

Mycorrhiza and Biochar for Remediation and Plant Production in Soils Polluted with Arsenic

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Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is currently not being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been fully acknowledged in this thesis.



Sahar Al-Shamma

Dedication

I dedicate my thesis to

The spirit of my father

and

My mother, brother, sisters, their sons and daughters,

and friends : my support in life

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Abstract

Arsenic (As) negatively affects the soil, and at high concentrations can cause biomass decrease, inhibition of photosynthesis and death of plants. Arsenic can enter the food chain via plant uptake and can be harmful to human health. Biological As-remediation is a process of using soil organisms or natural materials to reduce As concentration in soil and As toxicity in plants. Arbuscular mycorrhizal fungi (AMF) have a known role in enhancing plant growth and minimising effects of toxins. Magnetic biochar has recently gained interest for its capacity to adsorb pollutants, however research on its influence on accumulation of contaminants by plants and plant growth in contaminated soils is limited. Mycorrhizal fungi from different sources were tested for remediation of As in soil in two experiments. Commercial mycorrhizal inoculum was used to inoculate 8 species of vegetable plants (tomato, bean, capsicum, eggplant, lettuce, maize, okra and spinach) to study the effects on growth and on arsenic concentration and uptake in field contaminated soil. The plants were found to range in sensitivity to As. Arsenic reduced the growth of all species and the commercial mycorrhizal inoculum failed to improve the growth in contaminated soil. In another experiment mycorrhizal communities from contaminated and uncontaminated soil were propagated in pot culture and applied to maize in soil spiked with rates of arsenate from 0-75 mg kg⁻¹. Both sources of mycorrhiza reduced arsenic concentration and uptake of maize, but inoculum from uncontaminated soil resulted in arsenic concentration and uptake that were half of those in plants inoculated with mycorrhiza from contaminated soil, although there was little difference in growth. Magnetic biochar, made from steam-activated coconut husk biochar and iron precipitate, and untreated biochar were applied to

contaminated soil ($156 \text{ mg kg}^{-1} \text{ As}$), to determine effect on maize growth. The magnetic biochar adsorbed 3.8 times as much As per weight as raw biochar. Magnetic biochar reduced As concentration in shoot tissues by 42% and increased shoot dry weight by 40%. Raw biochar increased arsenic concentration in tissues and reduced shoot dry weight in contaminated soil. However, magnetic biochar reduced plant growth in uncontaminated soil, possibly due to excess iron. This study showed that, despite expectations, mycorrhiza from uncontaminated sites could be more effective for remediation than mycorrhiza from contaminated soils. It also showed for the first time that magnetic biochar can be used to remediate soil polluted with As. Mycorrhiza and magnetic biochar have potential roles in risk management for contaminated soil and in reducing arsenic concentration and uptake in plants, but further work is needed to improve methods of application.

1 General Introduction

1.1 Project overview

Environmental contamination by organic and inorganic pollutants has noticeably increased in recent years, due to the population expanding and to the different human activities (Chaudhry et al., 1998; Mandal & Suzuki, 2002). Some pollutants negatively affect plants and their growth, causing decrease in biomass and inhibition of photosynthesis (Gutierrez-Gines et al., 2012; Panyakhan et al., 2006). Pollutants also affect the soil, causing degradation in its structure and fertility (Ben-Moshe et al., 2013). Heavy metals such as Pb, Cr, As, Cu, Cd, and Hg, added to our soils through industry, agriculture and domestic sewage, persist in soils and can either be adsorbed in soil particles or leached into ground water. Human exposure to these metals through ingestion of contaminated food or uptake of drinking water can lead to their accumulation (Davamani et al., 2010).

Arsenic (As) pollution has become a serious environmental problem in many countries especially in Bangladesh, Vietnam, and India (Meharg, 2004). This metalloid element is now recognized as a serious threat to human health. It is a known human carcinogen and causes excess pigmentation and thickening of the skin (Singh & Ma, 2007; Smith et al., 2010). Arsenic is naturally occurring, but can be present on some sites at very high concentration as a result of human activities such as mining industry and the use of agricultural products (pesticides and herbicides) (Frankenberger & Arshad, 2002). In Iraq, farmers have used pesticides and

herbicides intensively in agriculture, and that has caused pollution of the soil and drinking water, which leads to transfer of the pollutants to plants then to humans through food chain (personal observation). On other hand, arsenic is found in Australia in mining areas (Ashley & Lottermoser, 1999), and there is concern too about pollution of the soil and plants growing in the areas near these mines and effects on health of people working and living within these areas. There is a need to develop simple technologies that alleviate arsenic toxicity in crops and soil, and find a biologically safe options for remediation of contaminated soils to mitigate the harmful effects on humans and our environments.

In this thesis I examine the efficacy of mycorrhiza and biochar in the biological remediation of soils contaminated with arsenic. Arbuscular Mycorrhizal Fungi (AMF) are known to improve plant growth on nutrient-poor soils and enhance uptake of P, Cu, Ni, Pb and Zn (Davamani et al., 2010; Gutierrez-Gines et al., 2012). Mycorrhizas have an important role in the biological remediation of contaminated soils. There is evidence that AMF can withstand potentially toxic elements and can work as a filtration barrier to reduce transfer of heavy metals to plant shoots (Davamani et al., 2010). Although there are few studies on mycorrhiza and its relation with arsenic-polluted soils, some research has suggested that AMF may have a role in arsenic tolerance of host plants (Smith et al., 2010).

Biochar is the product of heating a biomass in the absence of oxygen (Lehmann et al., 2011). It is found to be biochemically inert and potentially promotes the long-term soil carbon pool compared to uncharred organic matter (Lehmann et al., 2006). It can also improve soil structure, soil fertility and soil microbial activity if added to

the soil (Lehmann et al., 2011). Recently people found that biochar has the ability to alleviate the effects of pollutants in the soil due its sorption surface area (Beesley et al., 2011). Organic matter fertilizer is used often in farms and as a compost. These raw materials for biochar production are available at cheap prices, so biochar could be a suitable low cost soil treatment. For this reason biochar was chosen as a biological material in this study.

1.2 Objectives

This thesis includes several experiments that were conducted to investigate the effect of using mycorrhiza and biochar to mitigate the effects of arsenic in As-contaminated soil and in soil spiked with arsenic, and to examine effects on plant growth. The form of As used was arsenate [As(v), AsO_4^{3-}] as inorganic As pollutant because this form is most dominant in soils and plants take up As mostly as arsenate (Singh & Ma, 2007). Maize plants were used as an indicator to study the effect of arsenic on the plant and on As plant concentration and accumulation. It was found that maize survived sufficiently in As-contaminated soil to enable this. Experiments on mycorrhiza used different sources (commercial mycorrhiza, inoculum from contaminated and uncontaminated soils). Activated biochar was also modified with iron precipitates to increase As adsorption, based on recently published work on removing As from water (Sun et al., 2015).

The overall aim of this thesis work was to compare the use of mycorrhiza and modified biochars as simple and environmentally friendly methods to remediate arsenic pollution.

1.3 Structure of the thesis

The Literature Review (Chapter 2) discusses information on arsenic pollution, mycorrhiza and biochar and their benefits, as well as highlighting specific research relevant to the use of each for arsenic remediation.

The experiment in Chapter 3 tested the growth of 8 crops with and without commercial inoculum of mycorrhiza in As-contaminated soil and in similar uncontaminated soil. In addition, it included a comparison of mycorrhizal communities in the two soils.

Chapter 4 describes an experiment that tested the efficacy of mycorrhizal inoculum from As-contaminated soil and from uncontaminated soil on maize growth and on arsenic concentration, uptake and translocation in soil spiked with different rates of arsenic.

The experiment in Chapter 5 was based on the hypothesis that magnetic biochar has a high sorption area for arsenic in solution and in the soil, and it promotes the growth of plant. In this chapter, a magnetic biochar was manufactured from an activated biochar modified with iron precipitate. The capacity of magnetic biochar, iron precipitate, and biochar to adsorb As were compared. The effects of the materials on

the growth of maize in As-contaminated soil and uncontaminated soil were tested. Magnetic biochar has not previously been tested for its effect on growth when applied to contaminated soil.

The general discussion Chapter 6 integrates the key findings from each chapter and identified the gaps in knowledge and future research needs arising out of this study.

2 Literature Review

2.1 Introduction

Arsenic (As) pollution has become a big concern in many countries due its harmful effects on humans, soil and plants. Many trials have used different techniques of remediation, all aiming to alleviate the harmful effects of As. Biological remediation is one of these processes which uses soil organisms or natural material. This chapter will review previous work on As contamination and remediation, with a focus on two natural techniques (mycorrhiza and biochar). Other aspects directly relevant to the project will be reviewed and opportunities for research identified.

2.2 Arsenic overview

2.2.1 Arsenic and humans

Arsenic (As) is an environmental toxin that is found naturally in all soil. Arsenic enters our environment through natural sources (wind, volcano emissions, weathering etc.) and through human activities (pesticides, disposal of domestic waste, mining, manufacturing etc.) (Mandal & Suzuki, 2002; Smith et al., 1998). In Australia the main sources of As are mining and metal manufacturing (Ashley & Lottermoser, 1999). Arsenic contamination has become a global issue with many countries impacted such as West Bengal (India), Bangladesh, Mexico, Vietnam and China (Meharg A. A. & Jeanette Hartley-Whitaker, 2002; Meharg, 2004). People in these countries are at risk from consuming water contaminated with As or crops grown in As contaminated media (Singh & Ma, 2007).

Arsenic is a toxic metalloid that can be harmful to health. It is a human carcinogen, as well as causing disease (Ferguson & Gavis, 1972; Ortowska et al., 2012). High exposure to As causes a variety of harm such as dermal changes (pigmentation, hyperkeratosis, and ulceration), respiratory, pulmonary, cardiovascular, gastrointestinal, hematological, hepatic, renal, neurological, developmental, reproductive, immunologic, genotoxic, and mutagenic effects (Mandal & Suzuki, 2002).

Arsenic contamination of the human food chain is a world –wide concern (Finnegan & Chen, 2012). Although fruits and vegetables contain organic As, less than 10% of As in these foods exists in the inorganic form. As content of many foods such as milk and cereals, beef and poultry are mainly inorganic, typically 65-75%, and a recent study reported 85-95% inorganic arsenic in rice and vegetables (Mandal & Suzuki, 2002). Rice grain is one example of an important crops in the human food chain found to have high concentration of As in samples from some countries such as China and Bangladesh. Rice with arsenic levels of 1.8 mg kg^{-1} contribute approximately 30% of dietary arsenic intake in Asian diets (Meharg, 2004).

2.2.2 Arsenic in soil

Arsenic pollutes the soil and ground water and can be transferred from soil to plants and then to animals and humans through the food chain. Arsenic concentration varies from below 10 mg kg^{-1} in non-contaminated soils to $30,000 \text{ mg kg}^{-1}$ in contaminated soil (Garg & Singla, 2011). Uncontaminated soils usually contain $1-40 \text{ mg kg}^{-1}$ with lowest concentrations in sandy soils (Mahimairaja et al., 2005; Mandal & Suzuki, 2002), while in some areas As reached up to 9300 mg kg^{-1} in a strongly contaminated soil (Ashley &

Lottermoser, 1999). Arsenic concentration reached 16,000 and 21,000 mg kg⁻¹ in mine tailings area in Thailand (Visoottivisetha et al., 2002). The permissible limit of As in agricultural soils is 20 mg kg⁻¹ (Garg & Singla, 2011). The World Health Organization recommends As concentration at maximum 10 µg L⁻¹ for drinking water (Qafoku et al., 1999). However, the recommended maximum concentration in many countries, including Bangladesh and the United States, is still 50 µg As L⁻¹.

Arsenic occurs mainly as inorganic species As(III) and As(V) and as organic forms (Mahimairaja et al., 2005; Mandal & Suzuki, 2002). Arsenite As (III) is more toxic than As (V) and relatively mobile in contaminated soils, whereas arsenate As (V) is relatively less toxic. In contaminated soils, under oxidizing conditions generally As (V) dominates over As (III) and is stable and strongly sorbed onto clay, iron and manganese oxides /hydroxides and organic matters (Mahimairaja et al., 2005; Mandal & Suzuki, 2002) and is also strongly associated with Al, Ca, Mg and Ni (Wenzel et al., 2001). Under reducing conditions As (III) is the predominant As compound (Mandal & Suzuki, 2002).

There are some factors that affect soluble As concentration in the soil such as soil particle size, redox conditions, pH, type and nature of constituent minerals, biological activity, and adsorption reactions (Chiu & Hering, 2000; Smith et al., 1998). Phosphate addition and soil pH are the most important factors that control the adsorption of As. Part of the As adsorbed onto soil constituents is desorbed and released into the soil solution (Qafoku et al., 1999). Arsenic is subject to both chemical and biological transformations in soils, resulting in the formation of various species.

Both As and P are related to the same chemical group and they have some similar properties, therefore H_2AsO_4^- and H_2PO_4^- compete for sorption sites in the soil (Smith et al., 1998).

The pH as well affects the existence of As in soil. The soluble humic substances reduce the availability of As in soil at specific pH. The adsorption of As on humic acids depends on the pH. (Thanabalasingam & Pickering, 1986) found that the highest adsorption of As (V) on humic acids happened about pH 5.5 while the adsorption of As (III) on humic acid was maximum at pH 8. In general, adsorption of As (V) decreases with increasing pH. In contrast, adsorption of As (III) increases with increasing pH (Mahimairaja et al., 2005)

Arsenic concentration in the soil and under-ground water is affected by the type of soil. Arsenic is retained for a long time in clay and fine-texture soil. It is adsorbed onto the clay and organic particles and combines with the available minerals that usually dominate in this type of soil such as Fe and Al to form arsenic compounds. The leaching of As in this soil is very low (Sharma & Kappler, 2011; Sheppard, 1992).

Soil microorganisms show wide variation in resistance to arsenic. Some microorganisms contribute to transfer of As in soil and water through the redox process, oxidation of As (III) to As (V) or reduction of As (V) to As (III) (Mukhopadhyay et al., 2002). (Philips & Taylor, 1976) found *Alcaligenes faecalis* (a species of gram-negative, rod-shaped bacteria commonly found in soil) resistant to the toxic effects of 0.01 M sodium arsenite, was isolated from raw sewage and shown to be capable of oxidizing arsenite to arsenate. In addition microorganisms can also accumulate As in their tissues (Ferguson & Gavis, 1972; Singh et al., 2015). (Singh et al., 2015) analyzed physicochemical characteristics of

contaminated paddy soil, and 3 bacterial isolates amongst 11 were screened and selected for their study. Of these, *Lysinibacillus* sp. strain SS11 displayed arsenic tolerance of 3256 mg L⁻¹ for arsenate and 1136 mg L⁻¹ for arsenite. Additionally, it showed bioaccumulation capacity of 23.43 mg L⁻¹ for arsenate and 5.65 mg L⁻¹ for arsenite. Also, some microorganisms can release arsine gas via the release of organic methyl arsenic compounds (Mukhopadhyay et al., 2002). Fungi dominate the microbes that produce volatile, garlic-smelling trimethylarsine (Craig, 1989), although bacteria and animal tissues also have this potential (Aposhian, 1997). On other hand, a high concentration can negatively affect the microbial community. As (III) is more toxic than As (V) to the soil microorganisms, while fungi have higher tolerance to both As (III) and As (V) than bacteria (Maliszewska et al., 1985; Tabatabai, 1977). (Sharples et al., 1999) found EC50s for arsenate, based on growth inhibition, for the endomycorrhizal fungus *Hymenoscyphus ericae* and the ectomycorrhizal fungus *Hebeloma crustuliniforme* to be 99.6 mg As (V) L⁻¹ (1.33 mol m⁻³) and 24.7 mg As (V) L⁻¹ (0.33 mol m⁻³) respectively. The presence of phosphate (0.01 mol m⁻³) in the media ameliorated the toxic effects of arsenate.

(Burton, 1987) collected heterotrophic bacteria from a variety of contaminated sites and found that < 0.21% were resistant to arsenite at 750 mg As (III) L⁻¹ (10 mmol L⁻¹). Resistance to arsenite was much lower than that reported for selenite (54%) at the same sites (WHO., 2001).

2.2.3 Arsenic in plants

Arsenic is a toxic element while phosphorus is essential for plants. They are both group V_A elements. Therefore arsenate competes with phosphate for soil-sorption sites. In soil competition between arsenate and phosphate for soil-sorption results in a reduction in their sorption by soil and increased concentration in soil solution (Smith et al., 2002). Such

competition may help to alleviate arsenate toxicity and improve phosphate nutrition (Sneller et al., 1999). Arsenate competition with phosphate for the phosphate uptake system has been observed in many organisms, including plants and fungi (Beever & Burns, 1981; Singh & Ma, 2007).

Arsenic contamination affects both plants and animals especially at high concentration. Arsenic concentration of 25 mg kg^{-1} in soil is a threshold value which over the element causes some toxic change in plants (Miteva, 2002). Some studies related to As toxicity to the plant found that plant growth is stimulated at low As concentration (Carbonell-Barrachina et al., 1997; Garg & Singla, 2011; Miteva, 2002). (Miteva, 2002) studied the changes of growth and pigment content in tomato cultivated in soil spiked with As in different concentrations (15, 25, 50 and 100 mg kg^{-1}). He found that As concentration of 15 and 25 mg kg^{-1} stimulated synthesis of pigments and applied stress, while 50 and 100 mg kg^{-1} decrease the growth of both the vegetative and root system. While (Sheppard, 1992) found the threshold for toxicity is 40 mg kg^{-1} (Sheppard, 1992), but 5 mg kg^{-1} As in soil was found toxic to sensitive crops (Garg & Singla, 2011). (Yoon et al., 2015) studied the effects of inorganic and organic arsenic on the germination and seedling growth of 10 crop plants to explain the relationship between toxicity and the arsenic chemical states in two types of soils. They found that mung bean was the most sensitive species to the arsenic compounds with an EC50 value of 11 (9–15) mg kg^{-1} to As (III), 21 (13–33) mg kg^{-1} to As (V), and 8 (5–13) mg kg^{-1} to DMA. The sensitivity of the tested plant species to As (III) was mung bean > pea > cucumber > wheat > kale > barley > sorghum and to As (V) was mung bean > pea > cucumber > wheat.

Arsenic may accumulate in plant tissues and at high concentration will prevent metabolism and stop growth, leading to the death of the plant (Marques & Anderson, 1986; Tu & Ma, 2002). Toxicity may result from the binding of metals to SH groups in proteins, leading to inhibition of activity or disruption of structure, or from displacing of an essential element resulting in deficiency effects (Delnomdedieu et al., 1994; Van Assche & Clijsters, 1990). As well arsenic can reduce crop production (Carbonell-Barrachina et al., 1997).

Arsenic uptake in plants is influenced by many factors including As concentration and forms in the soil, plant species (Singh & Ma, 2007), the presence of other ions (Khattak et al., 1991), and soil properties (Akkari et al., 1986). Plant uptake is affected by the high concentration of As but also on its species because As (III) is more soluble and mobile than As (V) (Marin et al., 1992). The accumulation in edible parts commonly is low, as some plants restrict the As uptake by roots while some others prevent translocation of the As from roots to shoots, or it may be that bioavailability in soil is low (Mahimairaja et al., 2005).

In general the highest concentration of absorbed As in most plants growing in As-contaminated soil is found in roots, while it is lower in leaves and stems with the lowest levels found in fruit and seeds (Carbonell- Barrachina et al., 1995; Carbonell-Barrachina et al., 1997). A range of 10 to 50 times higher As accumulation in roots than shoots has been reported for some annuals or perennials, including lentil, maize tomato and white clover (Ahmed et al., 2006; Ultra et al., 2007a; Wang et al., 2008; Yu et al., 2009; Zhao et al., 2009). However, As accumulation is higher in shoots than roots for a number of ferns (Gonzalez-Chavez et al., 2002; Zhao et al., 2009). The relative distribution of As in the plant may be related to whether the plant is tolerant to As or nontolerant (Singh & Ma,

2007). (Meharg & Macnair, 1991) showed in *Holcus lanatus* that in tolerant plants about 75% of the As was transported to the shoots, but only 50% in nontolerant plants.

2.2.4 Remediation of arsenic in contaminated soil

Remediation is the process of removal of contamination from soil. There are three processes to remediate metalloids such as arsenic which may remove the arsenic partly or completely: physical, chemical and biological processes (Mahimairaja et al., 2005). All these processes aim to reduce the harmful effects of As and its bioavailability. The physical processes include containment, capping, soil washing (Tuin & Tels, 1991), soil mixing and solidification (Mahimairaja et al., 2005). Chemical remediation includes adsorption, immobilization (Naidu et al., 2008), liming (Bothe & Brown, 1999) and precipitation. Although chemical remediation is widely used and can be successful, biological remediation is widely acknowledged for its cost efficiency and because most of the involved materials are microorganisms and natural products such as compost, animal waste and plant residues (Smith et al., 1998). Biological As-remediation is a process of using soil organisms or natural material to remove As from As-contaminated soil. Biological remediation can be applied by enhancing and sustaining the microbial communities in soil to improve microbial activity to reduce arsenic risk in contaminant in soil.

