9025 Portable pH meter on 30 September (commencement) and 20 December 2012 (completion). The pH values were measured for the 5 different solutions (SH, SL, FH, FL, GW) and for the tap water used in the glasshouse trial (Table 4-3). Over the trial, pH increased by about 2 pH units for all solutions.

Table 4-3 Solution pH before and after the Third Trial

	pH			**pH**	
	Before	**After**		**Before**	**After**
SL-1	7.84	8.31	FL-1	7.83	10.34
SL-2	7.84	9.11	FL-2	7.83	10.21
SL-3	7.84	9.47	FL-3	7.83	10.13
SH-1	7.78	9.57	FH-1	7.72	10.43
SH-2	7.78	9.89	FH-2	7.72	9.79
SH-3	7.78	10.07	FH-3	7.72	10.33
GW-1	8.31	9.98	W-1	7.85	9.43
GW-2	8.31	9.85	W-2	7.85	9.33
GW-3	8.31	9.79	W-3	7.85	9.41

4.3.2 Shoots growth in the third trial

The height variation at the beginning and completion of the third trial (Table 4-4) and measurement of the dry weight at the end of the trial (Table 4-5) showed a difference between treatments with the greatest growth from addition of straw at high concentration (Figure 4-2).

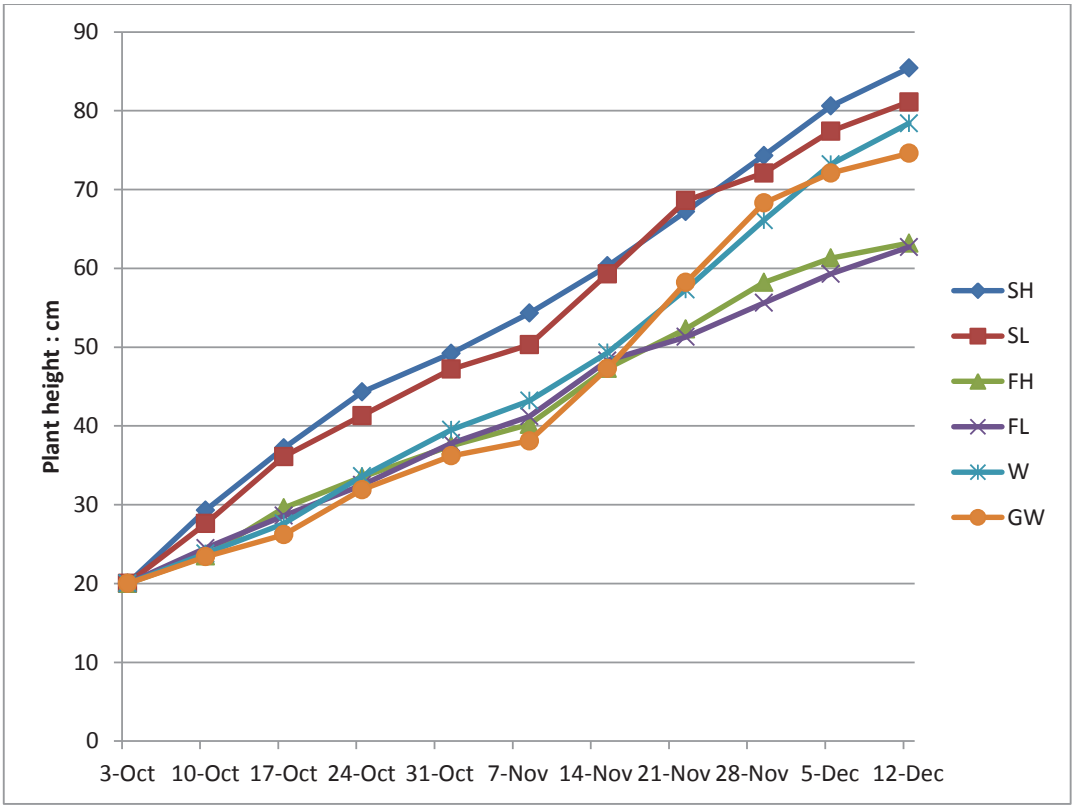

Figure 4-2 The *Vetiveria* mean shoots growth rate in the third trial:

1) FH= high concentration fulvic acids;

2) FL= low concentration fulvic acids;

3) SH= high concentration straw water;

4) SL= low concentration straw water;

5) GW= control group A only groundwater;

6) W=control group B only tap water (n=3).

Table 4-4 The mean shoots height in the third trial (unit: cm; n=3)

	3/Oct	10/Oct	17/Oct	24/Oct	1/Nov	8/Nov	15/Nov	22/Nov	29/Nov	13/Dec
SH	20.0	29.3	37.2	44.3	49.2	54.3	60.3	67.2	74.3	80.6
SL	20.0	27.6	36.1	41.3	47.2	50.3	59.3	68.6	72.1	77.4
FH	20.0	23.5	29.6	33.5	37.5	40.2	47.3	52.3	58.2	61.3
FL	20.0	24.5	28.6	32.5	37.8	41.2	48.3	51.3	55.6	59.3
W	20.0	23.8	27.6	33.6	39.5	43.2	49.3	57.3	66.1	73.2
GW	20.0	23.4	26.2	31.9	36.2	38.1	47.3	58.2	68.3	72.1

Table 4-5 Effect of amendments on dry weight of *Vetiveria* (mean± SD, n=3) grown in 'pot & pail' at the end of the third trial

	Dry Weight (g)	
Treatment	Above-ground part	Underground part
0.01 % Fulvic acid	4.84 ± 0.31	2.07 ± 0.67
0.1% Fulvic acid	5.19 ± 0.56	2.14 ± 0.37
0.01 % straw water	6.78 ± 0.97	3.11 ± 1.34
0.1% straw water	6.98 ± 1.15	3.46 ± 0.98
Groundwater No amendment	4.94 ± 0.68	1.95 ± 0.33
Tap water	5.85 ± 1.24	2.85 ± 0.24

4.3.3 Arsenic concentration analysis by ICP-MS in the third trial

The concentrations of arsenic in the roots and shoots were measured using the ICP-MS for the six treatments. Table 4-6 shows the arsenic concentration in the roots on the 3

October and 18 December 2012, with the highest As concentration recorded with addition of straw water (high concentration SH).