Three mechanisms could be used in bioremediation of As-contamination sites:

- 1- Immobilize As in the cell of the organisms (bioaccumulation).
- 2- Toxic species such as As (III) could be oxidized to less toxic As (V).
- 3- As compounds could be removed by volatilization from the soil

(Mahimairaja et al., 2005).

Fungal species are known to accumulate As in their tissue (bioaccumulation) (Granchinho et al., 2001) and could be used for bioremediation.

2.3 Mycorrhiza for remediation

2.3.1 Arbuscular mycorrhiza fungi (AMF) overview

In natural conditions, soil fungi colonize the roots of most land plants to form mycorrhizas, which are usually mutualistic associations. Arbuscular mycorrhizal fungi (AMF) are the most common type, occurring in approximately 80% of plant species (Khan et al., 2000; Smith & Read, 1997). AMF are endomycorrhiza where the fungus grows inside the host root with the development of intracellular structures in the cortical cells (Bonfante & Perotto, 1995). During AMF life cycle the fungus produces spores which germinate and give rise to a vegetative mycelium that contacts the host root surface and produces appressoria (flattened and thickened hyphal tips that facilitate penetration of the host plant). These give rise to hyphae which initiate infection of root tissues, where they form inter- and intracellular hyphae, coils, highly branched arbuscules (nutrient exchange structures) and in some cases vesicles (storage structures) (Smith & Read, 1997). The relationship of AMF with their host plants is an obligatory biotrophic status. In the absence of the host, their growth is limited to relatively short time (20-30 days), but the presence of plant roots allows the mycelium to develop and to colonize up to 60-90% of the length of the root system (Bonfante & Perotto, 1995; Rillig, 2004).

Mycorrhizas have the ability to increase the growth and production of plants through the adsorption of nutrients which they then deliver to the plants (Watts-Williams & Cavagnaro, 2012). They increase the uptake of nutrients such as P, Cu, Ni, Pb, Zn and water by plants (Khan et al., 2000; Watts-Williams & Cavagnaro, 2012). Mycorrhizas have a role too in enhancing plant growth, persistence and tolerance in contaminated sites (Elahi et al., 2010; Hegg & Angle, 1990; Watts-Williams & Cavagnaro, 2012).

A number of studies showed that mycorrhiza have a role in As uptake by plants, and translocation in plants, and could work as a barrier to transport of As to plant roots. For example, the influence of inoculating white clover and ryegrass with AMF on As uptake and translocation were investigated by (Yan et al., 2008). They found that AMF affected As translocation. It decreased root to shoot As ratio up to 12% and decrease shoot As concentration up to 66% in inoculated plants compared with non-inoculated. (Wang et al., 2008) got increased root As efflux when maize was inoculated with AMF and they found that AMF had no significant effect on As concentrations of shoot or roots of maize grown in different concentrations of As in soil except at 150 mg kg⁻¹, where root As concentrations were significantly lower than in controls. (Gonzalez-Chavez et al., 2002) reported that *Glomus mosseae* suppressed As uptake into shoots of *Holcus lanatus*, and (Elahi et al., 2012) found there was significantly lower As concentration in chili inoculated with AMF in comparison with un-inoculated plants. Decreases in As concentration due to mycorrhiza were also found in lentil (*Lens culinaris* L. cv. Titore) (Ahmed et al., 2006), and in *Medicago sativa* (Chen et al., 2007). For these reasons mycorrhizas might be used to remediate contaminated sites or risk management strategy.

However, conflicting results have been obtained regarding the effects of AMF inoculation on plant uptake of As in some species. Inoculating *Pteris vittata* (a hyperaccumulator of As) with AMF in As-contaminated soil showed enhanced As accumulation in the shoots (Leung et al., 2005). The effects of AMF on As uptake varied between studies to a large extent depending not only on plant species, and metal concentration in the soil, but also on fungal species, isolates and their origins (Nandita & Lena Q., 2007; Yu et al., 2010).

In addition, mycorrhiza has a role in uptake of other metals by plants. Some research has indicated that there are high concentrations of heavy metals in plants due to mycorrhizas, others have found a low concentration in mycorrhizal plants for example Zn and Cu (Hegg & Angle, 1990; Joner & Leyval, 1997). A great amount of heavy metals (Zn) was found in mycorrhizal root structures and in spores (Chen et al., 2001).

2.3.2 Mechanisms for effect of AMF on pollutants

A number of different mechanisms have been proposed to account for the effects of mycorrhiza on immobilization of pollutants and their uptake by plants. Glomalin is one of them. Glomalin is a protein produced on hyphae of AMF. It has been hypothesized that glomalin plays role to immobilize heavy metals in the soil before entry into the fungal-plant system (González-Chávez et al., 2004) .

Another mechanism is immobilization in fungal biomass. Mycorrhiza works as barrier to transfer of metals through plant roots. Uptake into hyphae may be influenced by absorption on hyphal walls as chitin has an important metal-binding capacity. There are also free amino, hydroxyl and carboxyl groups which exist in the cell walls of fungi and can combine with toxic elements such as Cu, Pb, and Cd (Kapoor & Viraraghavan, 1995).

Another mechanism is that mycorrhizal fungi could confer both As tolerance and accumulation ability on their host plant (Ortowska et al., 2012). Recent studies have shown that AMF can protect host plants under As contamination even for the As-tolerant plant species. (Liu et al., 2005) studied the effect of arbuscular mycorrhizal fungi (*Glomus mosseae*) on the biomass and arsenate uptake of an As hyperaccumulator, *Pteris vittata*. Two arsenic concentrations (0 and 300 mg As kg⁻¹) were used. They found that mycorrhizal colonization increased frond dry matter yield, lowered the root/frond weight ratio, and decreased frond As concentration by 33-38%. Nevertheless, transfer of As to fronds showed a 43% increase with mycorrhizal colonization at the higher soil As level.

The possible mechanism of As tolerance in mycorrhizal plants might be one or a combination of the following. First, AMF enhance P nutrition and plant growth, resulting in a higher P/As ratio and a relative reduction in As content in tissues of mycorrhizal plants (Liu et al., 2005; Ultra et al., 2007a; Ultra et al., 2007b). Second, As-tolerant mycorrhizal fungi exclude As(III) to external media and reduce As(V) uptake from As-contaminated soils and thus will reduce As uptake by AM plants (Gonzalez-Chavez et al., 2002; He & Lilleskov, 2014).

AMF inoculation not only reduces arsenic toxicity to the plant, but also can enhance plant growth, plant protection against various environmental stresses, and promote nutrient uptake of host plants by stimulating the growth of their root systems (Akhter et al., 2011; Lenoir et al., 2016; Smith & Read, 2008). Mycorrhizal hyphae can explore beyond the root-hair zone of the plant, so they can increase the absorptive surface area of the plant, and can enhance the capacity for uptake of nutrients and affect their translocation from root to shoot (Watts-Williams & Cavagnaro, 2012; Yu et al., 2010).

However, the nature of accumulation and mechanisms involved require further studies in order to better understand the participation of AMF in plant tolerance and its ecological significance in polluted soils (Mahimairaja et al., 2005).

2.3.3 The origin of mycorrhizal inoculum

To test the effect of mycorrhiza on plant growth and on As accumulation in soil and plant, researchers usually isolate AMF from As-contaminated soil (Smith et al., 2010). Isolating AMF spores from As-contaminated soil and production in trap cultures in As-contaminated soil will enable the inoculum mycorrhiza to have a reasonably diverse population of AMF (Gonzalez-Chavez et al., 2002), and the spores from As-contaminated soil will be less sensitive to As than those isolated from uncontaminated sites (Smith et al., 2010). Adapted fungi (Leung et al., 2005) will also enable AMF to survive and persist in As-contaminated soil then resulting in colonization of plants (Meharg & Cairney, 2000). However, although the mycorrhizal fungi from contaminated soils will be able to infect roots in these soils, their effectiveness for remediation in comparison with AMF from uncontaminated soils has not been thoroughly explored yet. Most investigations used inoculum that comes from contaminated soils (Smith et al., 2010).

Although there have been few studies on the area of origin of AMF, AMF originating from different environments appear to respond differently to As (Yu et al., 2009). (Gonzalez-Chavez et al., 2002) found that AMF isolated from mine soil have developed arsenate resistance and enhanced the tolerance of *Holcus lanatus* in contaminated soil. As well, they assessed spore production in trap cultures in As-contaminated mine-spoil and showed that a reasonably diverse population of AMF was present. They found that spores from the

mine-site populations were less sensitive to arsenic than those isolated from uncontaminated sites, leading to the conclusion that AMF may become adapted to As contamination, which partially explained their survival in contaminated soils (Gonzalez-Chavez et al., 2002). (Ortowska et al., 2012) studied the role of indigenous and non-indigenous AMF on As uptake by *Plantago lanceolata* growing on substrate originating from mine waste rich in As in a pot experiment. They found that inoculation with an indigenous isolate resulted in increased transfer of As from roots to shoots, while AMF from a non-polluted area apparently restricted plants from absorbing As to the tissue. Further studies are required to understand if this is a general consequence of mycorrhizal fungi from contaminated and uncontaminated sites. It is known that different AMF species from different environments appear to affect As uptake differently. (Yu et al., 2010) found that inoculation of maize with *Glomus mosseae* or *Glomus etunicatum* provided less shoot As accumulation than *Glomus constrictum*. However, very limited work has been carried out to investigate the effects of different origin and species of AMF on As uptake and translocation by plants.

2.3.4 Mycorrhiza in As-contaminated soil

Arsenic does not appear to adversely affect the colonization of roots in historically contaminated soil or in soil spiked with arsenic solution. Most experiments showed that As did not reduce the percentage of the root length colonized by AMF. (Gonzalez-Chavez et al., 2002) found that *Holcus lanatus* became equally colonized in As-contaminated and uncontaminated soils, and (Al Agely et al., 2005) found an increase in colonization in the hyperaccumulating fern *Pteris vittata* growing in As-contaminated soil. (Leung et al., 2005) also found an increase in percent root length colonized in *P. vittata* and in *Cynodon*

dactylon as arsenic concentration increased. In soil spiked with As different plant species (*Medicago sativa*, barley, *P. vittata*) and several different AMF showed no reductions in percentage of colonization (Chen et al., 2007; Christophersen et al., 2009; Trotta et al., 2006). On the other hand, (Liu et al., 2005b) reported a reduction in colonization of tomato roots in soil spiked with 150 mg kg⁻¹ As. However, there have been very few reports of reduced colonization due to arsenic (Smith et al., 2010). In naturally contaminated soil the cause of any reduction in colonization may be a result of other elements that may be present such as Pb (Smith et al., 2010).

2.4 Biochar overview

Biochar is biomass heated under oxygen-limited conditions or absence of oxygen to capture gases and create a porous, low density black carbon rich material (Beesley & Marmiroli, 2011). It is differentiated from charcoal in the broad sense by usage: derived principally from waste products and generally intended as a soil treatment.

2.4.1 Properties of biochar

Biochar is produced industrially in the absence of oxygen (Warnock et al., 2007). There are many varieties of raw materials used to produce biochar including farm waste, crop remains, wood, urban and industrial wastes, animal manures and sewage sludge (Kookana et al., 2011). Biochar can be produced under different temperatures, low-temperature (< 550 °C) and high pyrolysis (>550 °C). The chemical and physical properties of biochar may vary according to the temperatures that are reached during burning, and the raw materials that are used in the process (feedstocks). For example, if the biochar is prepared at lower

temperatures (350°C), it will have higher content of available nutrients than biochar heated at higher temperatures. Moreover, plants which have large diameter cells, if heated at high temperatures will form a higher quantity of macro-pores in biochar particles than plants heated at lower temperatures. Large numbers of macro-pores are essential to improve the ability of adsorbing the larger particles of organic compounds (Warnock et al., 2007).

2.4.2 Biochar and environmental applications

Biochar has some important properties which make some specialists pay attention to it. Biochar has an important role in climate change mitigation as it reduce gas emissions (Waters et al., 2011). Biochar can sustainably sequester carbon in soil and enhance crop yields (Namgay et al., 2010a). In addition it is one of the important sources of renewable energy (Waters et al., 2011). Moreover, biochar has the ability to adsorb micronutrients and toxic metals (Kookana et al., 2011) and is a good source of nutrients for soil and plant growth. The nutrients come from the raw materials (animal manures and plant wastes) which form the biochar. The values, quantities and kinds of nutrients depend on the source of the material (animal source or plant source) and on the temperatures used to produce biochar (Kookana et al., 2011).

In addition, biochar has important effects on soil fertility, physical, biological properties and remediation of the soil due to its characteristics. Different types of biochar added to the soil may affect its properties in different ways. Some biochars improve soil fertility and influence its physical properties (texture, structure, porosity, particle size and density), while others will change its chemical properties (pH, EC and CEC). It has also been noted that biochar has improved soil biological properties (Atkinson & Fitzgerald, 2010;

Lehmann et al., 2011; Mukherjee & Zimmerman, 2013; Singh et al., 2010), as discussed in the following section.

2.4.3 Biochar and soil properties

The structure of biochar influences the binding of cations and anions. It could improve the availability of essential elements for plants such as N and P (Atkinson & Fitzgerald, 2010). (Mukherjee & Zimmerman, 2013) demonstrated that biochar contains a variety of nutrient elements with different release rates. In addition (Mukherjee & Zimmerman, 2013) found that both soil nutrient sorption by biochar and biochar nutrient sorption by soil depended on biochar and type of soil. For example (Revell et al., 2012) found that biochar made from poultry feedstock has many advantages as a soil amendment. Evidence shows that bioavailability and plant uptake of nutrients increase in response to biochar application, particularly when in the presence of added nutrients (Atkinson & Fitzgerald, 2010).

Biochar has an important role in enhancing the physical properties of soils such as aggregation, water holding capacity, hydraulic conductivity and porosity (Waters et al., 2011). Biochar has been found to enhance soil structure and soil aggregation, giving good porosity, decreased strength and high water holding capacity (Namgay et al., 2010b) leading to increased plant productivity. The interaction between soil particles and biochar particles may help to increase aggregation of the soil (Waters et al., 2011). However, there is little direct evidence for this (Waters et al., 2011). In addition biochar has been shown to increase soil water reserves depending on the type of soil, increasing porosity and water conductivity (Waters et al., 2011). Many studies have shown that biochar has ability to increase water-holding capacity (WHC), Cation Exchange Capacity (CEC) and influence the pH of the soil. Revell et al. (2012) carried out a greenhouse trial to grow pepper in pots

by using two different soil textures (sandy loam and silt loam) mixed with biochar. They showed increase in the water-holding capacity due to the addition of biochar, for example adding 15% biochar nearly doubled the WHC of the sandy loam from 15% to 27% (Revell et al., 2012). Biochar, which had a pH of 9.3, increased pH in both soils. (Atkinson & Fitzgerald, 2010) also found there were improvements in soil field capacity after biochar addition.

2.4.4 Biochar and soil microorganisms

Although the impact of biochar amendments on soil organisms remains unclear. (Beesley & Marmiroli, 2011; Warnock et al., 2007) found that biochar has a positive effect on the biological communities in soil. Biochar found in many studies increased microbial biomass and affects positively microbial community and enzyme activities (Lehmann et al., 2011). The organic matter particles and nutrients that are adsorbed by biochar and concentrated on its surface may attract soil organisms to these materials (Kookana et al., 2011; Waters et al., 2011). The abundance and activity of the microorganisms is affected by the varieties of organic nutrients released from biochar. (Kookana et al., 2011) stated that the percentage of bacteria with nitrogen fixing ability increased with an increase in the amount of biochar addition.

(Warnock et al., 2007) mentioned four mechanisms by which biochar could influence mycorrhiza abundance and or function: (a) alteration of soil physio-chemical properties; (b) indirect effects on mycorrhiza through effects on other soil microbes; (c) plant–fungus signaling interference and detoxification of allelo-chemicals on biochar; and (d) provision of refuge from fungal grazers. Mycorrhizal colonization can be affected negatively or

positively by biochar according to the feedback. It is increased by adding plant biochar feedstocks, while it is decreased by adding poultry biochar feedstocks (Kookana et al., 2011). (Lehmann et al., 2011) found that biochar decreases the abundance of mycorrhizal fungi and they suggested that may be because of the increase in nutrient availability, reducing the need for symbionts. However more research is needed here to focus on the impact of biochar and its interaction with microorganisms and the environment because of its important role in plant production and soil health.

2.4.5 Biochar and soil remediation

Recently it has been demonstrated that biochar addition to soil is one solution for soil remediation (Namgay et al., 2010b). (Beesley et al., 2011) mentioned that the large surface areas and cation exchange capacity of biochar improves sorption of both organic and inorganic pollutants that will lead to reducing the mobility of pollutants when added to soil as amendment. Other reports remarked that biochar is very effective in the absorption of many natural and organic compounds. For example, chars and ashes produced from burning of wheat and rice residues were reported to be up to 2500 times more effective than soil in adsorbing the herbicide Diuron (Kookana et al., 2011).

The biochar adsorption ability depends on the feedstock (agricultural or animal sources). (Kookana et al., 2011) stated that the biochar produced from agricultural crop wastes is effective in absorbing organic contaminants and the biochar produced from dairy-manure is efficient in absorbing both heavy metals and organics. However, research here is limited. (Choppala et al., 2012) used black carbon and biochar to test their effect on reducing Cr (VI) in acidic and alkaline contaminated soil. The result showed that black

carbon had a greater effect in reduction of Cr (VI) than biochar. This was because of the differences in dissolved organic carbon and functional groups that provide electrons for the reduction of Cr (VI). (Gomez-Eyles et al., 2011) used biochar and earthworms to reduce the bioavailability of toxic hydrocarbons in calcareous contaminated soil. Biochar reduced the total hydrocarbons from 449 to 306 mg kg⁻¹ and also reduced water soluble Cu (60 to 37 mg kg⁻¹). (Namgay et al., 2010b) in their factorial trial used wood biochar pyrolysed at 550 °C applied at rates of 0, 5, and 15 g kg⁻¹ with 3 concentrations (0, 10, and 50 mg kg⁻¹) of As, Cd, Cu, Pb, and Zn separately to a sandy soil at pH 7. They found that biochar significantly affected the availability of trace elements in the soil. They showed that concentrations of extractable As and Zn increased with biochar application while Pb decreased, Cu was not changed and Cd showed inconsistent behavior. On the other hand, some authors note that biochar is a potential source of toxins that arise from the feedstock that is used to produce it, because this feedstock may come from mining or industrial waste, and contains contaminants (Kookana et al., 2011).

2.4.6 Biochar and arsenic

Some studies showed that biochar has the ability to immobilize and retain As in contaminated soil. (Beesley et al., 2011) in their leaching experiment with scanning electron microanalysis found that biochar can rapidly reduce the mobility of As, Cd and Zn in contaminated soil, especially Cd. (Zhang & Gao, 2013b) also found that biochar showed strong sorption of aqueous arsenic. In contrast, (Namgay et al., 2010a) found that extractable As increased with biochar application. (Zhang et al., 2012) in their experiment of how biochar can affect the mobility of Cd, Zn, Pb and As in rice seedlings showed that biochar produced from different parts of rice (straw, husk and bran) increased As

concentration in rice shoots up to 327%, while decreasing the concentration of Cd, Zn and Pb. As a conclusion biochar seems have low ability in sorption of As compared with other metals and has inconsistent effects on plant uptake of As.

2.4.7 Modified biochar: magnetic biochar

Although biochar has shown great ability to remove heavy metals from aqueous solution (Ahmad et al., 2014), the surfaces of most biochars are net negatively charged (Mukherjee & Zimmerman, 2013; Yao et al., 2012) and so their sorption of As which is in anionic form of As (v) is often low (Beesley & Marmiroli, 2011).

Iron oxide particles are a highly effective for As removal (Aredes et al., 2012; Chen et al., 2011). Although they have high surface area, iron oxide nanoparticles have a tendency to form aggregates when in contact with solution and that will decrease the surface area and adsorption abilities (Zhang et al., 2013a). Therefore several workers have combined iron oxide with biochar to increase stable surface anion exchange capacity and absorption abilities (Han et al., 2015). Magnetic biochar is a powder material that can be separated from aqueous solution for reuse. Magnetic activated carbon (Mohan et al., 2011) or magnetic biochar (Chen et al., 2011) may provide an alternative way to remediate anionic pollutants from soil and water. Magnetic activated carbon has been used for organic and inorganic pollutants removal from wastewater (Oliveira et al., 2002).

(Sun et al., 2015) in their study developed a type of biochar coated with magnetic Fe_3O_4 nanoparticles to test its ability to remove the anionic dye crystal violet from solution. They found that adding Fe_3O_4 nanoparticles to the biochar significantly enhanced the adsorption capacity from 80.36 to 99.19 mg g^{-1} . Other work done by (Zhang et al., 2013a) modified a biochar with nano sized $\gamma\text{-Fe}_2\text{O}_3$ particles and used it as an As sorbent in solution. They

found that the composite had a strong sorption ability for aqueous As. In addition, for the purpose of finding low-cost adsorbents to remove As from aqueous solution (Wang et al., 2015) modified a biochar by pyrolyzing a mixture of naturally occurring hematite mineral and pinewood biomass. Their result showed the hematite modified biochar not only had strong magnetic properties but also showed much greater ability to remove As from aqueous solution compared with unmodified biochar.

All these methods developed a biochar for As sorption from solution. However, using modified biochar (magnetic biochar) in agriculture to alleviate As contamination in soil and reduce As concentration and uptake by plants has not yet been tested.

2.5 Conclusion

Arsenic remains a pollutant of major concern in most of the world's countries. It is toxic to humans and causes other diseases. It affects plant growth and can transfer from the soil to the plant and humans within the food chain. Biological remediation is one way to clean the environment safely. To use this kind of remediation there is need to find natural and safe material with low-cost to alleviate As and to reduce its uptake by plant. Mycorrhiza and biochar both are biological and safe for the environment and humans and at the same time are useful for the soil and plants. Different investigations reported an effect of mycorrhiza to reduce pollutant concentration and uptake inside the plant. However other works found that mycorrhiza had no effect to reduce the pollutant and As in soil and inside the plant. There are many factors that affect on the efficient use of mycorrhiza and soil remediation and on the reduction of As concentration and uptake inside the plant. One of these factors is the origin of mycorrhiza isolates if it comes from As-contaminated soil or from

uncontaminated soil. On other hand biochar has been to clean the wastewater from pollutants but its efficiency to remove As from solutions is low compared with other pollutants due to the negatively charge of biochar surfaces which showed a weak sorption for arsenate and arsenite. To increase the efficacy of its surfaces, researchers have tried to modify biochar with materials that have a high ability to sorb As from solution, for example with iron to produced magnetic biochar. But magnetic biochar has not been tested yet in agriculture to alleviate As in soil and As concentration and uptake inside the plant.

3 Tolerance of different mycorrhizal plants to arsenic in soil

3.1 Introduction

Mycorrhiza are known to reduce As uptake and concentration in many plants (Smith et al., 1998). The effects of AM colonization on plant P status and growth are highly variable, ranging from very large positive increases (in so-called ‘responsive’ plants) to neutral or negative (in ‘non-responsive’ plants) (Smith et al., 2009; Smith & Read, 2008) Mycorrhiza-responsive plants usually show reduced As toxicity and higher P/As ratios when they are in a mycorrhizal symbiosis, while non-responsive plants have more variable outcomes (Smith et al., 2010). If mycorrhizas are to be used in a practical way to manage As risk, then more needs to be known about the As and mycorrhizal responses of common crop plants.

Vegetables are common crops on small farms that may be associated with areas of local-scale pollution. With elevated As levels in the soil, As can accumulate considerably in certain vegetables and in particular in plants from the Asteraceae and Brassicaceae families (Ramirez-Andreotta et al., 2013). It is urgently necessary to clean and remediate As from areas, where crops, vegetables, fruits and pastures have been grown, in order to protect the health of human beings and animals.

Arsenic causes wilting and yield reduction, and accumulates in the edible portions which will be harmful for health (Rahman & Naidu, 2009). (Smith et al., 2009) found the highest

concentrations of total As (35-278 mg kg⁻¹) in the roots of vegetables and lower concentrations (3-7 mg kg⁻¹) in the aerial portions of plants growing in arsenate-spiked nutrient solution. Total As accumulation in the edible portions of the vegetables decreased in the order radish > mung bean > bean > lettuce = chard. Arsenic was present in the roots of radish, chard and lettuce as arsenate As (V) and comprised between 77 and 92% of the total As present, whereas in mung beans, arsenite As (III) comprised 90% of the total As present (Smith et al., 2009).

In recent years, the use of commercial arbuscular mycorrhizal inoculants has grown for plant growth promotion. There has been less work on using them for remediation. However the result from using commercial arbuscular mycorrhiza inoculum on plant growth and mycorrhizal colonization can be variable. (Corkidi et al., 2004) used 10 commercial mycorrhizal inoculants to test the infectivity of those inoculants on plant growth. They found that only 6 of the products promoted mycorrhizal colonization, which ranged from 0-50%, but there was no increase in plant growth compared with non-inoculated controls. (Cozzolino et al., 2010) got increase in plant biomass when adding commercial mycorrhizal inoculum with P together in a greenhouse pot experiment. (Baum et al., 2015) found that AMF can be effective to increase drought and salt stress tolerance when they used commercial mycorrhizal inoculum with vegetable crops.

The aim of this experiment was to determine whether inoculation with a commercial inoculant may increase mycorrhizal colonization and promote the growth of a representative range of vegetable crops in As contaminated soil. Eight plant species, including a non-mycorrhizal comparison species, were tested. A secondary aim was to

choose a mycorrhizal plant that can survive in As-contaminated soil but shows sufficient sensitivity to be used in further experiments to investigate applications in remediation.

3.2 Methods

3.2.1 Soil samples

Contaminated soil (sandy clay loam) and uncontaminated soil (loamy sand) were collected on 14th of August, 2013 from Jennings, in northern New South Wales. Jennings is located in the New England region, latitude 29.05 °S longitude 152.02 °E. The As contamination occurred from the manufacture of As oxide as a pesticide on the site. The uncontaminated site was located 500 m from the contaminated site, but not impacted by the As processing and pesticide manufacture. Both locations were under native vegetation.

Litter and plant growth was removed from the surface and soils were collected to a depth of 25 cm. The two soil samples were mixed well and distributed separately in trays and left for three days to air dry. Roots and other large plant parts were removed. Soil samples were crushed by vertical force with a soil crusher and passed through a 2 mm sieve. Soil samples were stored in plastic bags with labels for analysis. Some samples were kept in a cold room (4 °C) for microbiological measurements.

Physical analysis (moisture content and particle size analysis) and chemical analysis (pH, electric conductivity (EC), carbon and organic matter, As and other elements) were determined in the laboratory of the university as follows.

3.2.2 Soil analysis and measurement

3.2.2.1 Field capacity and moisture content

Samples were oven dried at 105 °C overnight to determine moisture content. Field capacity of both soils was determined by saturating with excess water and leaving overnight to drain (Black, 1965).

3.2.2.2 Particle size analysis (PSA)

The two air –dried soil soils were analysed for particle size distribution by the hydrometer method using sodium hexametaphosphate (Calgon) to suspend the soil. A blank and the soil suspensions were measured by hydrometer for periods after 4 , 48 minutes and after 8 hours, and the temperature recorded (Day, 1965) .

3.2.2.3 pH and electrical conductivity (EC)

Air-dried soil samples passed through a 2 mm sieve were measured for EC and pH using a soil suspension of 1:5 soil: deionized water at 25 °C (Rayment & Higginson, 1992).

3.2.2.4 Arsenic and elements measurements

Total arsenic (As), phosphorous (P), and percentage of magnesium (Mg) and iron (Fe) were determined in the soils by a microwave digestion technique using aqua regia in a Milestone UltraWAVE instrument. The Standard reference material used in this analysis was “Montana II Soil” (National Institute of Standards and Technology, 2009) . Acid digestion of 0.50 g soil in 9 mL of hydrochloric acid (36%) and 3 mL nitric acid (70%) was done in a closed vessel rotor using temperature controlled microwave heating in a one

step process. The solution was made up to 25 mL total volume with deionised water. Subsequent element determination in extracts was carried out using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Agilent 725).

3.2.2.5 Cation exchange capacity (CEC)

A mixture of 2 g of soil with 40 mL of 1 M NH₄Cl was centrifuged and the obtained extraction was filtered through Whatman paper no. 42 and analysed for Ca, Mg, Na, K on the ICP-OES to estimate CEC (Rayment & Higginson, 1992).

3.2.2.6 Carbon and nitrogen

Total carbon and nitrogen were detected in the soil samples using the LECO TruSpec which is a dry combustion type with infrared detection for carbon and thermal conductivity detection for nitrogen. Approximately 0.2-0.5 g of soil sample was used for the analysis. Table 3.1 shows the analysis result for both soils.

Table 3.1 Selected characteristics of the soils used the experiment

Parameters measured	Uncontaminated soil	Contaminated soil
Sand (%)	83.50	68.80
Silt (%)	6.00	10.50
Clay (%)	10.50	20.50
Soil texture	Loamy Sand	Sandy Clay Loam
Arsenic (mg kg ⁻¹)	4.68	155.98
Phosphorous (ug g ⁻¹)	21.16	10.11
Nitrogen (%)	0.01	0.02
Carbon (%)	0.38	0.71
Ca (cmol+kg ⁻¹)	3.04	2.02
K (cmol+kg ⁻¹)	0.17	0.22
Mg (cmol+kg ⁻¹)	1.87	2.43
Na (cmol+kg ⁻¹)	0.06	0.57
EC (μS cm ⁻¹)	34.33	30.50
pH	7.93	8.42
Fe (%)	2.04	2.16
Mg (%)	0.25	0.18
Ca (%)	0.16	0.05
Na (mg kg ⁻¹)	13.20	131.40
Mn (mg kg ⁻¹)	341.00	194.84
Cu (mg kg ⁻¹)	1.77	23.55

3.2.3 Glasshouse experiment

The experiment was carried out at the glasshouse of the School of Environmental and Rural Science, University of New England, NSW, Australia. The plants were grown under controlled environmental conditions with temperature of 25/15 °C day/night. 128 plastic pots were used in the experiment (12 cm diameter and 1 kg capacity). Plastic bags lined the pots and half were filled with air-dried uncontaminated soil, while the other half of the pots were filled with As-contaminated soil. Half of the pots received commercial mycorrhizal inoculum (ZeoVAM, BioCoat, Melbourne Australia) it is (*Rhizophagus intraradices* spores pellets in a zeolite carrier) added at a rate of 4 g kg⁻¹ soil (according to the instruction for the product) mixed with the top 2/3 of soil in each pot. Nutrients N, K, S, Mg and Ca were applied in solution form as N (NH₄NO₃ 13.6 g L⁻¹), K and S (K₂SO₄ 8.4 g L⁻¹), Mg (MgCl₂.6H₂O 11.9 g L⁻¹), Ca (CaCl₂.H₂O 20.9 g L⁻¹). 100 mL of the nutrient solution was added to each pot before planting. All pots received another 100 mL of N (NH₄NO₃ 13.6 g L⁻¹) 30 days after sowing. Once all the experiment units received the treatments, the pots were irrigated with 100 mL of deionized water and allowed to equilibrate for 3 days. Five seeds from each of eight plants (Tomato, Dwarf Bean, Lettuce, Eggplant, Okra, Capsicum, Spinach and Maize) (Table 3.2) were sown in each pot at 1 cm soil depth. This was thinned to one plant per pot after emergence. The pots were watered by weight to field capacity with deionized water regularly and according to the requirements of the plants.

The experiment was arranged in Randomized Complete Block Design (RCBD). Three factors As (uncontaminated soil, contaminated soil), Mycorrhiza (inoculum, without inoculum), plants (8) were crossed in four replications.

Table 3.2 Plants used in the experiment

Plant name	Species
Tomato	<i>Solanum lycopersicum</i> – San Marzano
Dwarf Bean	<i>Phaseolus vulgaris</i> – Tender Delight
Lettuce	<i>Lactuca sativa</i> – All Year
Eggplant	<i>Solanum melongena</i> – Black Beauty
Okra	<i>Abelmoschus esculentus</i> - Clemsons Spineless
Capsicum	<i>Capsicum annuum</i> - Californian Wonder Bell
Spinach	<i>Spinacea oleracea</i> - English Medania
Maize	<i>Zea mays</i> – Kelvedon Glory F1

3.2.4 Sampling

3.2.4.1 Plant and soil material

The height of the plants was recorded each 10 day after sowing and before harvesting. The plants were harvested 55 days after sowing (DAS) by being cut 1 cm up from the soil surface. Shoots (leafs, stalks) and cobs of maize were harvested separately. Fresh weight for each was recorded, then they were rinsed twice with deionized water and each part of plant chopped into small pieces separately and stored in weighed acid washed 70 mL specimen containers. The specimens were freeze dried to determine the dry weight.

The contents of pots were emptied into trays (separate trays for each pot), carefully shaking the roots from the bulk soil. Fresh soil from the rhizosphere was collected in a

plastic bag and stored in a cold room (4°C) for mycorrhizal spore counting and identifying. Soil was washed off from the root system first with water over a sieve and then in the sonicator bath, after that rinsed with deionized water. A portion of the root samples was stored in 50% ethanol for determination of mycorrhizal colonization (Wang et al., 2008). The remainder of the root samples were freeze dried to determine dry weight. The weights were adjusted for the portion removed for mycorrhizal staining according to the proportion of fresh weight removed.

3.2.4.2 Mycorrhizal colonization

The samples of root were placed in a container then filled up with 10% of KOH, and heated for 10-12 minutes in a water bath at 90 °C, and then rinsed several times with tap water. Cleared roots were boiled for 3 minutes in 5% ink-vinegar solution. The solution was prepared from black Pelikan ink with pure household vinegar (5% acetic acid). The roots were destained by acidifying with a few drops of vinegar, then rinsed with tap water 3 times. Finally, the stained roots were boiled in tap water for 5 minutes, rinsed with tap water and stored in a container with tap water (Vierheilig et al., 1998).

Stained roots were tested for mycorrhizal infection by the gridline intersection method (Brundrett et al., 1996) and the colonization percentage counted using a stereomicroscope. Slides of arbuscules and vesicles in plant roots were made and identified by compound microscope to confirm mycorrhizal colonization.

3.2.4.3 Mycorrhizal spores

Wet sieving is a process of sieving the soil sample (100 g or less) through different size of sieves (45, 100 and 250 μm) to obtain the spores from each sieve. Soil samples were wet with water for at least 30 minutes before sieving. Roots and coarse material were collected on a coarse screen (750 μm or 1 mm). The spores on each sieve were transferred to 50 mL centrifuge tubes. The supernatant from the first centrifugation (1 minute at 2000 rpm) was discarded, then 50% sucrose added to the tubes and vigorously shaken. The samples were then centrifuged for 1 minute at 2000 rpm to separate spores, then immediately after centrifugation the spores with the sucrose were poured on the finest sieve (45 μm) and carefully washed with water to remove the sucrose and washed onto a pre-wetted filter in a Buchner funnel before vacuum filtration.

Microscope slide preparations of spores were made by using polyvinyl alcohol-lactoglycerol (PVLG) (8.33 g poly vinyl alcohol, 50mL distilled water, 50 mL lactic acid, 5 mL glycerine) mixed 1:1 with Melzer's reagent (1.5 g iodine, 5 g potassium iodide, 100 mL distilled water). Fresh spores were picked up from filter paper on microscope slides, and some of spores squashed to reveal inner-wall layers. The spores were identified by dissecting microscope and compound microscope (Brundrett et al., 1996).

3.2.5 Data analysis

All results were expressed as means and the effects of AMF on As and plant growth were examined by using the two-way analysis of variance with statistical program SPSS version

22. The least significant differences (LSD) at the 5% level was calculated to compare the means within and between the treatments.

3.3 Results

3.3.1 Plant height

The heights of all plant species were significantly lower in arsenic contaminated soil than in uncontaminated soil (Figure 3.1). The height of spinach could not be measured because of its growth form. Spinach was a rosette plant and height was not meaningful. Arsenic had a highly negative effect on tomato height and the tomato plants in contaminated soil all died before the end of the experiment. Un-inoculated lettuce plants also all died after 25 days after sowing. The bean, maize, okra and eggplant were less sensitive toward As than tomato.

There were no significant differences in the height of the plants between the inoculated and non-inoculated plants for most species. However, addition of mycorrhizal inoculum significantly increased the height of capsicum in the contaminated soil (Figure 3.1).

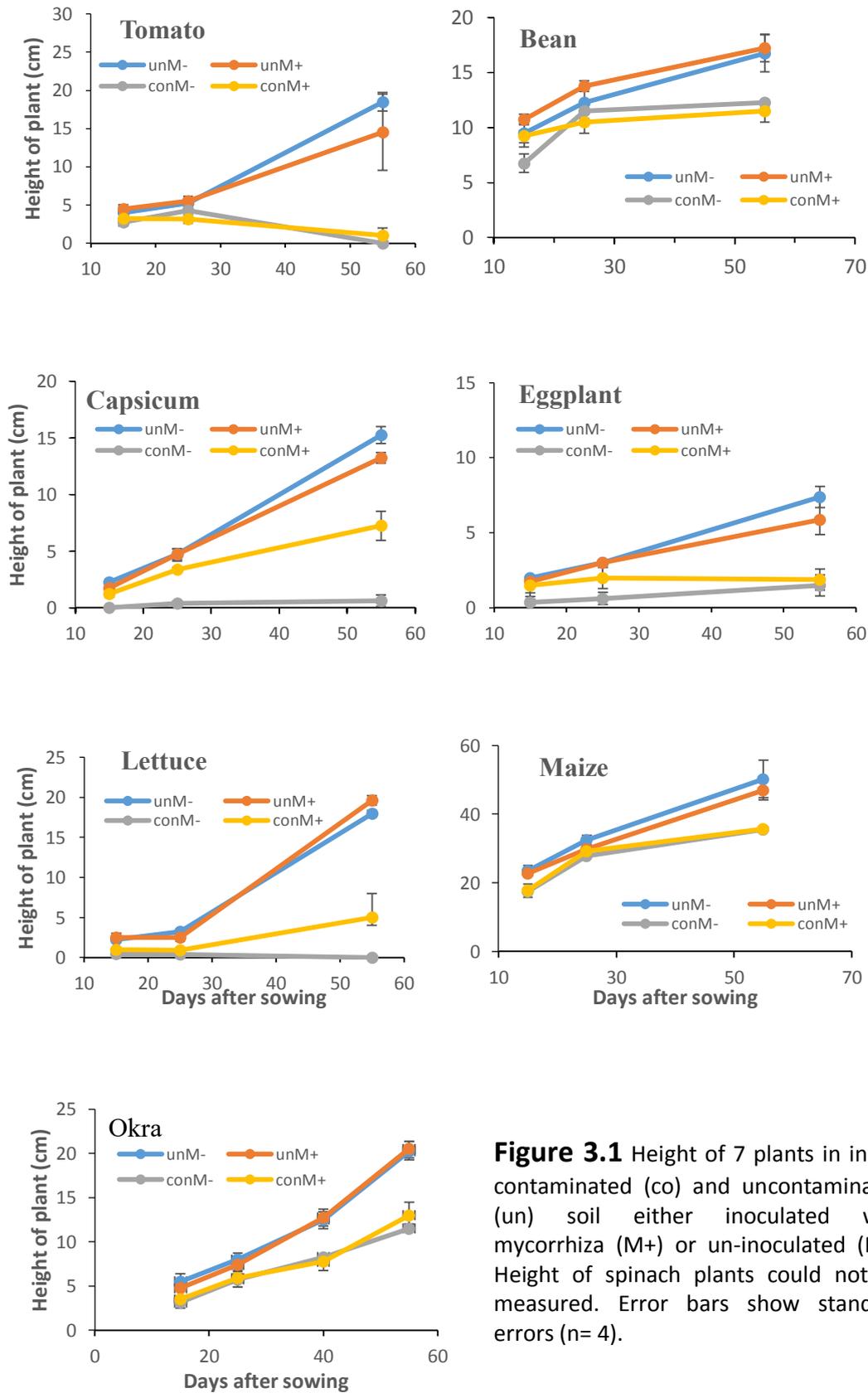


Figure 3.1 Height of 7 plants in in As-contaminated (co) and uncontaminated (un) soil either inoculated with mycorrhiza (M+) or un-inoculated (M-). Height of spinach plants could not be measured. Error bars show standard errors (n= 4).

3.3.2 Shoot dry weight

There was a significant reduction in the dry weight of shoots of all plants in contaminated soil compared with uncontaminated soil (Figures 3.2 and 3.3). The most sensitive species were tomato, lettuce and eggplant, and the most tolerant species were maize and beans (Table 3.3). Un-inoculated spinach plants did not germinate in contaminated soil.

In general, inoculation with mycorrhiza did not increase plant growth of any species in either soil. However, inoculation increased the number of plants of capsicum, lettuce and spinach that survived to the end of the experiment in the contaminated soil, and significantly increased the dry weight of capsicum plants in contaminated soil (Figure 3.2).

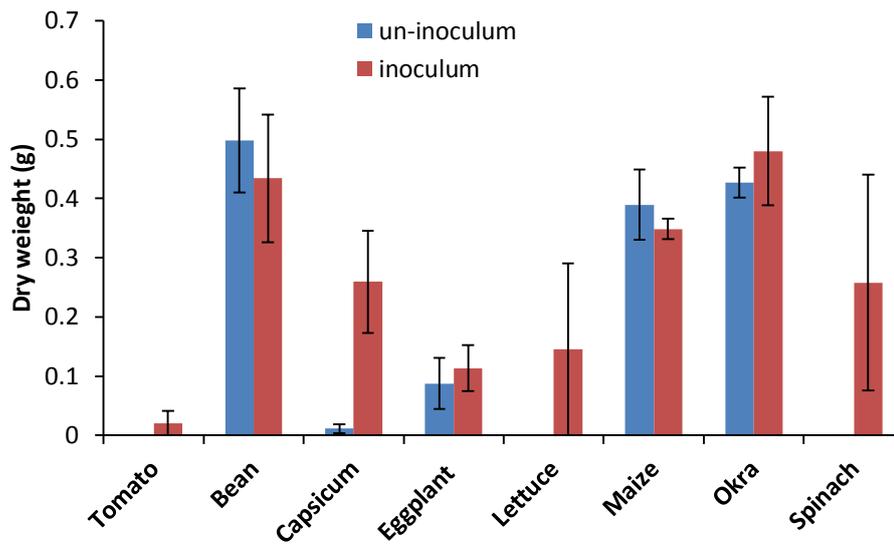


Figure 3.2 Dry weight of 8 vegetables in soil contaminated with arsenic, with and without inoculation with mycorrhiza. Error bars show standard errors (n= 4).