Arsenic concentration was measured every two weeks in shoots for the six treatments (Table 4-7, Figure 4-3, data in Appendix 7) the highest As concentrations recorded were in treatments with additions of straw water (high concentration SH).

Table 4-6 Arsenic concentrations in the roots at the beginning and end of the third trial

	As concentration (mg/kg)				As concentration (mg/kg)		
	Before[1] (03/10)	End[2] (18/12)	Increment		Before[1] (03/10)	End[2] (18/12)	Increment
SL-1	4.85	68.44	63.59	FL-1	4.71	84.99	80.27
SL-2	4.85	64.51	59.67	FL-2	4.96	83.64	78.69
SL-3	5.17	63.21	58.05	FL-3	4.78	79.32	74.54
SH-1	4.52	88.55	84.02	FH-1	4.40	65.32	60.93
SH-2	4.86	93.71	88.86	FH-2	4.61	62.50	57.89
SH-3	4.79	95.14	90.35	FH-3	4.49	61.03	56.54
GW-1	4.54	64.77	60.23	W-1	4.14	6.98	2.83
GW-2	4.38	60.63	56.26	W-2	4.46	5.69	1.23
GW-3	4.92	62.32	57.40	W-3	4.13	6.87	2.74

[1] Prior to addition of groundwater containing arsenic

[2] After final addition of groundwater containing arsenic

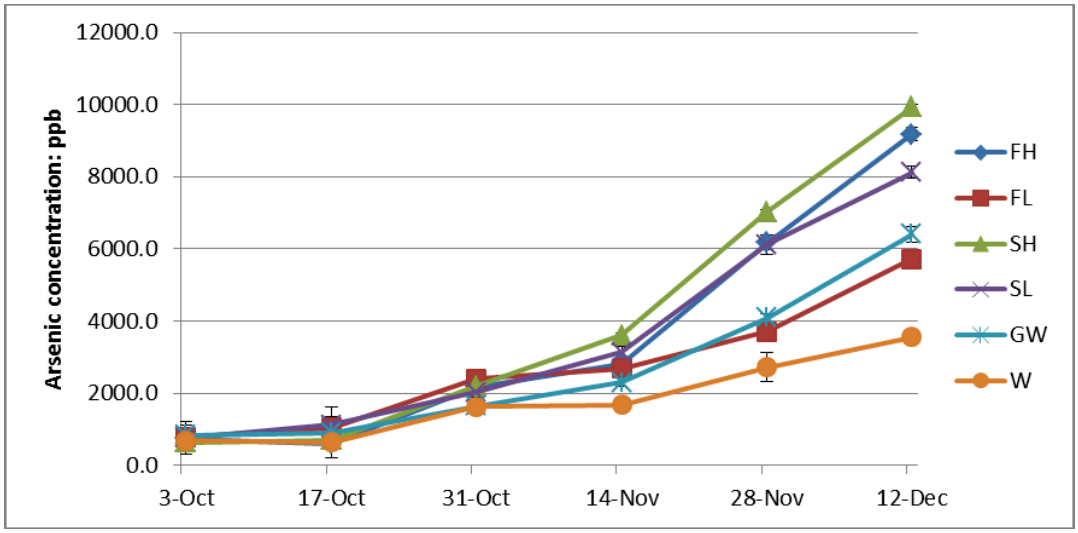

Figure 4-3 Mean arsenic concentration in the shoots in different treatments:

1) FH= high concentration fulvic acids;

2) FL= low concentration fulvic acids;

3) SH= high concentration straw water;

4) SL= low concentration straw water;

5) GW= control group 1 only groundwater;

6) W=control group 2 only tap water (n=3).

Table 4-7 The arsenic concentration in the shoot dry matter after the third trial (unit: mg/kg)

	3- October	17- Oct	31- Oct	14-Nov	28-Nov	12-Dec
FH	0.73 ± 0.05	0.61 ± 0.16	2.17 ± 0.23	2.82 ± 0.19	6.18 ± 0.89	9.18 ± 1.19
FL	0.78 ± 0.15	1.03 ± 0.28	2.39 ± 0.26	2.68 ± 0.18	3.72 ± 0.98	5.72 ± 2.24
SH	0.64 ± 0.32	0.70 ± 0.48	2.19 ± 0.28	3.61 ± 0.57	7.03 ± 1.07	9.93 ± 2.14
SL	0.76 ± .035	1.12 ± 0.49	2.03 ± 0.23	3.13 ± 0.77	6.13 ± 1.27	8.13 ± 3.17
GW	0.84 ± 0.36	0.90 ± 0.45	1.64 ± 0.23	2.30 ± 0.32	4.10 ± 1.12	6.40 ± 2.22
W	0.68 ± 0.16	0.64 ± 0.09	1.63 ± 0.15	1.68 ± 0.49	2.72 ± 1.39	1.56 ± 3.19

4.3.4 Comparison of the efficiencies of arsenic uptake

Using four different amendments (SH, SL, FH, FL), the average increments of arsenic concentration in the roots were recorded. The enhanced efficiencies of uptake arsenic from groundwater solution adding amendments by roots were calculated using Eq. 2,

$$\Delta E_r = \frac{C_1 - C_0}{C_C} \times 100\% \text{ (Eq. 2)}$$

The arsenic concentrations accumulated by the roots using different types of amendment and different concentrations of amendments are shown in Table 4-8.There was a 48% increase in arsenic uptake with the addition of straw water (high concentration SH) compared with the groundwater (GW control 1). There was only a 6.6% decrease in arsenic uptake with the addition of fulvic acid (high concentration FH) compared with the groundwater (GW control 1).