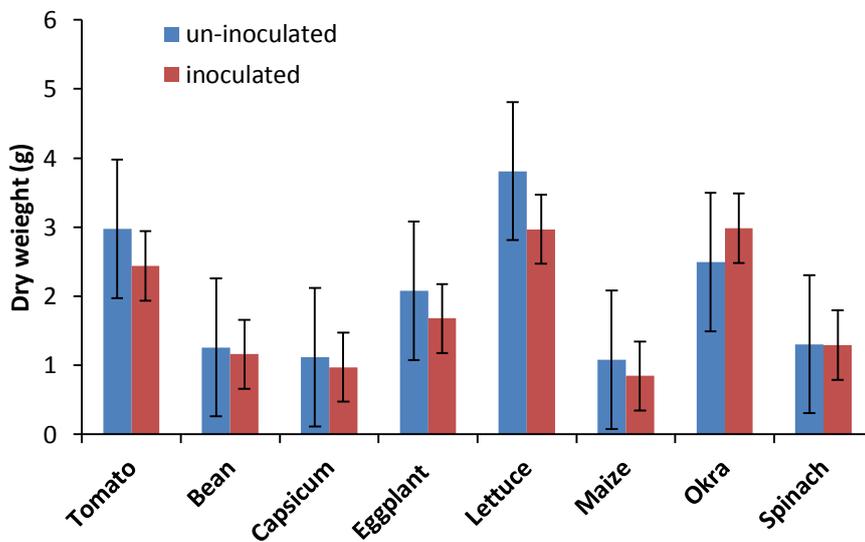


Figure 3.3 Dry weight of 8 vegetables in uncontaminated soil, with and without inoculation with mycorrhiza. Error bars show standard errors (n=4).

Table 3.3 Reduction in dry weight of shoots of 8 vegetables grown in arsenic contaminated soil compared with growth in similar uncontaminated soil with and without mycorrhizal inoculum

Plant	Reduction in dry weight (%)	
	With mycorrhizal inoculum	Without mycorrhizal inoculum
Tomato	99.2	100.0 ^A
Lettuce	95.0	100.0 ^A
Eggplant	93.0	95.8
Spinach	80.0	100.0 ^A
Okra	75.4	82.9
Capsicum	73.3	99.0
Bean	63.0	60.5
Maize	59.0	63.9

^A= plant died

3.3.3 Mycorrhizal colonization

Structures typical of arbuscular mycorrhizal fungal colonization were found in roots of all plants except spinach (Figure 3.4).

There was a significant reduction in the percentage of mycorrhizal colonization of roots of maize, bean, eggplant and okra in contaminated soil compared with un-contaminated soil (Figure 3.5). There was no significant effect of As contamination on mycorrhizal colonization of tomato or capsicum. Insufficient roots of lettuce could be obtained from contaminated soil to make a comparison, and there was no colonization of spinach roots in

any treatment. Inoculation with mycorrhizal fungi did not significantly increase the colonization of roots of any species.

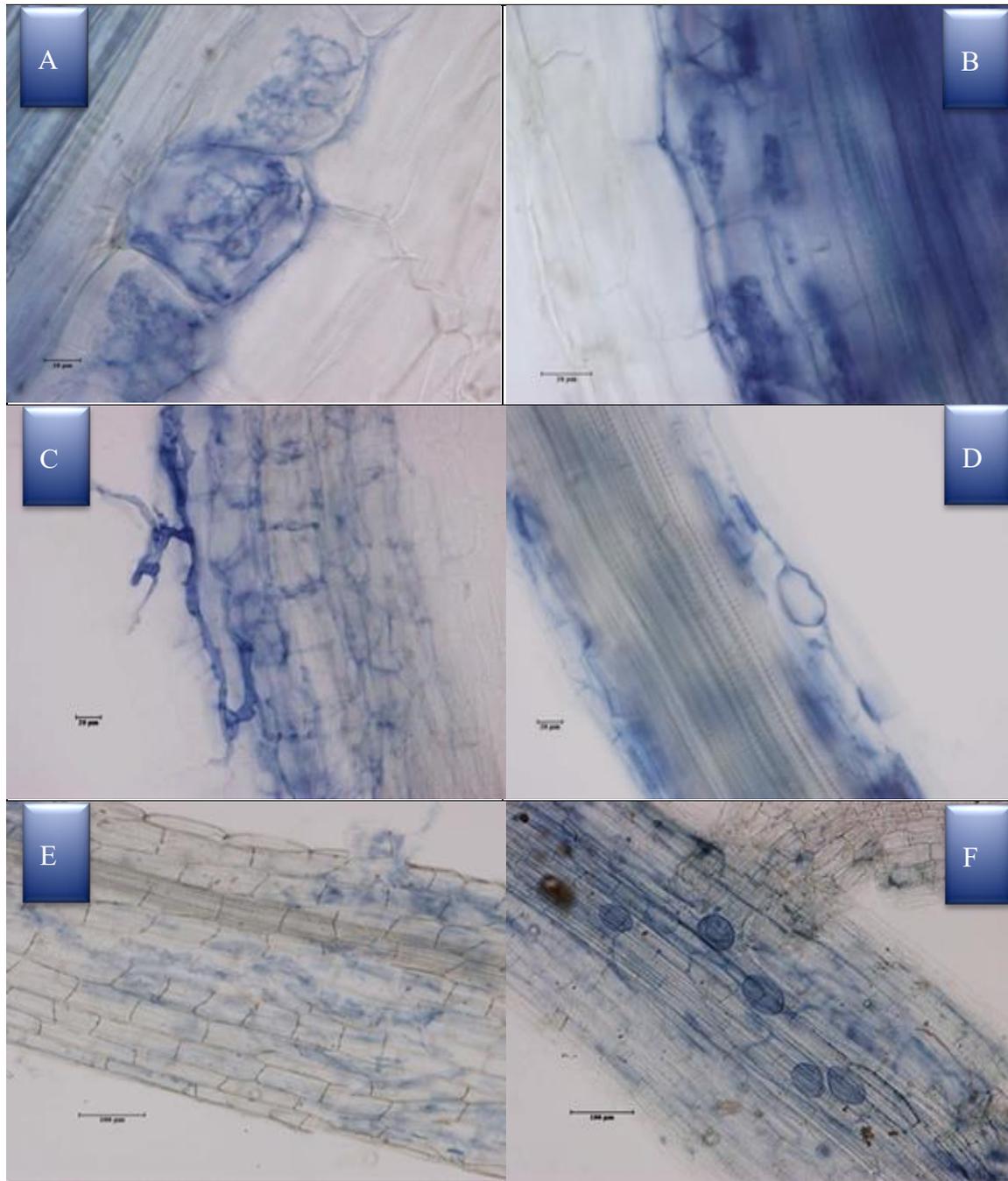


Figure 3.4 Typical mycorrhizal structures seen in roots of plants. A, B: Arbuscules in roots of bean; C: Hyphae in roots of okra; D: Vesicles in roots of okra; E: Colonies in roots of maize F: Vesicles

3.3.4 Morphological identity of AMF spores.

Spores recovered from the soils by wet sieving were identified to morphological species according to nature of hyphal attachment, wall thickness, colour and ornamentation. Nine common types were found (Figure 3.6). These were informally classified as three species of each of *Acaulospora*, *Gigaspora* and *Glomus*.

Due to time constraints, it was not possible to obtain counts for each spore type in each soil. A qualitative comparison of the soils was carried out according to the presence or absence of each spore type (Table 3.4). Some spore types were common to both contaminated and uncontaminated soil (*Acaulospora* 1, *Gigaspora* 1, *Glomus* 3). Other *Acaulospora* and *Gigaspora*-type spores were only detected in the uncontaminated soil. A *Glomus*-like spore with coarse reticulate patterning in the inner wall layers (*Glomus* 2) was particularly common in the contaminated soil, as were paler-coloured *Glomus*-like spores (*Glomus* 3).

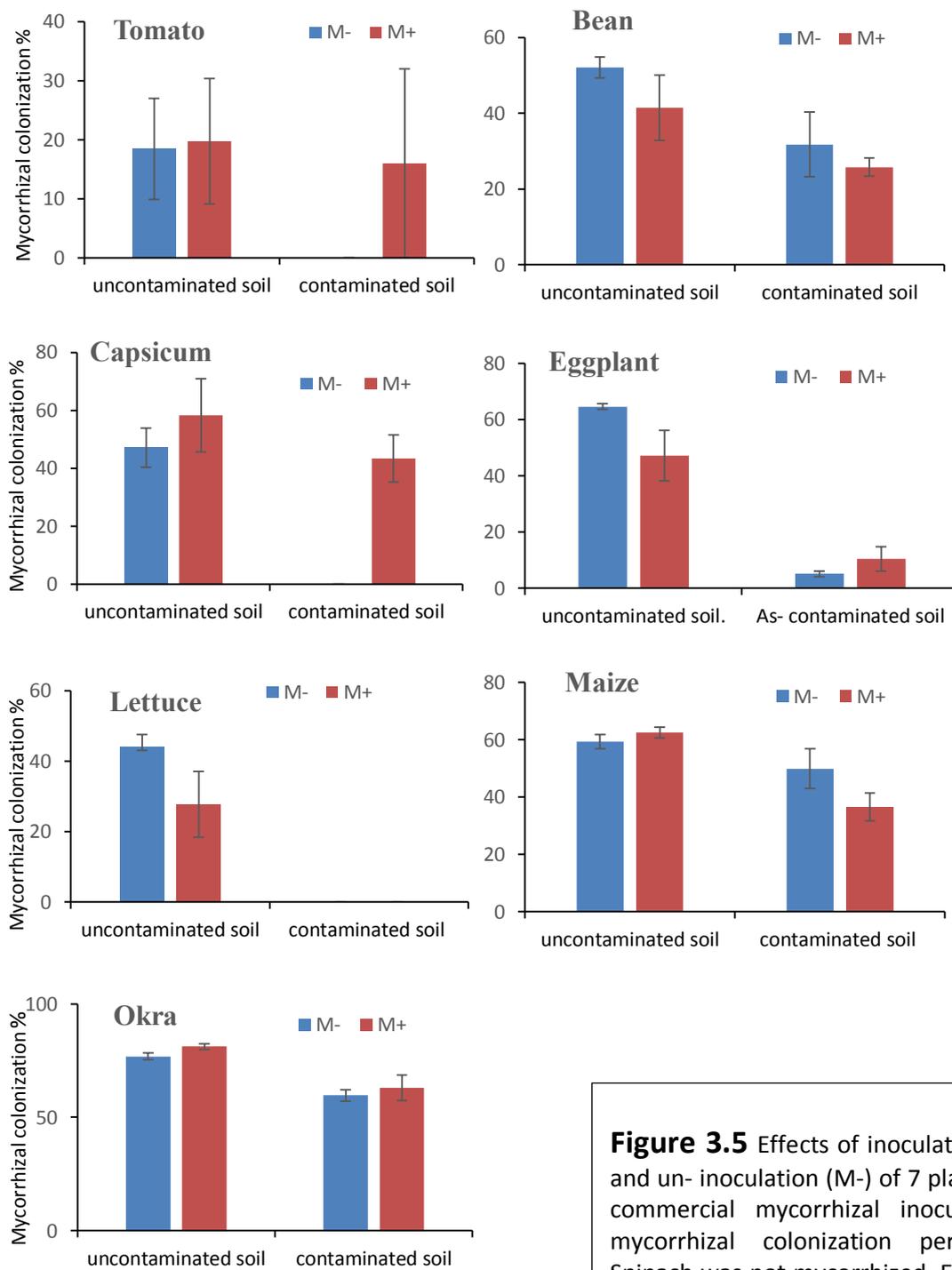


Figure 3.5 Effects of inoculation (M+) and un- inoculation (M-) of 7 plants with commercial mycorrhizal inoculum on mycorrhizal colonization percentage. Spinach was not mycorrhized. Error bars show standard errors (n= 4).

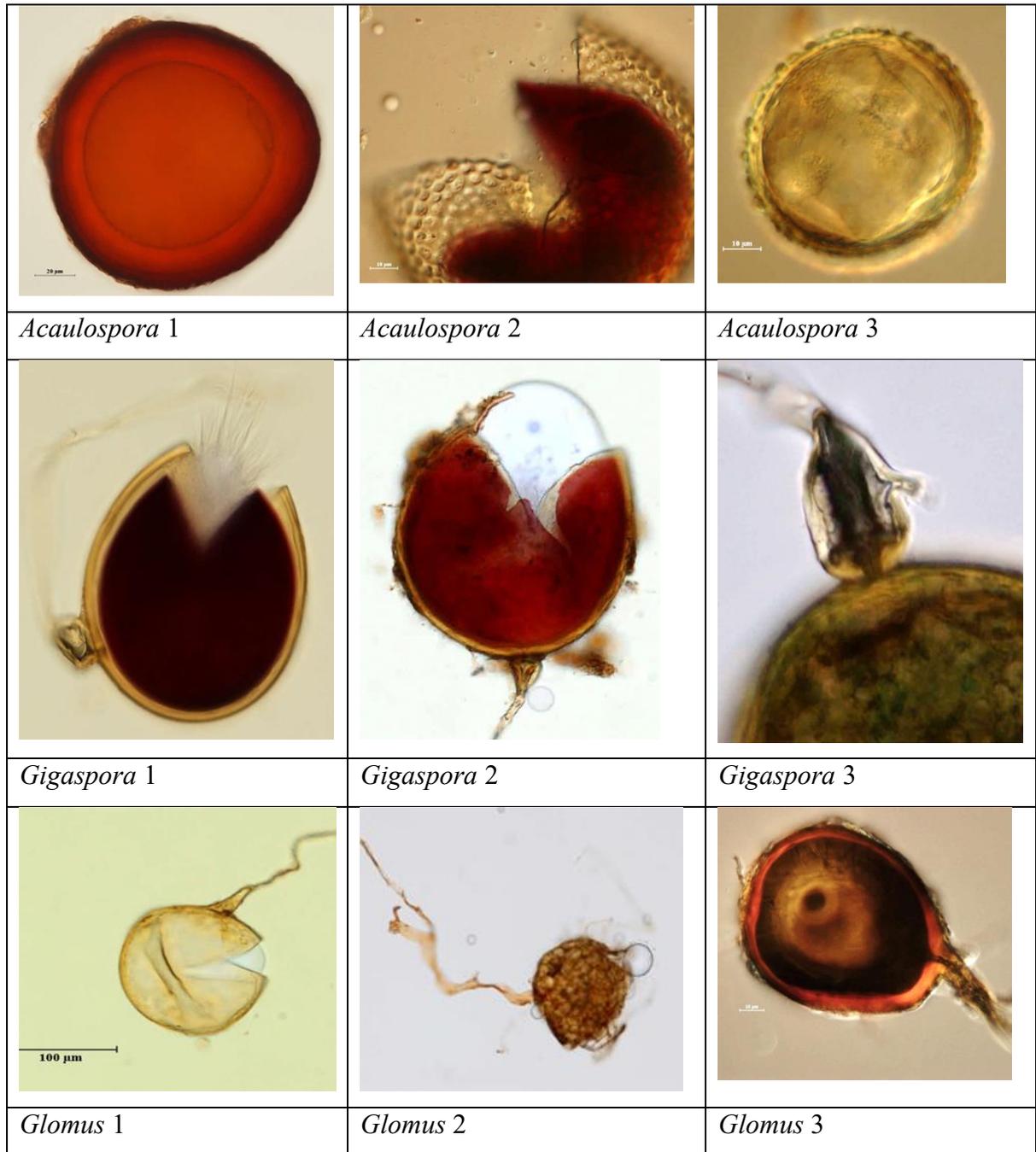


Figure 3.6 Morphological groups of AMF spores isolated from contaminated and uncontaminated soil samples from this study.

Table 3. 4 Occurrence of AMF spores morphospecies in uncontaminated soil and arsenic-contaminated soil.

Morphospecies	Uncontaminated soil	Contaminated soil
<i>Acaulospora</i> 1	✓	✓
<i>Acaulospora</i> 2	✓	
<i>Acaulospora</i> 3	✓	
<i>Gigaspora</i> 1	✓	✓
<i>Gigaspora</i> 2	✓	
<i>Gigaspora</i> 3	✓	
<i>Glomus</i> 1		✓
<i>Glomus</i> 2		✓
<i>Glomus</i> 3	✓	✓

3.4 Discussion

Although the two soils used in this experiment were not identical, the differences in growth and other parameters measured were much greater than would be expected based on differences in physical and chemical properties, apart from the arsenic concentrations. The concentration of contaminated soil that used in the experiment was 156 mg kg⁻¹ which was higher than the concentrations at which toxicity to plants is generally observed (25-40 mg kg⁻¹) (Miteva, 2002; Sheppard, 1992). This means that it is almost certain that the soil effect was mainly due to the As contamination. Arsenic reduced the growth (dry weight and height) and showed toxicity symptoms of all 8 crop plants.

One of the marks of toxicity of As to plant is a biomass reduction (Carbonell-Barrachina et al., 1997). The results showed plant height significantly decreased in As- contaminated soil compared with uncontaminated soil. The cause is that As interferes with plant metabolic processes and inhibits growth, leading to death (Jiang & Singh, 1994; Marin et al., 1992; Smith et al., 2010). (Carbonell-Barrachina et al., 1998) reported that bean leaf dry weight had an average reduction of 50% and fruit production of 84% in As solution compared with control. (Miteva, 2002) reported decrease in growth of both the vegetative and root system of tomato plants at higher As concentration.

Different plants showed different reactions towards the As in the contaminated soil. This may be because As uptake or translocation in the plants were different. Some of the plants could retain As in the root , while the others transfer more As to the aerial parts of the plants (Rahman & Naidu, 2009; Smith & Read, 2008). It may also be that the plants have different genetic and physiological capacity to accumulate and resist high amounts of metals (Ramirez-Andreotta et al., 2013). Because a major purpose of this experiment was to screen plants for response to As and mycorrhization, and not to explore mechanisms, arsenic uptake was not measured.

Inoculating with commercial mycorrhiza did not increase mycorrhizal colonization associated with native fungi in the soils. This meant it was not possible to test the interaction between mycorrhizal colonization and As toxicity. A different experimental design was developed to do this in the next chapter. However, some plants (Spinach, Capsicum, and Lettuce) showed higher survival, height or dry weight in As-contaminated soil when mycorrhizal inoculum was added. Because this was seen in spinach, which was not mycorrhizal, the effect was not due to mycorrhizal colonization itself. The reason may

be that the commercial mycorrhizal inoculum contained other promoters in the formulation (Corkidi et al., 2004). The particular product used was based on a zeolite carrier.

Arsenic reduced mycorrhizal colonization in most plants. (Smith et al., 2010) claimed that most experiments did not show a reduction in root length colonized due to As, although some authors have shown a negative effect of As on colonization in some plants (Liu et al., 2005b).

Different plants showed different reactions towards the As in the contaminated soil. The most sensitive toward the As was tomato and the high most tolerant was the maize. This result different from that of (Carbonell-Barrachina et al., 1997) who found that tomato was more tolerant to As than bean at As concentration of 0 - 10 mg L⁻¹. In this study tomato died after 25 days of growth in contaminated soil and the bean could survive in the same soil until the end of the experiment. This result is in agreement with (Miteva, 2002) who found that As at 25 mg kg⁻¹ caused reduction in dry weight and death of tomato.

Different native communities of AMF were found in the contaminated and uncontaminated soil. Some AMF can survive in contaminated soil at high concentration of As. This may be because the spores and the mycorrhizal communities found in the soil for a long time become adapted to the contaminated environment and are selected for resistance to As toxicity (Gonzalez-Chavez et al., 2002; Ultra et al., 2007a). Because there were different communities of fungi in contaminated and uncontaminated soils that may differ in their adaptation to As, inoculum from these two soils was compared in the next experiment.

The other purpose from this experiment was to identify a good plant to use for more experiments that can survive in contaminated soil, but still showed significant growth reduction. Maize was most suitable, also maize is a good mycorrhized plant and responds quickly to inoculation and can get high levels of infection. Maize also grows fast and is less sensitive to P deficiencies than plants like tomato.

In a conclusion, commercial inoculation failed to increase colonization, and failed to promote the growth of plants in As-contaminated soil, although there was evidence that inoculation improved growth of spinach, okra, capsicum, lettuce and eggplant in contaminated soil by increasing dry weight compared with un-inoculum plants. However, it seems that was not due to mycorrhizal inoculation but probably some other component of the inoculum. Further studies are needed to test mycorrhizal inoculums originating from different soils contaminated with different As concentration instead of one concentration because different plant showed different reaction toward the As. Different communities of AMF were present in the contaminated and uncontaminated soil, and their effectiveness in presence of As should be compared.

4 Effect of origin of AMF in arsenic remediation

4.1 Introduction

In the previous chapter, it was shown that vegetable crops differed greatly in their sensitivity to As. Maize (sweet corn) showed significant reduction in growth at typical concentrations of As contaminated soil, but was able to survive for a period sufficient for experimental use. Different communities of mycorrhizal fungi were found in contaminated and uncontaminated soil, but it is not known what the implications of this are for their function in the presence of As. Because inoculation had little effect on mycorrhizal colonization percentage, it was not possible to determine any interaction between mycorrhization and As.

Propagating AMF by growing them with living host plant in soil pot culture can be used as inoculum for experiments. Pot cultures consist of soil, spores, root pieces and hyphae (Brundrett et al., 1996; Leung et al., 2005). (Gonzalez-Chavez et al., 2002) found that propagules of AMF in As-contaminated soil were able to colonize plants. (Zhao et al., 2009) found that the spores produced in trap cultures in As-contaminated soil were less sensitive to As than those isolated from un-contaminated soil. Most workers appear to have assumed that AMF from contaminated soil should be more effective in protecting plants from As, but this has not been widely assessed.

This experiment in this chapter was carried out to investigate the effects of mycorrhizal inoculum from two different origins propagated in pot culture, on As concentration and As

uptake by maize and the tolerance of host plant to As in autoclaved soil spiked with different levels of As.

4.2 Methods

4.2.1 Mycorrhiza inoculum

Mycorrhizal inoculum from As contaminated soil and un-contaminated soil (as used in Chapter 2) was produced in pot culture. The soils were mixed 1:1 with river sand and placed in 12 cm pots. One plant per pot of maize cv. Kelvedon Glory was grown for 10 weeks. Roots of maize were tested for the percentage of colonization to confirm a high level of infection. Spores were extracted from the soil as described in Chapter 2. The number of spores was in the range of 86-240 spores per 100 g soil in uncontaminated soil and 128-246 in the contaminated soil. The plants were removed from the pots, and the root systems with adhering soil were chopped coarsely to produce the inoculum. Part of each inoculum was autoclaved for use in control experiments.

4.2.2 Pot experiment

Uncontaminated soil from Jennings, near Tenterfield (Chapter 3) was mixed 1:1 with river sand. The soil-sand mix was sieved to less than 2 mm and autoclaved on two successive days for 1 hour at 121 °C. The arsenic content of the sand-soil mix determined by acid digestion and ICP-OES (Chapter 3) and was 2.7 mg kg⁻¹. 900 g of sand-soil mix was placed into pots lined with plastic bags.

Arsenic solutions were prepared from sodium arsenate (Na₂HAsO₄·7H₂O) and added to the pots to give nominal rates of 0, 5, 10, 25, 50 and 75 mg kg⁻¹. These As concentrations are

typical of the range of As concentrations in contaminated agricultural soil (Ahmed et al., 2011).

Each pot received 100 g of live or autoclaved mycorrhizal inoculum spread in a thin layer, then 100 g of autoclaved soil: sand spread over the inoculum. Measurement of arsenic levels in soil of 36 representative pots at the end of the experiment showed that inoculum from the contaminated soil increased the average arsenic concentration of the whole pot by 3 mg kg⁻¹.

Seeds of sweet corn Kelvedon Glory F1 were pre-germinated for 2 days at 25 °C. Three seeds were sown in each pot and then thinned to one after emergence.

The pots were watered regularly to 80% of the field capacity. All pots received weekly an application of soluble complete fertiliser (Aquasol). The fertilizer content of elements is shown in Table (4.1). Each pot received 16 mg Aquasol per pot dissolved in deionised water to support the plant.

Table 4.1 Elemental composition of Aquasol fertilizer

Elements	% w/w	mg / pot
Nitrogen (N) as ammonium	1.700	0.272
.Nitrogen (N) as urea	21.300	3.408
Total nitrogen (N)	23.000	3.68
Total phosphorus as water soluble	3.950	0.632
Total potassium (K) as sulphate	14.000	2.24
Sulphur (S) as sulphate	6.600	1.056
Magnesium (Mg) as sulphate	0.150	0.024
Manganese (Mn) as sulphate	0.130	0.0208
Copper (Cu) as sulphate	0.060	0.0096
Iron (Fe) as sodium ferric EDTA	0.060	0.0096
Boron (B) as sodium borate	0.010	0.0016
Molybdenum (Mo) as sodium molybdite	0.001	0.00016

The factorial experiment was arranged in a Randomized Complete Block Design (RCBD) in the glass house of University of New England on 13 February 2015. Three factors were used in the experiment: mycorrhizal inoculum from two origins, contaminated soil (co)

and uncontaminated soil; live (M+) or autoclaved (M-) inoculum; and 6 concentration of As. There were 5 replications.

4.2.3 Sampling

Measurements for height of the plant, number and width of the largest leaf and width of the stem were measured each 10 days during the plant growth period.

4.2.3.1 Maize shoots and cobs

The plants were harvested 75 days after sowing, by cutting 1 cm above the soil surface. Shoots (leaves, stalks) and cobs were harvested separately. Fresh weight for each was recorded, then they were rinsed twice with deionized water and each part of plant chopped into small pieces separately and stored in weighed acid washed (10% HCl) 70 mL specimen containers. The samples were frozen then freeze dried to determine dry weight and for analysis of As and phosphorus content.

4.2.3.2 Maize roots and rhizosphere soil

The contents of each pot was emptied into a tray (separate trays for each pot), and the soil carefully shaken from the roots. Soil was washed off the root system first with water over a sieve and then in a sonicator bath, then roots were rinsed with deionized water. The fresh weight of roots was obtained, and a portion of the root samples was stored in 50% ethanol for determination of mycorrhizal colonization (Wang et al., 2008). The rest of the root system was placed in an acid-washed 70 mL specimen container and freeze dried to determine dry weight and for use in measuring As and phosphorus content. Dry weights

were adjusted according to the amount of material removed for mycorrhizal determination. Soil from 3 replicates of each treatment at 0, 50 and 75 mg kg⁻¹ of As was collected from the pots, oven dried (40 °C) for 3 days, then sieved through a 2 mm sieve and kept for arsenic and phosphorus analysis.

4.2.4 Mycorrhizal colonization

Mycorrhizal colonization was measured using ink-vinegar staining and the line intercept method as described in Chapter 3.

4.2.5 Arsenic uptake and concentration

The freeze dried shoot, root and cob samples from 3 replicates (because of cost, not all treatments or replicates could be analysed) of each treatment at 0, 50 and 75 mg kg⁻¹ of As were milled in a coffee grinder. Arsenic and phosphorus contents were measured by ICP-AES after acid digestion by ALS Environmental, Sydney, Australia. Plant samples were analysed by a commercial laboratory because it was expected that As concentrations in some treatments would be below the limit of detection of the techniques available at the University.

4.2.5.1 Arsenic in soil analysis

0.5 g of ground soil sample was digested with a mixture of hydrochloric acid and nitric acid in a microwave digester at 175 °C, for 40 minutes, then the solution was made up to 25 mL total volume with deionised water and mixed well prior to analysis. The extract was

analysed for total As by using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) in the University of New England laboratories.

4.2.5.2 Phosphorous in soil analysis

Available phosphorous in soil samples after harvesting was determined by the Colwell method. A sample of 0.4 g air-dried sieved soil was mixed with 40 mL 0.5 M sodium bicarbonate (NaHCO_3) solution at pH 8.5 and shaken for 16 hours at 20 °C, and filtered through Whatman No.42 filter papers. Phosphorus content in the extract was measured calorimetrically (Colwell, 1963).

4.2.6 Statistical analysis

All results were expressed as means and the effects of mycorrhizal inoculum on As and plant growth were examined by using the two-way analysis of variance with statistical program SPSS version 22. The least significant differences (LSD) at the 5% level was calculated to compare the means between and within the treatments.

4.3 Results

4.3.1 Mycorrhizal colonization

There was no mycorrhizal colonization in any plant inoculated with the autoclaved inoculum (M- treatment). Mycorrhizal colonization rate was significantly affected by As concentration in soil and by the origins of mycorrhizal inoculum. The mycorrhizal inoculum originated from contaminated soil (coM+) caused significantly higher percentage of colonization than mycorrhizal inoculum originated from uncontaminated soil

(unM+) (Figure 4.1). There was a significant effect of As on mycorrhizal colonization, which decreased as arsenic concentration increased (Figure 4.1). The interaction between As concentration and origin of inoculum was not significant.

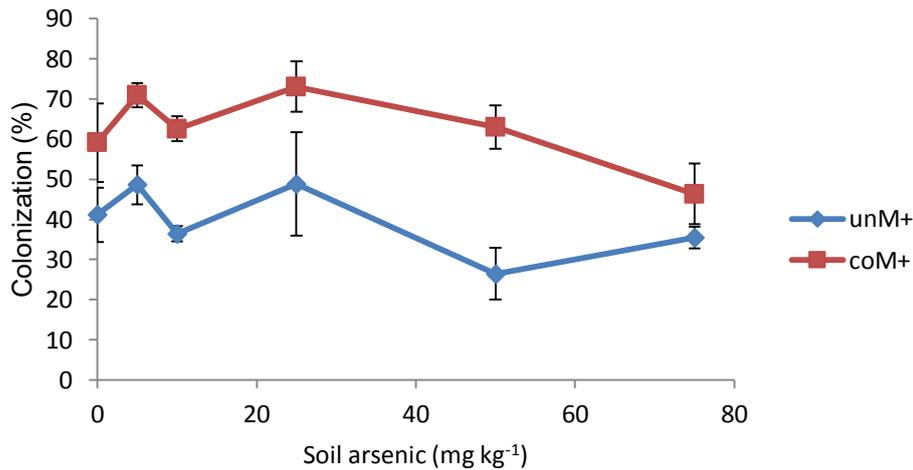


Figure 4.1 Effects of soil arsenic concentration on the percentage mycorrhizal colonization of roots of maize inoculated with mycorrhizal inoculum (M+) from uncontaminated soil (un) or soil contaminated with arsenic (co). Error bars show standard errors (n=5).

4.3.2 Height

Arsenic concentration, mycorrhizal inoculum, and the interaction between inoculum and As concentrations all had highly significant ($P < 0.001$) effects on the height of the plants. For all inoculum types, plant height decreased as arsenic concentration increased (Figure 4.2). For the live mycorrhizal treatments (M+), plants were significantly taller at the highest concentrations of As with mycorrhizal inoculum originated from contaminated soil (coM+) than from uncontaminated soil (unM+). In the non-mycorrhizal treatments (M-), plants grown in 75 mg kg⁻¹ As were taller when treated with autoclaved inoculum from uncontaminated soil (unM-) than inoculum from contaminated soil (coM-) (Figure 4.2).

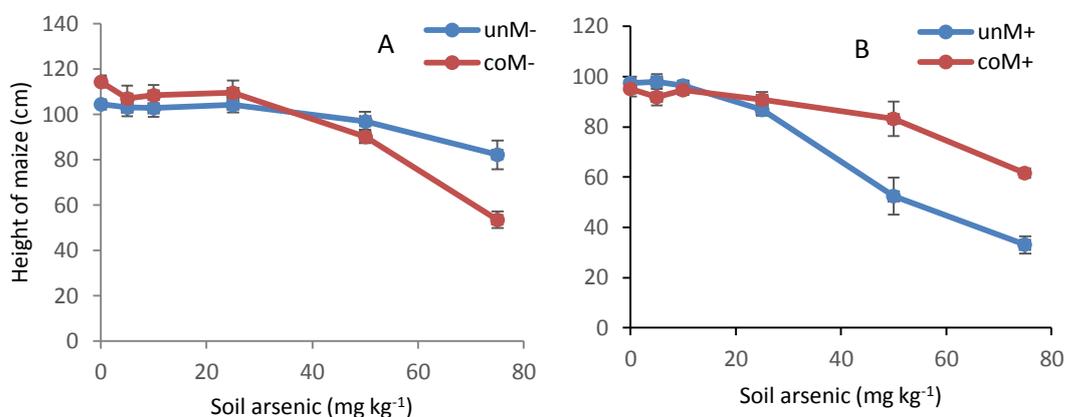


Figure 4.2 A: Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on the height of maize. B: Effects of live mycorrhizal inoculum (M+) on the height of maize. Error bars show standard errors (n=5).

4.3.3 Dry weight of shoots

Arsenic concentration, mycorrhizal inoculum, and the interaction between inoculum and As concentrations all had highly significant ($P < 0.01$) effects on the shoot dry weight of the plants. For all inoculum types, shoot dry weight decreased as arsenic concentration increased (Figure 4.3). Shoot dry weight was on average 23% higher for plants treated with autoclaved inoculum (unM-, coM-) than with live inoculum (unM+, coM+). For the live mycorrhizal treatments (M+), plants had significantly higher shoot dry weights at the highest concentrations of As with mycorrhiza originated from contaminated soil (coM+) than from uncontaminated soil (unM+). In the non-mycorrhizal treatments (M-), plants grown in 75 mg kg⁻¹ As had higher shoot dry weight when treated with autoclaved inoculum from uncontaminated soil (unM-) than from contaminated soil (coM-) (Figure 4.3).

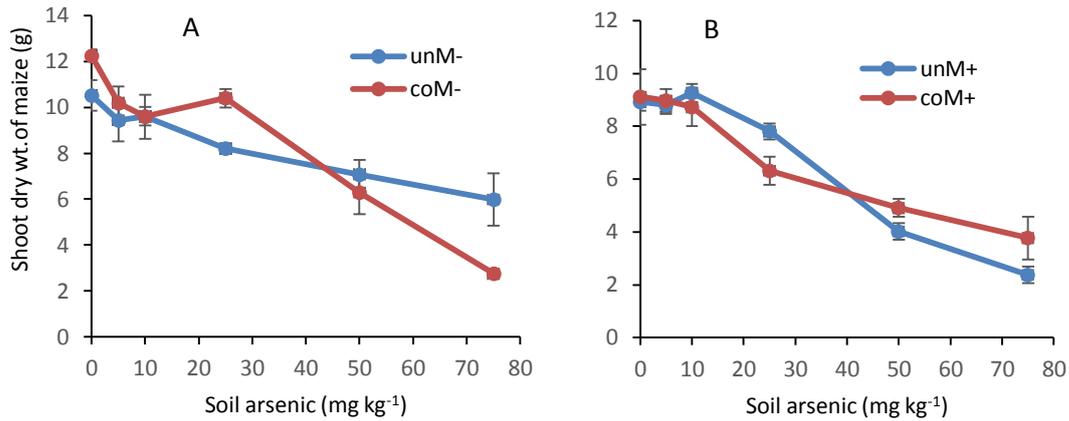


Figure 4.3 A: Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on dry weight of maize shoots. B: Effects of live mycorrhizal inoculum (M+) on dry weight of maize shoots. Error bars show standard errors (n=5).

4.3.4 Dry weight of roots

Arsenic concentration and inoculum type significantly affected the dry weight of roots of the maize. There was no significant interaction between As concentration and inoculum type. Root dry weight decreased as As concentration increased (Figure 4.4). There were no significant differences in root dry weight between the two origins of mycorrhizal inoculum (coM) and (unM), but for both root dry weight was higher with autoclaved inoculum (M-) than with live inoculum (M+).

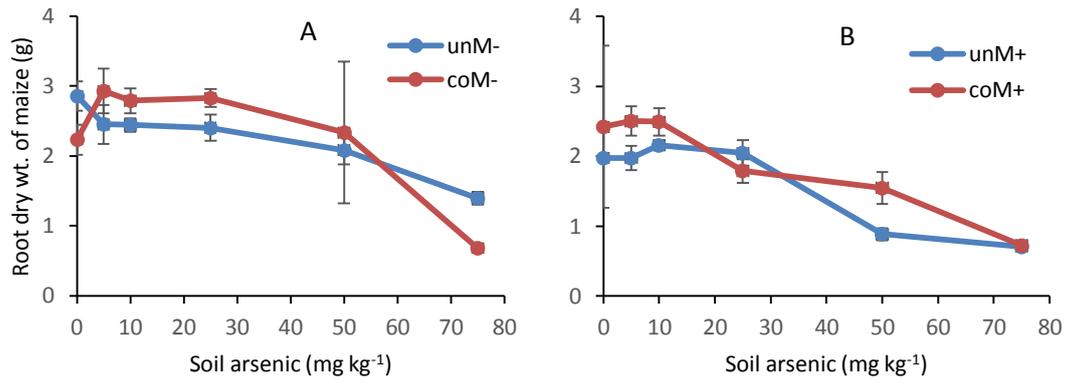


Figure 4.4 A: Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on maize root dry weight. B: Effects of live mycorrhizal inoculum (M+) on maize root dry weight. Error bars show standard errors (n=5).

4.3.5 Dry weight of cobs

There was no significant main effect of As concentration on the dry weight of cobs. The main effect of inoculum type on cob dry weight was significant, with cob weight tending to be higher in the autoclaved inoculum treatments (M-) (Figure 4.5). There was a significant interaction between As concentration and inoculum type, which was seen as a very high cob weigh in the unM- treatment at 25 mg kg⁻¹ of As (Figure 4.5).

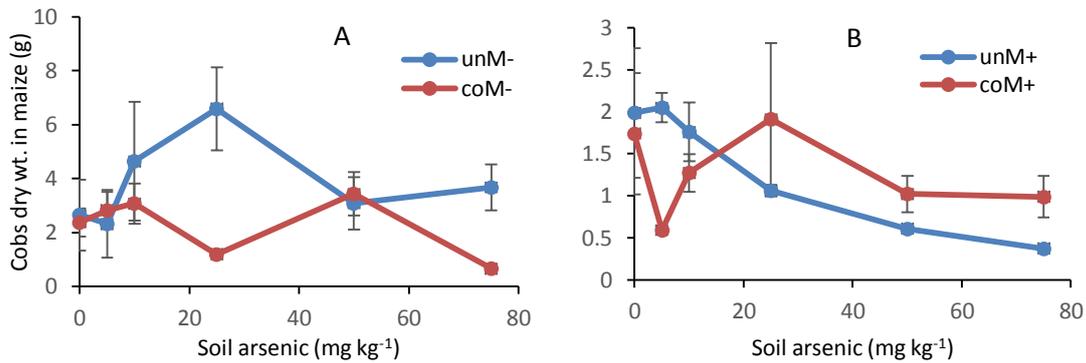


Figure 4.5 A: Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on maize cob dry weight. B: Effects of different live mycorrhizal inoculum (M+) on maize cob dry weight. Error bars show standard errors (n=5).

4.3.6 Arsenic concentration in shoots

The As concentration of shoots was significantly affected by As concentration in soil, mycorrhizal colonization, and origin of mycorrhizal inoculum. Shoot As concentration increased as soil As concentration increased (Figure 4.6).

The As concentration in the mycorrhizal plants (M+) was significantly lower than non-mycorrhizal plants (M-) (Figure 4.6).

Mycorrhizal inoculum originated from un-contaminated soil (unM+) gave significantly lower As concentration in comparison with mycorrhizal inoculum originated from contaminated soil (coM+).

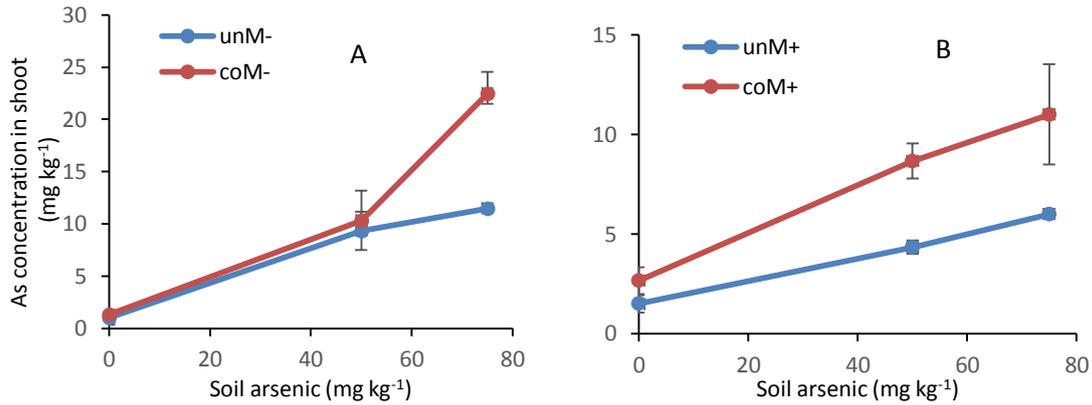


Figure 4.6 A: Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on As concentration in shoots of maize. B: Effects of live mycorrhizal inoculum (M+) on As concentration in shoots of maize. Error bars show standard errors (n=3).

4.3.7 Arsenic uptake in shoots

Arsenic uptake (concentration times dry weight) by the shoots was significantly affected by As concentration in the soil, mycorrhizal colonization, origin of mycorrhiza, the interactions between the origin of inoculum and mycorrhizal colonization, and the interaction between the concentration of As in the soil and mycorrhizal colonization.