Table 4-8 The variation for arsenic uptake efficiencies

	SL	SH	FL	FH	GW (control 1)	W (control 2)
Mean increment (mg/kg)	65.39	92.47	77.84	58.45	62.57	2.27
Enhanced efficiency: (Eq. 2)	4.5%	47.8%	24.4%	- 6.6%	None	None

Chapter 5. Discussion

The discussion addresses four issues: the growth of *Vetiveria* in sandy soil under glasshouse conditions, weekly water use by the plants, the growth of roots and shoots in the 'pot & pail' situation, and effects of the two amendments on arsenic concentration in the plant tissue.

The plants used in this research were grown in sandy soil containing a low concentration of arsenic (2 ppm As) (Table 4-1). Sandy soils that have been strongly leached often have very low levels of exchangeable calcium and magnesium, thus the plants growth may be limited (Abbott, 1989). The arsenic concentration was not too high even for most plants (the toxic content of arsenic threshold to plant growth between 1 to 10 mg/kg) (Truong, 1999). Datta et al. (2011) found that the *V.zizanioides* threshold for arsenic (arsenite) was 225 mg/kg. This tolerance of high concentration arsenic allows *V.zizanioides* to grow well in the field soil sample with no symptoms of plant toxicity observed. Furthermore, one characteristic of sandy soil is that it consists of large particles with aeration and drainage. Plants were watered regularly in sandy soil to provide enough water for plant uptake and to maintain high soil moisture content.

5.1 Growth in glasshouse under controlled conditions

Environmental conditions such as soil content, temperature and humidity determine the efficiency of phytoremediation as the survival and growth of plants are adversely affected by extreme environmental conditions, toxicity and the general conditions of soil in contaminated lands (Danh et al., 2009).

For the underground parts, root growth was also impacted by daily air temperature, soil moisture content/soil type. Moreover, the optimum air temperature for root development is between 20°C to 25°C. However, soil temperature is another important factor for the root elongation (Wang, 2000). In general, the optimum temperature for *Vetiveria* growth is between 21°C to 29°C (Wang, 2000). In the current case study, plants were grown under glasshouse conditions (air temperature approximately 25°C).

In the first trial from 23 May to 2 October, the height of the plants increased rapidly for the first five weeks and then slowed down until reaching the maximum height of 145.8

cm (Figure 4-1). All the 20 plants grew well and no adverse symptoms were identified. These results are in agreement with those of Dudai et al. (2006) who found *Vetiveria* could grow in a wide range of susbtrates. Utilizing perennial plants to reduce soil arsenic to an acceptable level by phytoextraction is affected by plant above-ground biomass and regrowth capacity (Fayiga and Ma, 2006). The shoot growth rate obtained from the first trial implies that *Vetiveria* could grow well in the project site of EESI.

At the end of first trial, the longest root was 68.1 cm and the shortest is 43.6 cm. Truong (2000) found that *Vetiveria* has no stolons and an extensive roots system, often reaching 3 to 4 metres in depth. It is not an invasive species (Erskine, 1992). It seemed that the root elongation was restricted by the confined volume of the pot. This result is similar to that found by Roongtanakiat and Chairoj (2001). The restricted elongation of the roots in the pot maybe has been due to the plant root system's ability to adapt to the soil depth (Feddes and Raats, 2004).

The root growth rate was illustrated by the duration of the roots crossing of the 'nylon gauze' (Table 4-1). The average duration was 13 days. The fastest elongation was found in Plant No.1, which took only nine days to cross the 'nylon gauze'. However, the slowest was observed in Plant No. 6 and No.18, both of which took 16 days. Once the new roots dipped into the solution, they started to accumulate various elements directly from the bathing solution.

Table 4-2 shows the water volume consumed in the 'pot & pail' system in one week. The average water consumption was 202.4 gram per week for each 'pot & pail'. This water consumption includes the total water evaporation, plant growth and transpiration losses. This information from second trail determined the volume of water to be used for the third trial.

Figure 5-1 The roots developed new roots successfully crossing the 'nylon gauze' in the second trial

Figure 4-2 and Table 4-4 refer to chapter 4 show the *Vetiveria* growth in the third trial which includes the use of arsenic in aqueous solutions with amendments of straw water and fulvic acid at two concentrations (high 0.1% and low 0.01%).

There is substantial enhancement for the growth of the shoots by the straw water high concentration (SH) treatment and the mean height of the shoots reached 80.6 cm on 13 December. It was also observed in the straw water low concentration (SL) treatment that the straw water was able to enhance shoot growth, resulting in increased above-ground biomass. However, the two fulvic acid treatments inhibited shoot growth. At the end of third trial the mean shoot height in fulvic high concentration (FH) and fulvic low concentration (FL) were 61.3 cm and 59.3 cm respectively.

At the end of the third trial, the mean shoot height in the two control groups (arsenic contaminated groundwater GW and tap water W) was similar (72.1cm and 73.2 cm). The finding of inhibition of shoot elongation is similar to that of Rauthan and Schnitzer (1981), in which they found the fulvic acid could limit plant growth and uptake of nutrients when the arsenic concentration was above 1000 ppm.

The results show that the growth of *Vetiveria* was optimal in groundwater containing straw water at high concentration (SH). The least growth occurred in groundwater with

low concentration of fulvic acid (FL). The inhibition of the shoot elongation by fulvic acid is in agreement with Poapst et al. (1970), who found 500 to 4000 ppm fulvic acid can inhibit stem elongation in peas.

From Figure 4-2, all of the plants in the six groups grew at a similar rate. Also in the third trial for the six treatments there are no plant symptoms of toxicity observed such as red-brown necrotic spots on old leaves, yellow browning of roots and overall growth reduction (Cuypers et al., 2010).

Figure 5-2 Root system at the end of third trial. The green part is the exudate of root

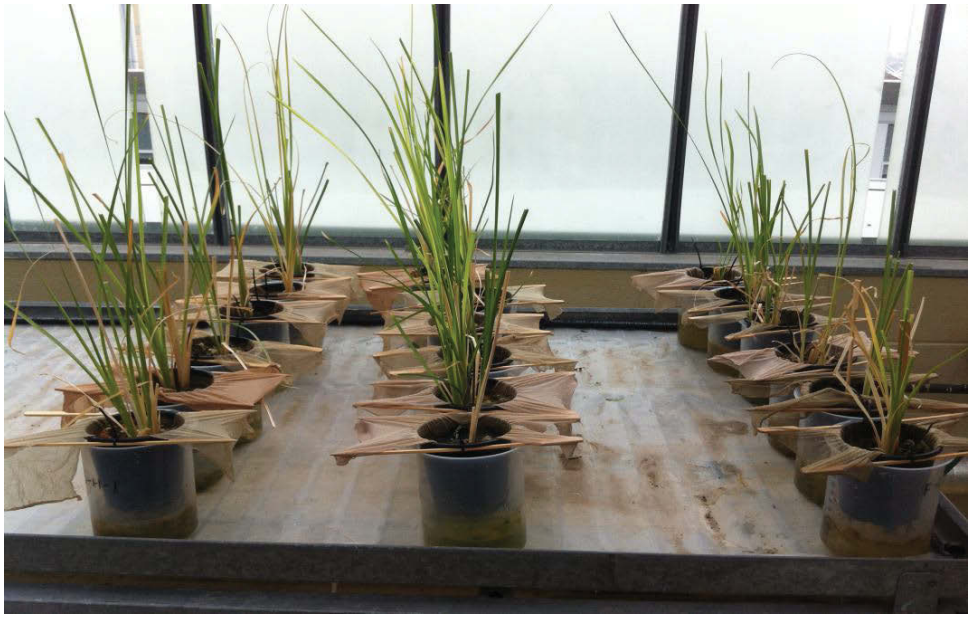

Figure 5-3 18 *Vetiveria* in the 'pot & pail' system in the third trial

The effects of amendments on plants dry weight (biomass) in the third trial are presented in Table 4-5. The best result occurred in the straw water high concentration (SH) treatment, in which the straw water not only improved the dry weight of shoots, but also enhanced the dry weight of roots. However, no substantial enhancement of dry weight was achieved by using either concentration of fulvic acid. Compared with the control 1(arsenic-contaminated groundwater) and the control 2 (tap water), *Vetiveria* had higher dry weight yield growing in tap water. One possible reason is that the tap water content more abundant nutrients. But more probably the arsenic may have suppressed the plant growth.

It should be noted that a fertilizer (Nitrosol) was used in the growing stage (first trial), as there was no access to the glasshouse to water the plants for 10 days. There was one application only of fertilizer per pot required.

5.2 Effect of fulvic acid and straw water

The 18 *Vetiveria* plants used in the first trial were grown in sandy soil with 2 ppm As from May to September. The rooted shoots also planted in the 'pot & pail' in sandy soil in the second trial had already accumulated arsenic from the soil. Table 4-6 (in the column of 'before') shows the amount of arsenic accumulated in the roots of *Vetiveria*

in the first two trials. These data were used as the initial root arsenic concentration for the third trial. The arsenic concentration accumulated in the roots is 4 mg/kg to 5 mg/kg dry weight. The arsenic concentration in the shoots was analysed by ICP-MS on 3 October. These results showed arsenic translocation from under-ground parts to above-ground parts by the plant's transpiration mechanism through the xylem.

The current theories for the translocation of metals plant roots to shoots propose that the responsible chelators are phytochelatins and organic acids, such as citric acid and malic acid, the latter translocating via the xylem (Senden et al., 2006). The predominant form in most water is inorganic arsenic which includes trivalent arsenic and pentavalent arsenic (Raab et al., 2007). In terms of inorganic arsenic translocation in plants, Pickering et al. (2000) explored pentavalent arsenic uptake mechanism, then claimed pentavalent arsenic uptake by plant mostly via phosphate transporters. Liu et al. (2004) argued trivalent arsenic is mainly taken up via glycerol transporters, which are independent of phosphate present.

Although Raab et al. (2007) argued that arsenic concentration in the roots was not related to arsenic concentration in the shoots after exposure to any of the arsenic species, heavy metals accumulated in the shoots can partially indicate the efficiency of a plant remediation potential for soil heavy metals (Chen et al., 2004). The arsenic concentration in the shoots of the 18 plants was <1 mg/kg (Table 4-7, Appendix 6). Thus, the root has a greater uptake capacity and accumulation capacity. This finding is similar to the study of Srisatit et al. (2003) which explored two speices (*V. zizanioides* and *V.nemoralis*) for 150 mg As/kg soil in a pot trial. They found that arsenic concentrations in the shoots only reached about 0.5 mg As/kg whereas 6 to 12 mg As/kg in the roots of the two species.