In non-mycorrhizal plants (unM- and coM-) As uptake increased as soil As concentration increased, and there was no difference between the two sources of inoculum (Figure 4.7). Mycorrhizal plants (unM+ and coM+) showed significantly lower As uptake compared with non-mycorrhizal plants at the highest soil As concentrations.

Plants inoculated with mycorrhizal inoculum from uncontaminated soil (unM+) had significantly lower As uptake than plants inoculated with mycorrhiza from contaminated

soil (coM+). In both live mycorrhizal treatments, As uptake was lower at 75 mg kg⁻¹ of soil As than autoclaved mycorrhizal treatment at 50 mg kg⁻¹ (Figure 4.7).

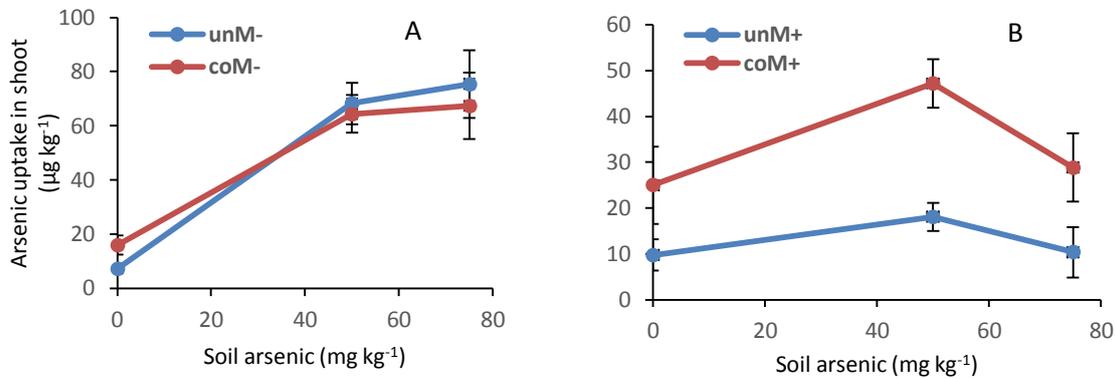


Figure 4.7 A: Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on As uptake in shoots of maize. B: Effects of live mycorrhizal inoculum (M+) on As uptake in shoots of maize. Error bars show standard errors (n=3).

4.3.8 Arsenic concentration in cobs

The concentration of As in the dry weight of cobs was very low compared with dry weight of root and shoot. It did not exceed 2 mg kg⁻¹ in any replicate. Because this was only slightly higher than the limit of detection (1 mg kg⁻¹), the data were not analysed statistically.

4.3.9 Arsenic concentration in roots

Arsenic concentration in the roots increased significantly as As concentration in the soil increased (Figure 4.8). Root As concentration was slightly lower in plants with live inoculum (M+). Plants inoculated with mycorrhizal inoculum originated from

contaminated soil (coM+) had significantly higher concentrations of As in roots than with mycorrhizal inoculum originated from uncontaminated soil (unM+) (Figure 4.8).

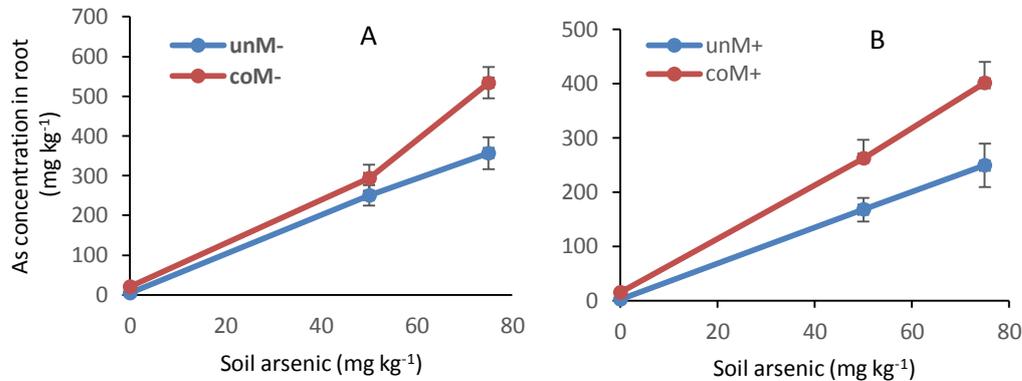


Figure 4.8 A: Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on As concentration in roots of maize. B: Effects of live mycorrhizal inoculum (M+) on As concentration in roots of maize. Error bars show standard errors (n=3).

4.3.10 Arsenic uptake in roots

There were significant effects of soil As concentration, mycorrhizal colonization, and interaction between source of inoculum and soil As concentration on As uptake by roots. Root uptake was higher in non-mycorrhizal plants (M- treatments) than mycorrhizal plants (M+ treatments) (Figure 4.9). Root uptake of As at 50 mg kg⁻¹ of soil As was higher in the treatments with inoculum from contaminated soil (coM-, coM+) than with the inoculum from uncontaminated soil (unM-, unM+) (Figure 4.9).

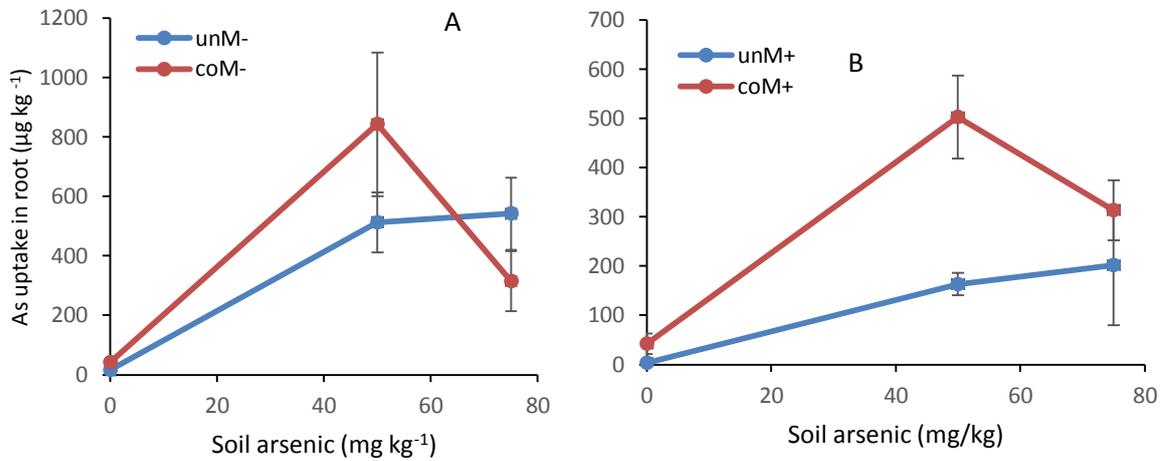


Figure 4.9 A: Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on As uptake in roots of maize. B: Effects of live mycorrhizal inoculum (M+) on As uptake in roots of maize. Error bars show standard errors (n=3).

4.3.11 Translocation factor (TF)

Translocation factor (the ratio of As concentrations in the shoot to As concentration in root) could only be calculated accurately for plants from the 50 mg kg⁻¹ soil As treatment due to very low plant As concentrations at 0 mg kg⁻¹ and lack of sufficient root material in some treatments at 75 mg kg⁻¹. The mean translocation factor at a soil As concentration of 50 mg kg⁻¹ was 0.034, and there were no significant effects of mycorrhizal source or colonization status on this.

4.3.12 Phosphorus concentration in shoots

Arsenic concentration in soil, mycorrhizal colonization, and the interaction between arsenic concentration in soil and mycorrhizal colonization had significant effects on phosphorus concentration in the shoot after harvest. Phosphorus concentration increased with the increase of As concentrations in the soil in the M⁺ treatments (Figure 4.10). Phosphorus concentration was higher in plants that were inoculated with live mycorrhizal inoculum (M⁺ treatments) (Figure 4.10). The effect of mycorrhizal colonization on Phosphorus concentration was greater at higher concentrations of soil As.

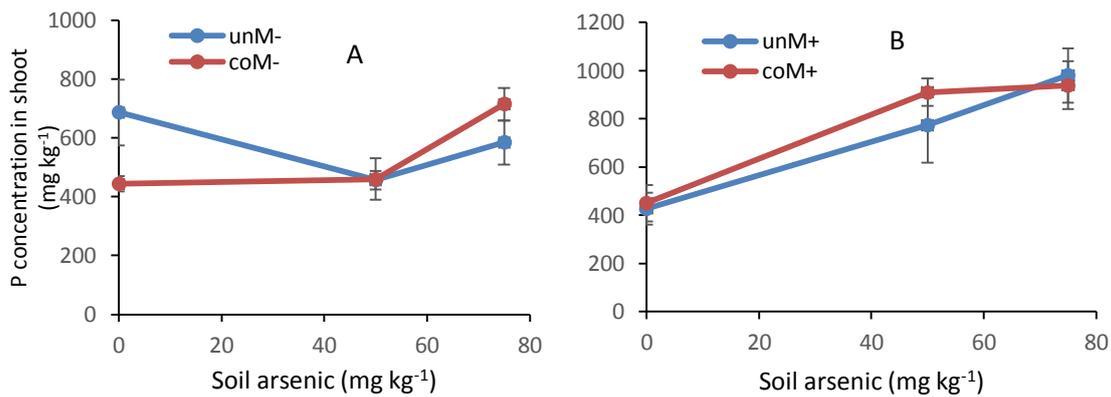


Figure 4.10 A: Effects of autoclaved mycorrhizal inoculum (M⁻) from contaminated (co) and uncontaminated (un) soil on P concentration in shoots of maize. B: Effects of live mycorrhizal inoculum (M⁺) on P concentration in shoots of maize. Error bars show standard errors (n=3).

4.3.13 Phosphorus concentration in roots

Inoculation with mycorrhizal inoculum increased the concentration of P in the roots of maize significantly compared with non-mycorrhizal plants (Figure 4.11). With increased As concentration in the soil phosphorus concentration in roots increased. The interaction

between the As concentration in soil and mycorrhizal colonization was significant, with the effect of mycorrhizal inoculum on root P concentrations being greatest at high concentrations of soil As concentration (50, 75 mg kg⁻¹).

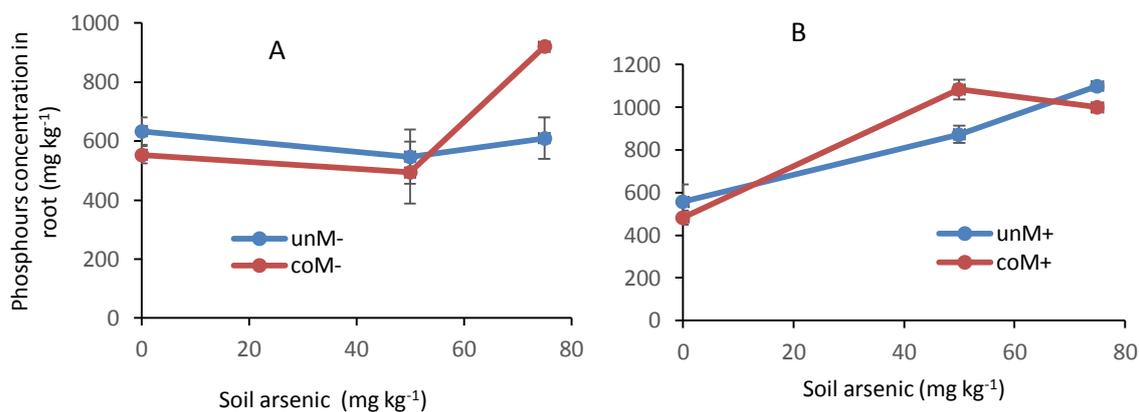


Figure 4.11 A: Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on P concentration in maize roots. B: Effects of live mycorrhizal inoculum (M+) on P concentration in maize roots. Error bars show standard errors (n=3).

4.3.14 P/As ratio in shoots

The ratio between the concentration of P and As in shoots could not be calculated reliably for soil As concentration of 0 mg kg⁻¹ because shoot As concentration levels were below or close to the limit of detection. For soil As concentrations of 50 and 75 mg kg⁻¹, the P/As ratio was significantly affected by origin of inoculum and whether it had been autoclaved. P/As ratio was higher in plants inoculated with live mycorrhizal inoculum (M+ treatments) than with autoclaved inoculum (M- treatments). The P/As ratio was significantly higher

with inoculum from uncontaminated soil (Figure 4.12). This was particularly evident for the live inoculum (unM+) although the interaction between inoculum source and autoclaving was not significant ($P = 0.093$).

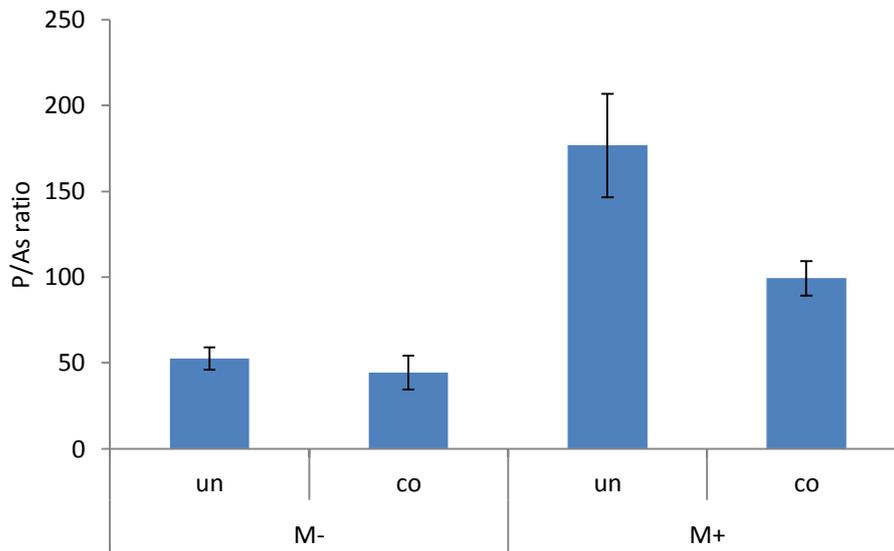


Figure 4.12 Effects of autoclaved mycorrhizal inoculum (M-) from contaminated (co) and uncontaminated (un) soil on P/As concentration ratio in shoots of maize. Error bars show standard errors (n=3).

4.3.15 Soil phosphorus concentration

Arsenic concentration in the soil and mycorrhizal colonization significantly affected the concentration of bicarbonate-extractable P in soil after harvest. With increased concentration of As in soil, extractable P concentration increased from 19.1 mg kg⁻¹ standard error (SE) = 2.2144 at As concentration of 0 mg kg⁻¹ of As, to 31.0 mg kg⁻¹

SE = 1.5901 at As concentration of 75 mg kg⁻¹. Soil after growing mycorrhizal plants had significant higher concentration of extractable P than after non-mycorrhizal plants. The P concentrations were 27.4 mg kg⁻¹ SE = 1.7493 and 22.9 mg kg⁻¹ SE = 1.7676 respectively.

4.4 Discussion

In this chapter, the effects of inoculating maize with mycorrhizal inoculum from two different origins at different soil concentrations of As on growth as well as As and phosphorous uptake were studied. As arsenic concentration increased, mycorrhizal colonization decreased, dry weight and height of plants decreased, As concentration in tissues increased, and phosphorus concentration in the tissues also increased. Mycorrhizal plants (treated with live inoculum) had lower As concentration and uptake, higher phosphorus concentration, and lower dry weights than non-mycorrhizal plants. Inoculation of plants with mycorrhiza from contaminated soil resulted in higher colonization of roots, and higher As concentration and uptake, than mycorrhizal inoculum from uncontaminated soil.

In mycorrhizal plants the concentration of As in the shoots of maize was decreased significantly compared with non-mycorrhizal plants. Several papers have mentioned that there are positive effects of mycorrhizal inoculum on growth in contaminated soil. Yu et al. (2010) showed that inoculation of maize with *Glomus mossae* or *G. etunicatum* reduced As accumulation in the shoots, although inoculation with other AMF had no effect. This benefit may be related to the fungal hyphae associated with host plant roots which may reduce or exclude the entry of contaminants into the plants (Ahmed et al., 2011; Meharg & Cairney, 2000). The possible mechanisms that may explain this result are that mycorrhizal plants might exclude As (III) to external media and reduce As (V) uptake from As-

contaminated soils and thus will reduce As uptake by AM plants (Gonzalez-Chavez et al., 2002; He & Lilleskov, 2014).

Mycorrhizal plants also showed lower dry weight than non-mycorrhizal. The reason might be that mycorrhiza decreases the rate of root respiration (Del-Saz et al., 2017), and might be that mycorrhiza expanded the root system of maize to allow the mycelium to develop and to colonize up to 60-90% of the length of the root system (Bonfante & Perotto, 1995; Rillig, 2004) . This needs additional amount of nutrition more than non-mycorrhizal plant, and due to the limited volume of the pot may lead to limited growth.

The origin of mycorrhiza affected the results. Mycorrhiza originated from contaminated soil (coM+) had higher As uptake and concentration in tissues, compared with mycorrhiza originated from uncontaminated soil (unM+). Gonzalez-Chavez et al. (2002) concluded that AMF from contaminated soil may become adapted to As contamination and show less sensitivity to As, partially explaining survival in contaminated soils, when they assessed spore production in trap cultures in As-contaminated mine soil. They showed that a reasonably diverse population of AMF was present, and studied effects of As on germination of a few selected species. Of these spores from the mine-site populations were less sensitive to As than those isolated from uncontaminated sites leading to their conclusion.

My results agree with (Ortowska et al., 2012) who inoculated *Plantago* with AMF of different origins produced in pot culture, and grown in substrate of extremely high content of As-contaminated soil. The soil was pasteurized to eliminate indigenous mycorrhizal fungi. They found there were significant differences in As concentration and uptake between different AMF isolates. Inoculation with an isolate indigenous to contaminated

soil resulted in increased transfer of As from roots to shoots. An isolate from a non-polluted area apparently restricted plants from absorbing As into the tissue. This finding is consistent with the results of this experiment when coM⁺ caused higher As concentration and uptake than unM⁺.

As well as, mycorrhizal inoculum originated from contaminated soil (coM⁺) had significantly higher colonization than mycorrhizal inoculum originated from uncontaminated soil (unM⁺) at all concentrations of As. The reason for this is not known, but may reflect the differences in species present in the two soils (Chapter 3). Rates of colonization decreased slightly but significantly for both sources of mycorrhizal inoculum as soil As concentration increased, but there was no difference between the mycorrhizal inoculum from contaminated or non-contaminated soil in the rate of decrease. This result is consistent with (Yu et al., 2010) who found the root colonization of maize decreased with As concentration in the range 0 to 100 mg kg⁻¹, and with (Ahmed et al., 2006) who found a significant reduction in mycorrhizal colonization due to the effects of As at concentrations of 0-10 mg L⁻¹ on lentil inoculated with *G. mosseae*. However, this result is opposite to the finding of (Liu et al., 2005) that there was no significant inhibition of mycorrhizal colonization of *Pteris vittata* by *G. mosseae* when arsenate was added at the rate of 300 mg kg⁻¹. However, this plant is a hyperaccumulator and highly tolerant to As.

Although in this experiment a limited amount of P was added to all pots, there was an increase in P concentration in the shoots of mycorrhizal plants compared with non-mycorrhizal. It is known that the importance of AMF in crop production comes from the ability to stimulate plant growth in soils with limited amounts of available P. Improving productivity in soil of low fertility by increasing the uptake of slowly diffusing ions such

as phosphate (PO_4^{3-}) (Jakobsen et al., 1992; Smith et al., 2010). Due to a large surface area of AMF external mycelium the nutrient uptake increases, especially P (Jakobsen et al., 1992). The increase in P concentration in mycorrhizal plants was opposite to the decrease in As concentration, leading to higher P:As ratios. Phosphate is generally considered as having the same chemical behaviour as arsenate and may compete with arsenate for sorption sites on the surface of soil particles or roots (Jackson & Miller., 2000). It is expected that As and P will restrict or inhibit uptake of each other because they are using the same uptake system (Ahmed et al., 2006), which suggests that P may play an important role in the interaction between AMF inoculation and As uptake by participating in and inhibiting As translocation into the shoots (Yu et al., 2010). Although P and As are thought to be taken up by the same transport system, there must be some selectivity in the maize-AMF symbiosis used here, because of the different effects of mycorrhizal inoculum on the two elements.

Higher concentration of P was found in shoots of maize inoculated with both sources of mycorrhizal inoculum compared with non-mycorrhizal plants in As-contaminated soil. This result is consistent with (Smith et al., 2010) who found that inoculating plants with AMF enhances plant uptake of P and improves the plant growth. In this study, colonization with AMF decreased growth despite the reduced uptake of As and increased uptake of P. This may be because the costs of supplying carbon to the AMF were greater than the benefits of higher P and lower As in the pot system was used.

Concentration of P in the maize roots and shoots was increased with increased As concentration in the soil. Bicarbonate extractable P in soil was also higher at high As

levels. This was probably because addition of arsenate caused release of phosphate due to competition for sorption sites in the soil (Jackson & Miller., 2000).

In conclusion, this experiment confirmed that mycorrhizal inoculum can reduce the uptake of As by plants. Mycorrhizal inoculum from uncontaminated soil had a larger effect on reducing As uptake than mycorrhizal inoculum from contaminated soil. No improvement in growth was found, presumably because of limitations of experiments in pots. The results show that use of mycorrhizal inoculum for allowing growth of food crops in contaminated soil may be effective. Further work needs to be done using different soils, different crops to understand the effects of AMF from different sources.

5 Magnetic biochar for arsenic remediation

5.1 Introduction

Biochar is a carbon-rich material produced from different types of biomass feedstock by heating in the absence of oxygen, used as a soil amendment. Recently biochar has gained attention for its high ability to adsorb pollutants including heavy metals (Kookana et al., 2011) due to its large surface area (Mukherjee & Zimmerman, 2013; Revell et al., 2012). However the surfaces of biochars are mostly negatively charged (Mukherjee & Zimmerman, 2013), and so have little capacity to adsorb negatively charged arsenic (As) ions. On other hand, iron is known to have a high ability to adsorb As (Aredes et al., 2012; Chen et al., 2011), but it tends to form aggregates when it contacts a solution, which reduces the surface area and reduces its adsorption capacity (Zhang et al., 2013a). Combining biochar with iron may create a low cost remediation material with high absorption capacity and with properties that cannot be achieved by any of components acting alone. (Chen et al., 2011) described a novel magnetic biochar made by adding iron salts to organic material before pyrolysis. made magnetic biochar by a different process, by precipitating and oxidising iron salts onto biochar, and using this to bind anionic pollutants. Magnetic biochars have been tested by several workers for removing arsenate from water, but not from soil (Oliveira et al., 2002; Wang et al., 2015; Zhang & Gao, 2013b). I hypothesised that adding a magnetic biochar to As-contaminated soil growing maize, will reduce As concentration and uptake by the maize due to the increased sorption capacity compared with non-magnetic biochar.

The objective of this experiment was to use activated coconut husk biochar combined with iron to form a modified magnetic biochar. The sorption ability to As of magnetic biochar

was measured in the laboratory through a batch sorption experiment. An experiment conducted in the glasshouse used magnetic biochar, iron and biochar at different rates to test their effects on As concentration and uptake by maize in As-contaminated and un-contaminated soil.

5.2 Methods

5.2.1 Magnetic biochar preparation

The magnetic biochar (MB) was prepared using activated biochar from coconut husks (untreated biochar)(Coconut carbon manufactured by steam activation process, pH 7, total ash 1%, surface area $1050 \text{ m}^2 \text{ g}^{-1}$), and magnetite (Fe_3O_4 nanoparticles) as the magnetic medium (Sun et al., 2015). Ferrous chloride (9.9 g) and ferric chloride (27 g) were dissolved in 100 mL of deionized water and 10.5 g of biochar was added. This was stirred vigorously with a magnetic stirrer at a temperature of $80 \text{ }^\circ\text{C}$, and then a 5 M NaOH solution was added drop wise until the iron solution precipitated. The stirring was then continued for 1 hour. The mixture was next separated by centrifugation at 3600 g for 10 minutes and the precipitate was oven-dried at $60 \text{ }^\circ\text{C}$ to a constant weight (Sun et al., 2015). Iron oxide precipitate (Fe) was also prepared by following the above protocol but omitting the biochar. (Sun et al., 2015) showed that the final product will be a biochar coated with magnetic Fe_3O_4 nanoparticles which have amorphous and crystalline phases, consisting of Fe_3O_4 , NaCl, and SiO_2 .

5.2.2 Sorption experiment

Sorption of As on magnetic biochar, iron oxide and untreated biochar were measured by a batch experiment (Namgay et al., 2010b). The treatments of 25 mL of 0.01 M $\text{Ca}(\text{NO}_3)_2$ solution were placed in centrifuge tubes with 1 g of each material in each tube (except the control treatment without material) and the suspension adjusted to pH 7 by adding dilute HCl. After obtaining the desired pH of the suspension materials, 1 mL of 0.25M sodium arsenate solution was added to the tubes. The material mixtures were equilibrated overnight at 21 °C on rotary shaker at 30 rpm. The suspension pH was recorded at the end of equilibration and then the samples were centrifuged at 3000 rpm for 15 min followed by filtration through Whatman No. 1 filter paper. The supernatant solutions were analysed for As by ICP-OES (Namgay et al., 2010b).

5.2.3 Glass house experiment

Two glasshouse experiments were carried out at the University of New England (UNE), Armidale, NSW, Australia to investigate the effects of magnetic biochar, untreated biochar and iron oxide on As concentration and uptake in maize shoots, and on maize growth in two soils (As contaminated soil and un contaminated soil) that were used in Chapters 3 and 4. The experiments with the contaminated soil and uncontaminated soil were conducted separately. For the experiment with contaminated soil the treatments consisted of three rates of magnetic biochar application (0, 5, and 15 g kg^{-1} soil) (which is similar to the rates of application of biochar in the literature) and the same application rates for untreated biochar and iron oxide. Control treatments had no additives. There were 6 replicates of each treatment. Plastic pots (12 cm top diameter) were lined with plastic bags to prevent

leaching. The required amount of each of magnetic biochar, untreated biochar and iron oxide crushed and sieved to <2 mm, was thoroughly mixed with a mixture of sieved (<2 mm) soil: sand (1:1 w/w) and 1 kg added to the pots. The pots were then irrigated with 100 mL of deionised water and allowed to equilibrate for 3 days. Three seeds of sweet corn *Zea mays* (Kelvedon Glory F1) pre-germinated for 2 days at 25 °C were sown in each pot then the seedlings thinned to one after emergence. All treatments received weekly application of soluble complete fertiliser (16 mg Aquasol per pot, dissolved in deionised water). The plants were watered regularly with deionised water to 80% of field capacity. The temperature in the glasshouse was adjusted to 25/15 °C day/night. After 10 weeks, the shoots of the maize plants was harvested. The harvested shoots were weighed and thoroughly washed in running water, rinsed with deionised water then chopped and stored in acid-washed containers in a freezer for at least 24 hours. The frozen samples were freeze dried and the dry weight of samples recorded. Freeze-dried shoot material from 4 replicates of each treatment in the experiment with contaminated soil was milled with a coffee grinder and assayed for As and phosphorous (P) content using acid digestion and ICP-AES by ALS Environmental, Sydney. Height and leaf number of maize were measured each 10 days, and mycorrhizal colonization were measured with the ink-vinegar (Chapter 3).

The experiment with uncontaminated soil (soil: sand) (w/w 1:1) was conducted similarly to the one with contaminated soil, except that the highest rates of magnetic biochar 15 g kg⁻¹ and iron oxide 15 g kg⁻¹ were not used because of their high toxicity to the plant in the first experiment resulting in death to plants at early stages of growth. As well, there were only 4 replicates of each treatment, and As concentration in the plants was not measured.

Data were analysed by one way ANOVA (analysis of variance) with statistical program SPSS version 22. The criterion for significance was $P < 0.05$. Log transformation was used when necessary to correct for non-homogeneity of variance. Duncan's multiple range test was used for mean separation at the 5% level.

5.3 Results

5.3.1 Sorption experiment

There were highly significant differences in As sorption between treatments (Figure 5.1). Untreated biochar (biochar) removed 20% of As in solution, magnetic biochar (MB) removed 75% of As in solution and iron oxide (Fe) removed almost 100%.

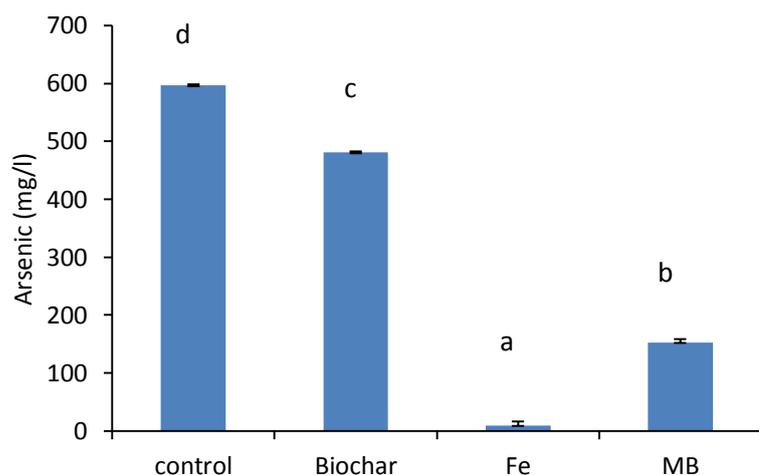


Figure 5.1 Arsenic remaining in solution after sorption with three materials. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different ($P = 0.05$).

5.3.2 Glasshouse experiment

5.3.2.1 Plant height

Growth of plants during the experiment was monitored by measuring the distance from the soil surface to the highest leaf tip ('height'). There were no significant differences between the height of plants treated with two rates of untreated biochar and the control (Figure 5.2).

The effect of two concentration of iron oxide on the height of the plants during the growth showed that 15 g kg⁻¹ of iron oxide caused the death of plants 15 days after planting (DAS) (Figure 5.2). However, 5 g kg⁻¹ of iron oxide led to an initial reduction, but the plants grew rapidly between days 25 and 57 reaching the same height as the control. At the end of the experiment (74 DAS) the height of plants in the 5 g kg⁻¹ iron oxide treatment was significantly lower than the control treatment (Figure 5.3).

The effect of two concentration of magnetic biochar 15g kg¹ and 5g kg⁻¹ on the height of maize during the period of growth showed that 15g kg⁻¹ of magnetic biochar failed to support the plant and caused death of the plants 15 days after planting (DAS), while at the concentration of 5g kg⁻¹ of magnetic biochar plants were significantly taller at the end of the experiment compared with the control treatment (Figure 5.3).

In the uncontaminated soil experiment, there were no significant effects of additives up until 50 DAS (Figure 5.4). At 62 DAS, the height of plants treated with 5 g kg⁻¹ of untreated biochar, iron oxide, and magnetic biochar were significantly less than the controls but were not significantly different from each other (Figure 5.5).

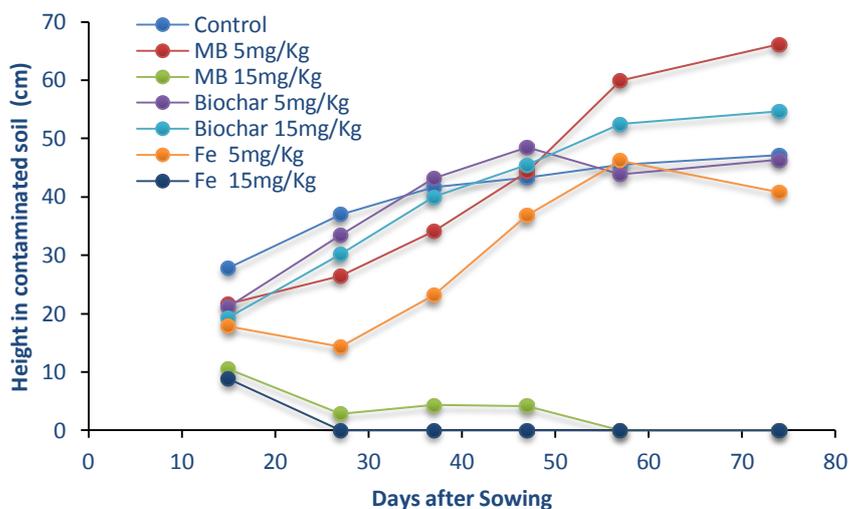


Figure 5.2 Effects of the treatments rates of untreated biochar (biochar), iron oxide (Fe) and magnetic biochar (MB) on height of maize in contaminated soil.

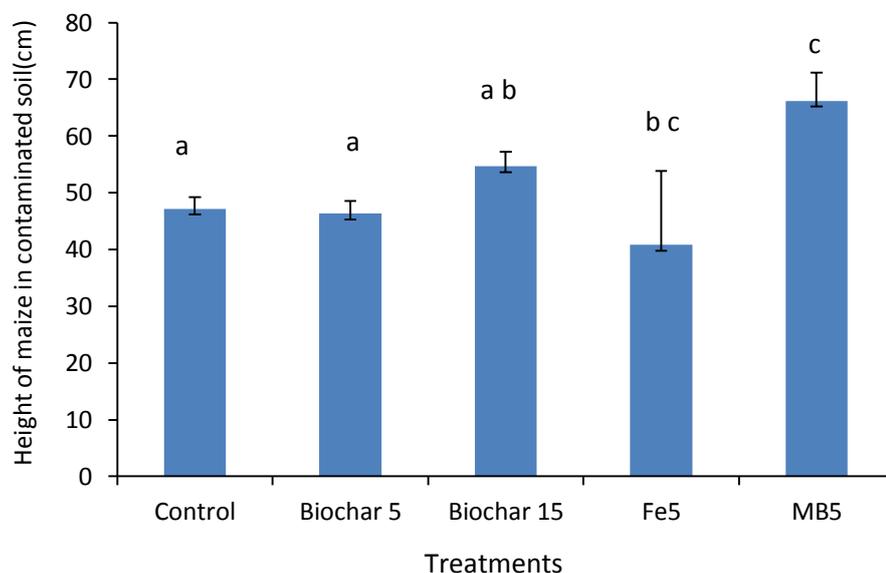


Figure 5.3 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on height of maize in contaminated soil at 74 DAS. The error bars show standard errors (n=6). Columns labelled with the same letter are not significantly different (P = 0.05).

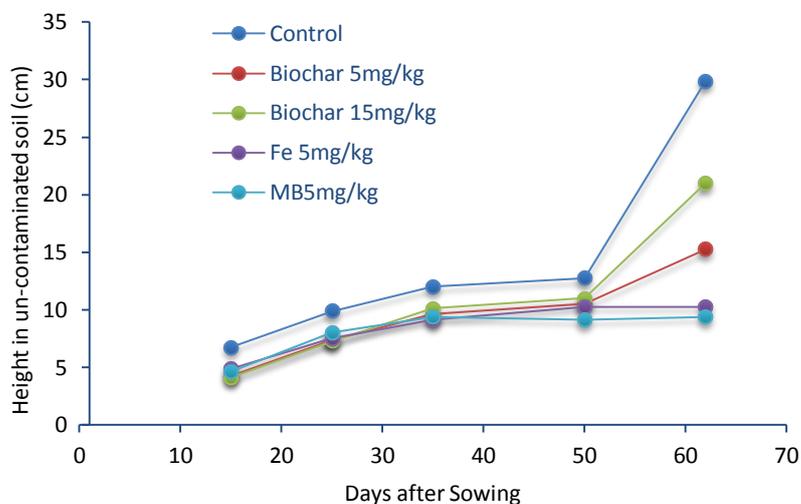


Figure 5.4 Effects of the treatments rates of untreated biochar (biochar), iron oxide (Fe) and magnetic biochar (MB) on height of maize in uncontaminated soil.

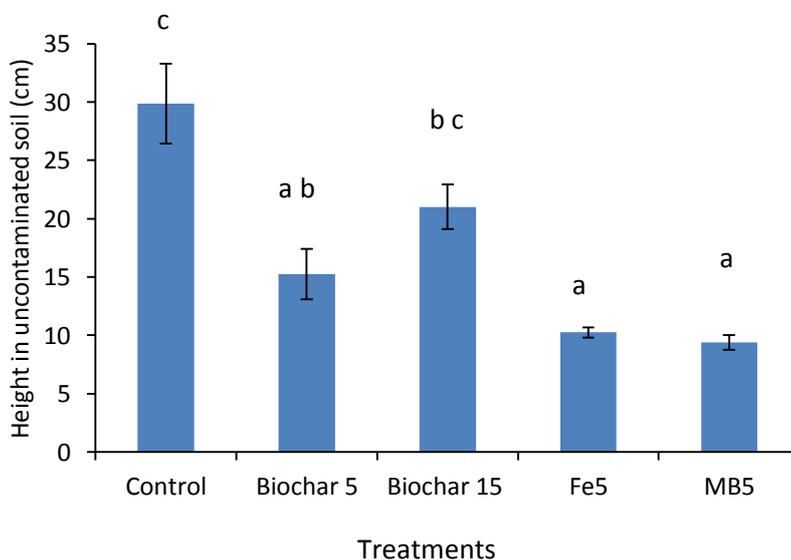


Figure 5.5 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on height of maize in un-contaminated soil at 62 DAS. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different (P = 0.05).

5.3.2.2 Shoot dry weight

In contaminated soil, magnetic biochar 5 g kg⁻¹ significantly increased dry shoot compared with the control treatment and the two rates of untreated biochar (5 g kg⁻¹ and 15 g kg⁻¹) (Figure 5.6) Iron oxide treatment at 5 g kg⁻¹ gave higher dry shoot weight than the untreated biochar treatments but was not significantly different from either the control or the magnetic biochar treatment. On other hand, in un-contaminated soil all treatments had significantly lower shoot dry weight than control (Figure 5.7). In magnetic biochar 5 g kg⁻¹ and iron oxide 5 g kg⁻¹ treatments the dry weight was significantly lower than untreated biochar treatments.

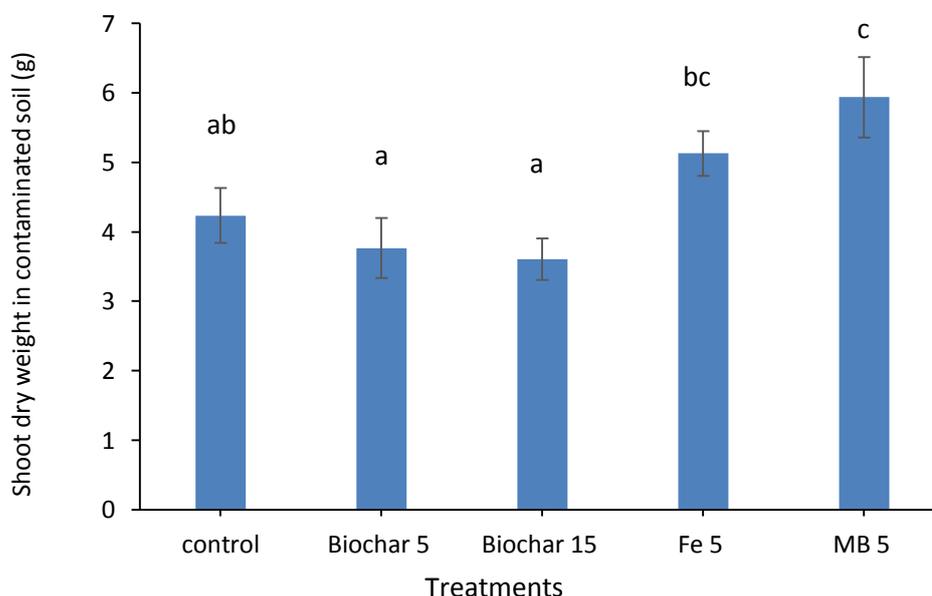


Figure 5.6 Effects of the treatments rates untreated biochar(biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on shoot dry weight of maize in contaminated soil. The error bars show standard errors (n=6). The Columns labelled with the same letter are not significantly different (P = 0.05).

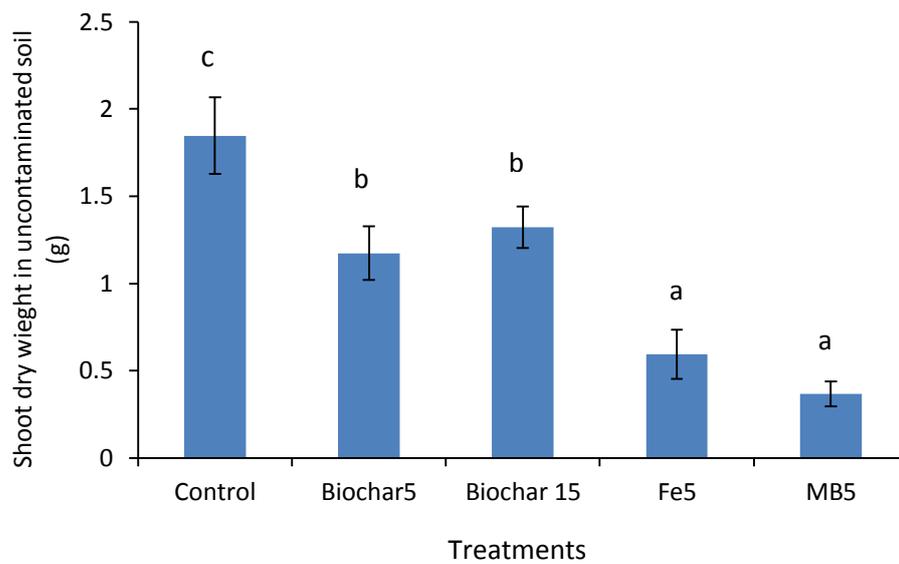


Figure 5.7 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on shoot dry weight of maize in uncontaminated soil. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different (P = 0.05).

5.3.2.3 Root dry weight

In contaminated soil, the root dry weight was significantly higher in magnetic biochar 5 g kg⁻¹ and Iron oxide treatment at 5 g kg⁻¹ than in the control (Figure 5.8). Root dry weight was not measured in the uncontaminated soil experiment.