Solution pH values in the six treatments were recorded in the third trial (Table 4-3). The initial pH value in groundwater with addition of either straw water or fulvic acid was about 7.8. Adding an organic acid to the groundwater solution slightly decreased the pH values at the beginning (pH 8.3 in groundwater and 7.9 in tap water). There was no substantial difference between two concentration amendments (0.1% and 0.01%) in terms of lowering the pH value. This was because humic substances mainly act as pH buffers in the solution. At the end of the third trial, all the pH values of the six groups'

treatments were increased to approximately pH 9.0, even for the control group using tap water. The highest pH value at the end of third trial was found in the treatment of adding a high concentration of fulvic acid (FH) with pH of 10.4 in FH-1 treatment. This increase in pH is presumably due to the greater uptake of anions (such as NO_3^-) than cations by the plant, which induces the release of HCO_3^- from roots in order to maintain electrical neutrality (Riley and Barber, 1969). According to Smith and Brauning (1995), there is a relationship between arsenic mobility and an increase in pH.

During the ten weeks of third trial, groundwater (containing 5.5 ppm As) instead of tap water was introduced in the 'pot & pail' system. There were two arsenic sources in the third trial: one the sandy soil and the other was the groundwater. The plants in the 'pot & pail' trial could take up arsenic from both soil and groundwater with only one source of arsenic for the control group (tap water) i.e. sandy soil. From the column of increment in Table 4-6, it indicated that the addition of high concentration (0.1%) of straw water showed the greatest enhancement for roots accumulating arsenic. This result was also found in the treatment by adding low concentration (0.01%) of fulvic acid. However, there was no substantial difference among FH, SL and GW treatments for enhancing arsenic accumulated in roots.

In terms of enhanced arsenic accumulation efficiency (Table 4-8), SH achieved 47.8% increment in root's arsenic accumulation compared with control 1, followed by FL (24.4%) and SL (4.5%). However, FH decreased the arsenic accumulation efficiency by 6% compared with control 1. One possible reason for the enhancement by adding straw water (SH & SL) or fulvic acid (FL) is that the functional groups (such as phenolic acid) can be involved with arsenic speciation due to 1) possible redox reaction of arsenic; 2) organic matter coating on inorganic adsorbents and 3) aqueous complexation of arsenic species (Redman et al., 2002).

A high concentration (0.1%) fulvic acid addition enhanced arsenic accumulation in above-ground parts (Table 4-7). Similar results were found in the groups adding a low concentration or a high concentration of straw water. These results are in agreement with those of Cieśliński et al. (1998) and Nigam et al. (2001) who found organic acids had a positive effect on metal extraction by plants. The low concentration of fulvic acid increased arsenic accumulation in roots (Table 4-6), but no improvement was observed

for arsenic accumulation in shoots. The presence of free metal ions and the regulation of their availability and mobility in soil and aquatic environments is impacted on by the chelation of cations (Sanyal, 2001; Sinha and Bhattacharyya, 2011). Humic anions can compete with arsenic for sorption sites on mineral phases, such as iron oxides, preventing arsenic sorption or inducing arsenic desorption, both leading to a higher arsenic concentration in the aqueous phase (Grafe et al., 2002; Martin et al., 2009). Sinha and Bhattacharyya (2011) argue that there are various functional groups on humic substances such as carboxyl and phenolic OH groups, the acidic functional groups, have high complexation capacities with metal ions, enabling humic acid or fulvic acid to bind heavy metals. Evangelou et al. (2004) argue that humic acid or fulvic acid could also form an 'enhancer' through their functional groups, which is not resorbed by plants and delivers the heavy metals in a more available form to the exudate of the plants.

Figure 4-3 shows the variation of arsenic levels in shoots in the third trial. During the first six weeks there was no substantial accumulation in the shoots in all the six different treatments. After six weeks, arsenic accumulation rates in shoots increased markedly in three treatments (SH, FH and SL). It is noteworthy that there was a slight inhibition in the treatment with the low concentration of fulvic acid compared with the control group 1.

In addition to plant growth (biomass and height) and arsenic concentration accumulated in plant tissues, a bioconcentration factor (BF), defined as the ratio of arsenic concentration in the plant tissue to that in the soil, was used in a current study to characterize the effectiveness of plant arsenic accumulation (Ma et al., 2001).The amendments were not added to the soil but into the groundwater. Therefore in this study, BF should be correlated with arsenic concentration in plants and the arsenic concentration in groundwater. However, it was difficult to measure the arsenic concentration in the groundwater used in the 'pot& pail' system at the end of third trial because the solution (groundwater or amended groundwater) was continuously added into the pail in order to keep the water level constant at 200 ml in the pail. This led to various concentrations in different 'pot & pail' systems at the end of the trial. Therefore, the bioconcentration factor was not applicable in the current study.

According to Bing (1996), hydroponically grown terrestrial plants such as sunflower are frequently used for rhizofiltration, because they tend to have an extensive root system and greater biomass compared to aquatic species, resulting in a higher uptake capacity for metals. Sunflower is a typical plant for rhizofiltration treatment and has been used to grow in radioactively contaminated pools (Dushenkov et al., 1997).

In a follow-up study, *Vetiveria* will be planted in the field. The roots will elongate into the groundwater phase (1.5 metre depth). Then the *Vetiveria* will be used to undertake two tasks. The first is to remediate the groundwater from arsenic contamination; the second is to reduce the water table in order to decrease the potential risks created by contaminated water. The 'pot & pail' system indicated that *Vetiveria* can grow well and it also demonstrated it could be used to treat a field site. *Vetiveria* mainly accumulates arsenic from groundwater in the root, and the method used in this project is rhizofiltration which is a sub-section of phytoremediation. Salt et al. (1995) argued that plants that do not readily transfer contaminants to the stem or the leaves are also preferred, if removal of most of the accumulated metal can be accomplished by harvesting the roots alone. Consequently, *Vetiveria* can be used as a rhizofiltration treatment not only for the sandy soil but also for the groundwater. Therefore, it is feasible to use *Vetiveria* on the project site. Amending with straw water (0.1%) in the groundwater can maximize the efficiency of arsenic accumulation.

The best amendment for arsenic uptake by plants tested here is the high concentration of straw water (SH). SH not only improved plant growth, but also enhanced arsenic accumulation in both roots and shoots. Compared with the control group 1 (arsenic-contaminated groundwater), groundwater amended with a high concentration of fulvic acid enhanced arsenic accumulation in shoots and low concentration of fulvic acid enhanced arsenic accumulation in roots. Both of the two treatments had negative impacts on plant growth, especially for shoot elongation.

Chapter 6. Conclusions

6.1 Conclusions

Glasshouse laboratory experiments were conducted to investigate the growth of *Vetiveria* in sandy soil from May to September. *Vetiveria* was cultivated in the first trial indicating that it was able to survive as well as accumulate arsenic from soil. The maximum height of the shoots was 145.8 cm at the end of the first trial (5 months). During five months' cultivation, the arsenic concentration in roots was 4.0 to 5.0 mg/kg dry weight and 0.64 to 0.84 mg/kg dry weight in shoots. The solution pH values in all the 'pot & pail' units gradually increased to approximately 9.0. This is presumably due to a greater uptake by the plant of anions (such as NO_3^-) than cations.

The 'pot & pail' system was designed to demonstrate that *Vetiveria* could take up metals from both soil and water phases in this system in the practical situation of the field treatment. *Vetiveria* grew well in this system. Once the roots regrew into the solution, it can accumulate arsenic directly from groundwater.

The potential for using fulvic acid and straw water in the remediation of arsenic-contaminated groundwater by *Vetiveria* was examined. The results showed that the high concentration of straw water used had the most substantial enhancement of arsenic uptake from contaminated groundwater. The highest arsenic concentration roots could reach was about 95 mg/kg dry weight with the amendment of high concentration straw water. It reached about 10 ± 2 mg/kg in the shoots. Compared with the control group 1 (arsenic-contaminated groundwater), the high concentration straw water amendment and the low concentration straw water treatments were able to increase arsenic accumulation in roots by 47.8% and 4.5% respectively. This enhancement was also found by adding low concentration fulvic acid which could increase 24.4% arsenic accumulation in the roots. However, there was a negative effect on arsenic accumulation in the roots by addition of the high concentration fulvic acid. In addition, fulvic acid could inhibit *Vetiveria* growth especially for shoot elongation.

Furthermore, *Vetiveria* can transpire an average of 202.4 g water in the 'pot & pail' per week. This result could be a guide for EESI to determine the quantity of *Vetiveria* needed in practical situations to lower the groundwater.

Consequently, judging from the arsenic concentration in the different organs and plant growth performance, the high concentration straw water is recommended for future site treatment.

6.2 Recommendations for further research

During the process of conducting this research work, recommendations for future research emerged including:

- Factors influencing the uptake of arsenic from the groundwater, such as pH and redox potential (Eh), need to be investigated. The results of these experiments will allow us to gain better understanding of the interaction between solution and plant.

- Arsenic speciation should be investigated for the solution during the third trial. The arsenic forms within the plant tissues should also be analysed.

- There should be one more control groups in the third trial, in which sandy soil containing no arsenic is used to determine whether the plant exclude arsenic by its detoxification.

- Straw water and fulvic acid should be amended directly into soil in order to determine the effects of these two organic substances on arsenic uptake by *Vetiveria* in the soil phase.

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Appendices

Appendix 1 Biosafety Approval Form for Arsenic

UNIVERSITY OF TECHNOLOGY SYDNEY

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28 August 2012

Dr Pamela Hazelton
CB02.05.524
Faculty of Engineering and Information Technology
UNIVERSITY OF TECHNOLOGY, SYDNEY

Dear Pamela,

2012-11-R-C – P. Hazelton, G. Armstrong, Z. Zhao – "Determine the efficiency of As removal by photoremediation"

PROJECT DETAILS	
Location	CB04.07.38
Involves	Cytotoxins
Type of Dealing	N/A
Approval period	28/08/2012 to 28/08/2017

Thank you for your response to my email dated 21/08/12. Your response satisfactorily addresses the concerns and questions raised by the Committee, and I am pleased to inform you that ethics clearance is now granted.

Your clearance number is UTS BIOSAFETY REF NO. 2012-11-R-C

The approval is for the maximum permitted period of five years. An extension of the approval will be considered by the Committee at the end of that time should it be required.

Please also note that you are required to notify the Committee if you wish to make any changes to the procedures described in your application, location, or the staff working on it.

If you have any queries about your Biosafety approval, or require any amendments to your research in the future, please do not hesitate to contact the Ethics Secretariat at the Research and Innovation Office on 02 9514 9772.

Yours sincerely,

Production Note:
Signature removed prior to publication.

Dr Maurizio Labatte
UTS Biosafety Committee

THINK.CHANGE.DO

Appendix 2 Glasshouse Condition from May to December

Note: because the glasshouse is shut down in December, no data available for that month.

	Max Temperature(°C)	Min temperature(°C)	Average temperature(°C)	Mean Relative Humidity (RH :%)
May	31.9	19.0	24.7	31.5
June	31.9	15.1	23.7	47.9
July	31.7	10.5	18.4	58.1
August	32.1	10.8	22.7	42.1
September	32.6	18.4	25.7	43.0
October	35.3	19.4	25.8	49.9
November	35.3	20.3	25.7	55.0

Comment: Relative humidity refers to the ratio of the partial pressure of water vapor present to the saturated pressure of water vapor at a given temperature.

Appendix 3 Selected Major Procedure and Instruments of EPA3050 method

Instruments:

1. Digestion Vessels - 250-mL
2. Vapor recovery (Figure 3-10)
3. Appropriate solvent handling system
4. Drying ovens - able to maintain 30EC + 4EC
   Temperature measurement device capable of measuring to at least 125EC with
5. Filter paper - Whatman No. 41 (Figure 3-10)
6. Centrifuge and centrifuge tubes
7. Analytical balance - capable of accurate weighings to 0.01 g (Figure 3-11)
8. Heating source - Adjustable and able to maintain a temperature of 90-95EC
9. Graduated cylinder
10. Volumetric Flasks - 100-mL

Procedures:

1. For each digestion procedure, weigh to the shoots part or roots part nearest 0.1 g (wet weight). Oven dries the samples at 105°C for 24 hours. Transfer to a digestion vessel;
2. Add 5 mL of 70% HNO_3. Heat the sample to 95EC ± 5EC and reflux for 10 to 15 minutes without boiling. Allow the sample to cool, add 5 mL of concentrated HNO_3, replace the cover, and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by HNO_3, repeat this step (addition of 5 mL of conc. HNO_3) over and over until no brown fumes are given off by the sample indicating the complete reaction with HNO_3 . Using a ribbed watch glass or vapour recovery system, either allows the solution to evaporate to approximately 1 mL without boiling or heat at 95EC ± 5EC without boiling for two hours;
3. After cooling, dilute to 50 mL with MilliQ water. Particulates in the digestate should then be removed by filtration; by centrifugation Centrifugation at 2,000-3,000 rpm for 10 minutes is usually sufficient to clear the supernatant. Then the samples are ready for ICP-MS analysis.

Appendix 4 *Vetiveria* Shoots Growth rate in FT (unit: cm)

Note: no records during June 10th to June 24th due to the temporary shutdown of lab system.

No. / Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
23/05	23.9	22.3	24.3	21.9	24.7	24.1	24.7	23.6	26.2	25.2	22.6	22.0	24.4	22.2	25.9	23.5	23.3	26.3	25.3	22.5
30/05	26.2	22.9	27.1	23.7	27.8	27.0	27.8	30.1	26.6	27.1	25.1	22.7	27.5	26.1	26.6	25.1	31.0	27.2	27.6	25.9
05/06	35.5	37.4	35.4	36.8	36.1	33.5	37.0	35.7	35.5	33.6	34.9	37.5	36.2	37.0	34.6	35.3	37.0	35.4	33.8	34.0
12/06	43.7	40.8	45.8	46.1	46.6	42.2	45.8	45.7	45.4	43.1	45.4	44.6	44.0	43.3	40.7	43.3	45.3	43.7	42.9	40.5
19/06	57.7	50.5	47.8	57.4	47.1	55.4	46.4	47.5	47.4	57.2	45.6	52.5	48.6	47.4	49.4	47.3	58.0	48.7	57.7	49.8
26/06	73.3	73.6	70.1	74.9	73.5	74.4	70.1	73.6	52.6	72.7	71.8	72.5	71.0	53.5	73.9	70.7	74.4	73.3	71.2	61.0
03/07	77.4	76.7	73.1	75.5	74.9	75.9	77.0	77.6	52.7	76.4	72.8	76.5	76.4	54.0	75.3	75.5	74.4	75.3	77.3	74.2
10/07	78.6	78.1	76.2	76.1	80.2	77.4	78.4	78.5	58.6	77.7	77.8	76.6	80.0	60.3	76.6	76.7	77.0	76.1	78.1	76.8
24/07	78.6	78.1	76.2	76.1	80.2	77.4	78.4	78.5	58.6	77.7	77.8	76.6	80.0	60.3	76.6	76.7	77.0	76.1	78.1	76.8
31/07	92.7	93.3	91.3	92.0	93.4	91.8	89.2	90.8	69.0	90.5	81.7	92.4	93.4	71.1	88.3	89.6	93.9	82.2	92.0	82.8
07/08	96.0	95.3	96.4	98.1	94.2	96.2	94.7	98.8	76.1	96.5	96.8	94.2	98.5	77.8	96.3	97.1	94.2	95.8	95.2	97.5
14/08	98.7	102.7	98.8	101.8	102.1	100.9	95.6	105.1	81.0	99.3	101.2	98.6	100.4	83.3	100.2	100.0	103.6	101.0	99.8	102.7
21/08	106.5	109.2	100.0	105.3	106.5	105.7	105.8	113.1	85.4	101.6	106.9	106.2	104.1	85.5	105.9	106.7	105.4	104.2	105.4	105.2
28/08	110.2	110.5	102.3	110.8	108.6	111.6	110.5	116.7	87.6	108.2	109.8	110.5	109.4	92.4	112.2	107.8	107.8	109.1	109.4	111.5
04/09	115.0	113.9	103.2	116.7	113.8	114.9	118.9	117.7	95.3	114.9	117.2	117.7	117.3	95.8	115.1	117.3	118.6	114.6	115.1	116.5
11/09	119.5	122.6	109.3	120.2	121.4	122.2	119.9	123.0	100.5	119.6	122.8	118.4	121.3	100.8	120.0	120.1	119.7	122.5	124.0	119.1
18/09	127.8	123.0	112.0	122.8	123.1	122.5	123.0	123.8	103.0	122.1	123.3	122.1	123.7	101.2	120.4	131.4	123.2	122.9	124.2	121.0
25/09	133.8	123.3	113.9	125.0	123.7	130.4	124.5	125.5	104.9	125.4	125.1	124.0	124.1	104.3	123.3	135.5	123.5	123.6	125.3	133.5
02/10	135.2	126.9	116.1	126.7	126.1	138.5	134.8	135.9	111.2	135.9	127.2	137.9	138.4	105.6	123.8	145.8	124.2	128.5	135.9	137.7

Appendix 5 *Vetiveria* Roots Variation before and after FT (cm)

No.	Before	End
1	18.2	45.8
2	16.9	49.3
3	20.3	59.1
4	19.3	47.6
5	22.3	55.2
6	17.4	43.6
7	15.6	57.6
8	19.6	42.3
9	19.4	45.8
10	12.6	55.3
11	15.6	58.3
12	18.5	50.2
13	18.6	47.6
14	17.9	61.2
15	19.7	56.3
16	24.8	68.1
17	20.6	60.7
18	19.1	47.4
19	16.3	46.8
20	16.7	49.2
Average.	18.5	52.4

Appendix 6 *Vetiveria* Shoots Growth Rate in the Third trial (unit: cm)

Date / No.	03/10	10/10	17/10	24/10	31/10	07/11	14/11	21/11	28/11	04/12	11/12
SL1	20	28.1	36.5	42.5	47.4	55.3	62.1	64.6	72.0	81.4	84.0
SL2	20	30.0	38.4	42.6	49.0	56.9	62.0	66.2	76.3	79.1	82.1
SL3	20	29.8	36.6	47.8	51.2	50.7	56.8	70.8	74.6	81.3	81.1
SH1	20	29.1	38.3	39.3	50.0	48.3	57.3	71.0	74.2	79.0	78.3
SH2	20	27.3	37.0	39.4	45.3	48.9	61.8	70.1	72.2	77.0	82.6
SH3	20	26.4	36.3	45.2	46.3	53.7	58.7	64.7	69.9	76.2	82.4
FL1	20	23.5	27.7	32.1	39.5	39.5	46.6	53.4	58.7	61.6	64.0
FL2	20	25.3	30.1	32.1	35.9	40.5	49.1	53.3	60.0	62.9	65.1
FL3	20	21.7	31.0	36.3	37.1	40.6	46.1	50.2	55.9	59.4	60.4
FH1	20	22.5	30.1	31.8	37.2	43.0	45.8	49.5	55.0	56.5	65.4
FH2	20	23.3	30.3	31.5	38.5	39.3	50.5	49.4	56.7	57.9	61.0
FH3	20	26.2	25.4	34.2	37.8	41.3	48.6	55.1	55.1	63.5	61.8
GW1	20	18.7	29.3	34.5	38.0	43.8	49.8	56.7	67.2	74.9	79.5
GW2	20	21.9	27.2	34.3	38.9	42.9	51.2	55.0	66.1	72.7	78.2
GW3	20	19.4	26.3	32.0	41.7	42.9	47.6	60.2	65.0	71.9	77.6
W1	20	24.3	25.6	30.0	38.5	40.1	49.8	60.5	68.6	74.2	77.7
W2	20	24.5	27.1	33.7	38.4	39.0	48.4	56.2	70.7	73.2	77.4
W3	20	21.4	25.9	32.0	31.6	35.2	43.7	57.8	65.5	68.8	80.8

Appendix 7 Arsenic Concentration Variation in Shoots during Third trial (mg/kg; ppm)

Date / No.	03/10	17/10	31/10	14/11	28/11	12/12
SL1	0.78	1.17	2.09	3.21	6.31	8.05
SL2	0.80	1.12	2.02	3.08	6.20	7.88
SL3	0.70	1.07	1.98	3.10	5.87	8.46
SH1	0.67	0.73	2.21	3.63	6.91	9.69
SH2	0.68	0.70	2.12	3.65	6.97	10.03
SH3	0.58	0.67	2.23	3.54	7.21	10.08
FL1	0.75	1.01	2.36	2.76	3.84	5.52
FL2	0.80	1.08	2.37	2.71	3.85	5.78
FL3	0.79	1.01	2.43	2.57	3.47	5.85
FH1	0.69	0.65	2.21	2.75	6.11	9.31
FH2	0.77	0.57	2.16	2.77	6.04	9.18
FH3	0.73	0.53	2.14	2.94	6.39	9.05
GW1	0.82	0.85	1.66	2.24	4.09	6.44
GW2	0.88	0.90	1.67	2.23	3.90	6.33
GW3	0.82	0.95	1.59	2.43	4.31	6.43
W1	0.66	0.60	1.58	1.72	2.74	3.54
W2	0.73	0.66	1.60	1.69	2.72	3.72
W3	0.65	0.66	1.71	1.62	2.70	3.41

Appendix 8 Straw Water Analyses

Type of Samples: Solutions

Analysis Sample No(s)	Phenolic acids (mg/L)
Day 0	14
Day 8	115
Day 15	47
Day 22	83
Day 29	86
Day 37	110
Day 45	220
Day 50	225
Day 58	220