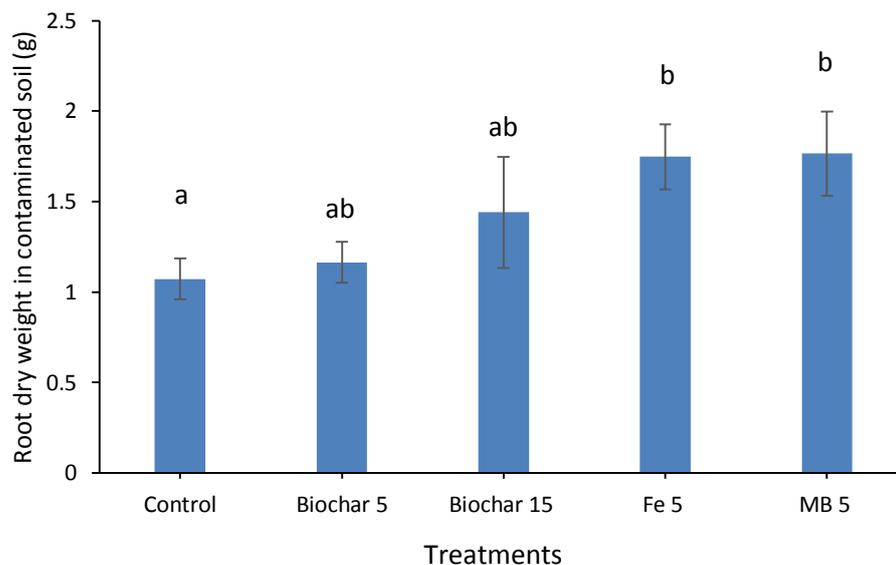


Figure 5.8 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on root dry weight of maize in contaminated soil. The error bars show standard errors (n=6). Columns labelled with the same letter are not significantly different (P = 0.05).

5.3.2.4 Number of leaves

In contaminated soil, the application of both magnetic biochar treatment 5 g kg⁻¹ and iron oxide treatment 5 g kg⁻¹ increased the number of leaves compared with the untreated biochar treatments and the control, while untreated biochar 15 g kg⁻¹ reduced the number of leaves compared with the control treatment (Figure 5.9). In contrast, in un-contaminated soil, all treatments except biochar at 5 g kg⁻¹ reduced the number of leaves compared with the control, but there were no significant difference between the treatments (Figure 5.10).

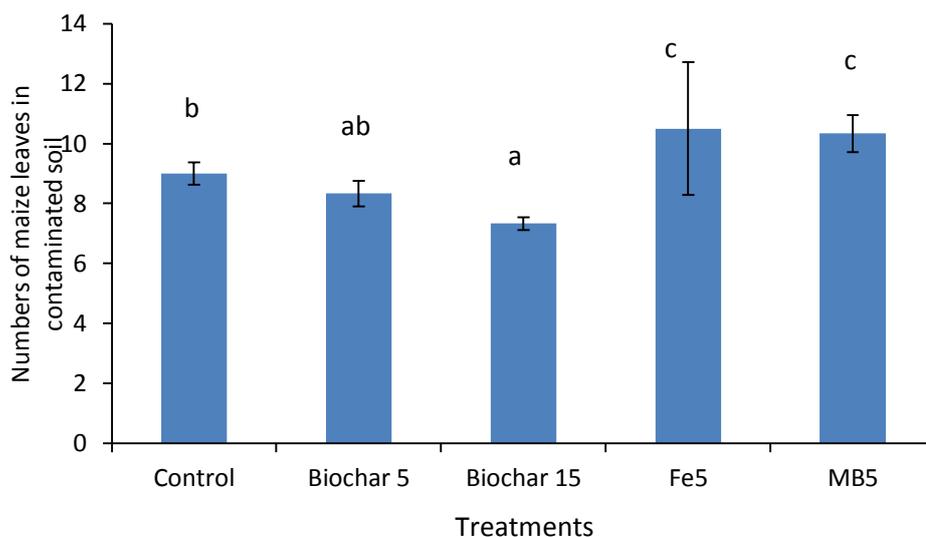


Figure 5.9 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on number of maize leaves in contaminated soil. The error bars show standard errors (n=6). Columns labelled with the same letter are not significantly different (P = 0.05).

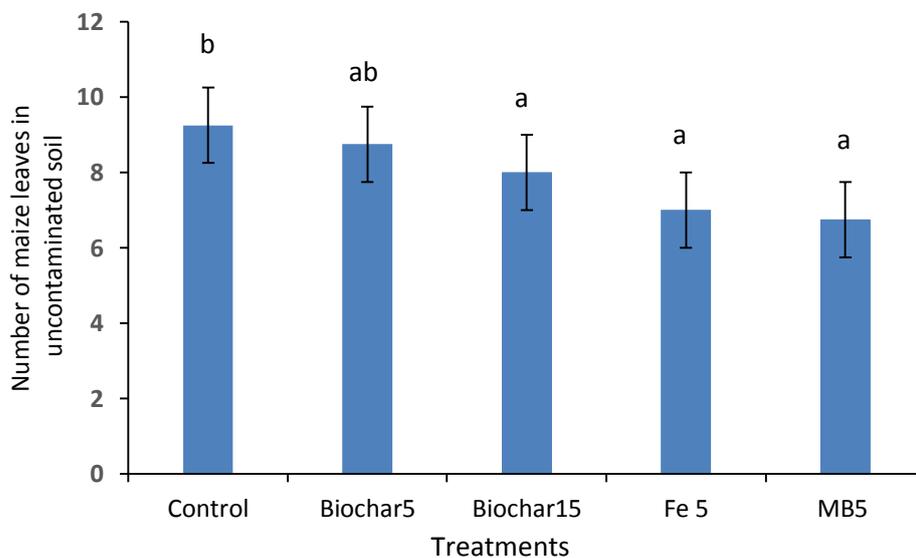


Figure 5.10 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on number of maize leaves in uncontaminated soil. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different (P = 0.05).

5.3.2.5 Mycorrhizal colonization

In contaminated soil, all treatments except untreated biochar at 5 g kg⁻¹ significantly increased mycorrhizal colonization compared with the control treatment (Figure 5.11). In un-contaminated soil both untreated biochar treatments significantly increased mycorrhizal colonization compared with the other treatments and control, while there were no differences between control, iron oxide 5 g kg⁻¹ and magnetic biochar 5 g kg⁻¹ (Figure 5.12).

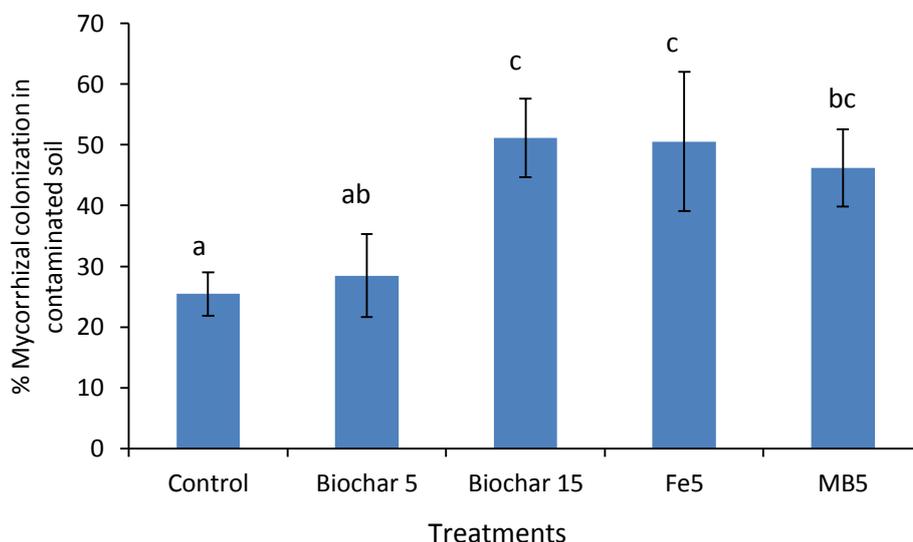


Figure 5.11 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on mycorrhizal colonization percentage of maize in contaminated soil. The error bars show standard errors (n=6). Columns labelled with the same letter are not significantly different (P = 0.05).

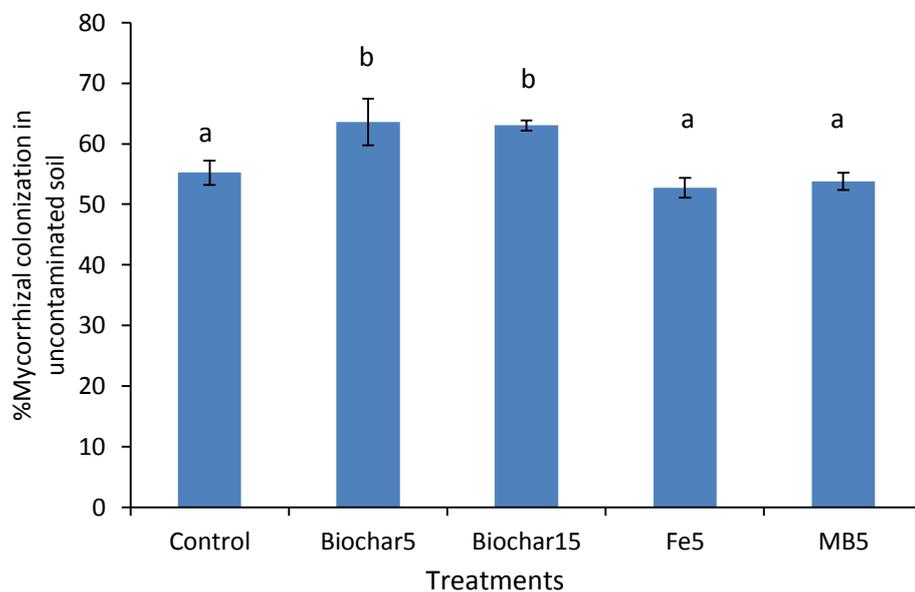


Figure 5.12 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on mycorrhizal colonization percentage of maize in uncontaminated soil. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different (P = 0.05).

5.3.2.6 Arsenic concentration in shoots

In contaminated soil, untreated biochar 15 g kg⁻¹ significantly increased the As concentration in shoots compared with control and all other treatments. In contrast, iron oxide 5 g kg⁻¹ and magnetic biochar 5 g kg⁻¹ treatments significantly reduced the concentration of As in shoot in compared with control. There was no significant difference between the untreated biochar 5 g kg⁻¹ treatment and the control (Figure 5.13).

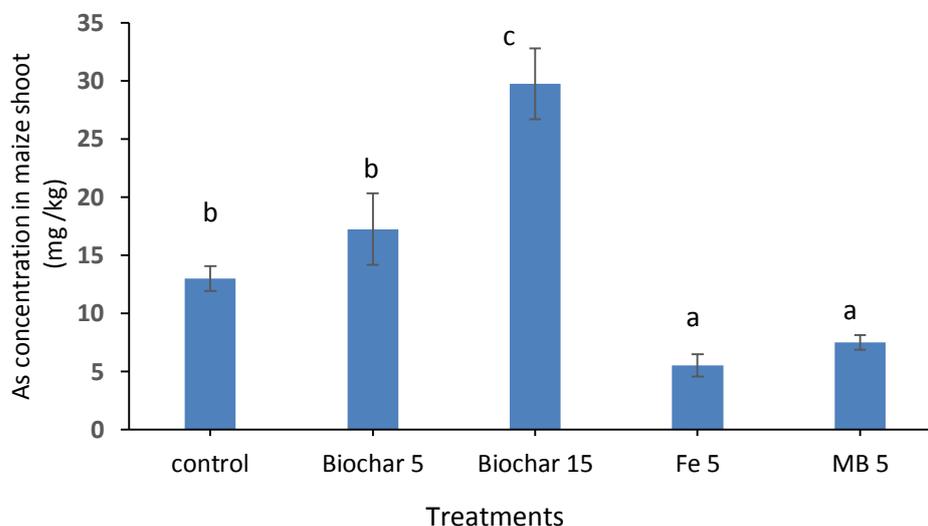


Figure 5.13 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on arsenic (As) concentration in maize shoot in contaminated soil. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different (P = 0.05).

5.3.2.7 Phosphorus concentration in shoots

There was no significant effect of treatment on phosphorus concentration in shoots of maize in contaminated soil (Figure 5.14).

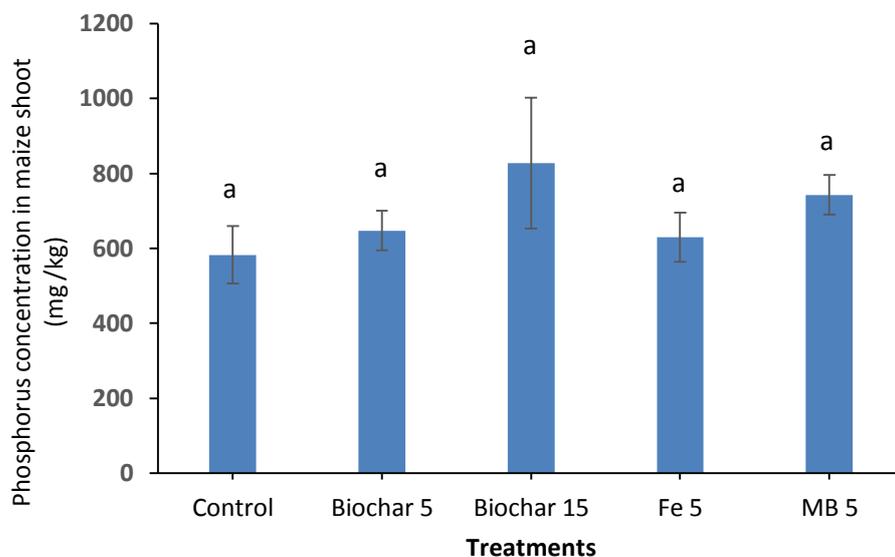


Figure 5.14 Effects of the treatments rates untreated biochar(biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on phosphorous (P) concentration in maize shoot n contaminated soil. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different (P = 0.05).

5.3.2.8 Phosphorous/Arsenic ratio

The ratio of P/As in shoot tissues was significantly increased by the iron oxide 5 g kg⁻¹ and magnetic biochar 5 g kg⁻¹ treatments (Figure 5.15). There were no significant differences between the untreated biochar (biochar) treatment and the control.

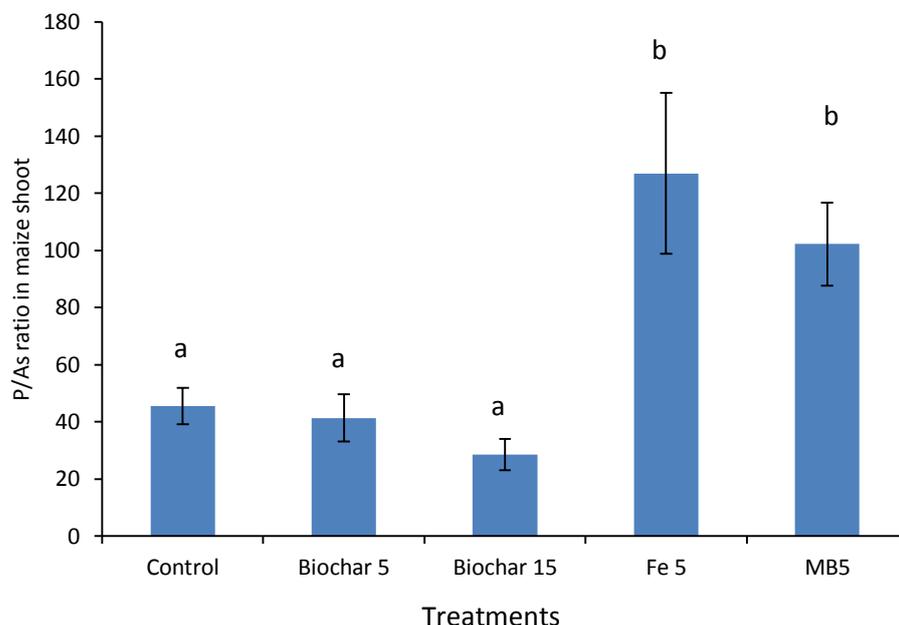


Figure 5.15 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on phosphorous/arsenic ratio (P /As) in maize shoot in contaminated soil. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different (P = 0.05).

5.3.2.9 Arsenic uptake in shoots

Arsenic uptake was affected significantly by the treatments. Untreated biochar 15 g kg⁻¹ treatment significantly increased As uptake in shoots compared with all other treatments and the control (Figure 5.16). On other hand, iron oxide 5 g kg⁻¹ showed reduced As uptake values in shoots in compared with all other treatments. As uptake in magnetic biochar 5 g kg⁻¹ and untreated biochar at 5 mg kg⁻¹ were not significantly different from the control.

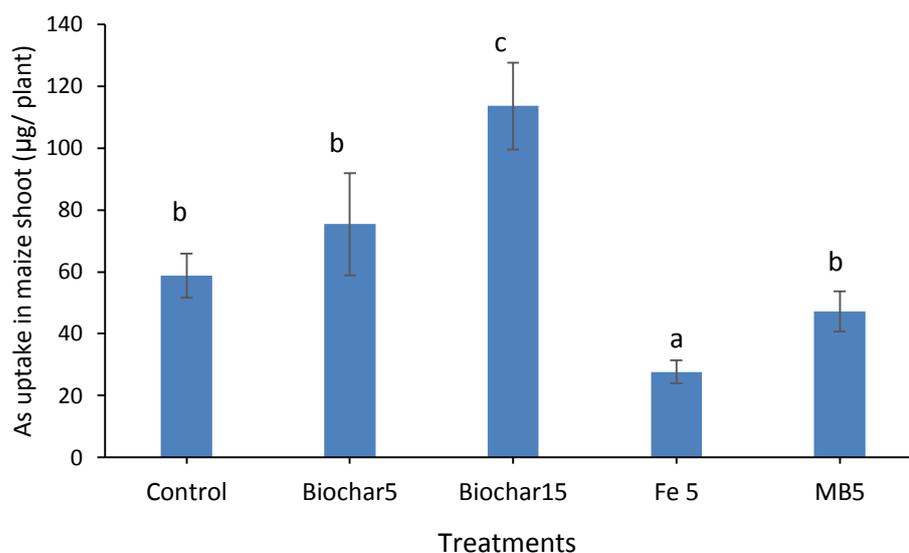


Figure 5.16 Effects of the treatments rates untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on arsenic (As) uptake in maize shoot in contaminated soil. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different (P = 0.05).

5.3.2.10 Phosphorus uptake in shoots

The magnetic biochar 5 g kg⁻¹ treatment significantly increased P uptake in shoots in compared with all other treatments and the control (Figure 5.17). There were no significant differences between untreated biochar 5 g kg⁻¹, 15 g kg⁻¹ and iron oxide 5 g kg⁻¹ treatments and the control on P uptake in shoot of maize.

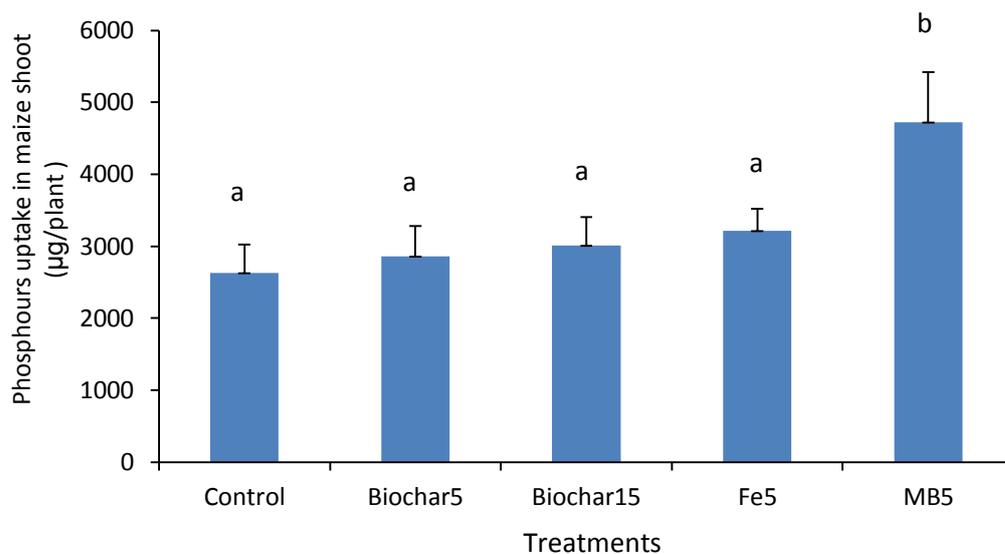


Figure 5.17 Effects of the treatments rates of untreated biochar (biochar 5, 15 g kg⁻¹), iron oxide (Fe 5 g kg⁻¹) and magnetic biochar (MB 5 g kg⁻¹) on Phosphorous (P) uptake in maize shoot in contaminated soil. The error bars show standard errors (n=4). Columns labelled with the same letter are not significantly different (P = 0.05).

5.4 Discussion

This experiment aimed to test whether magnetic biochar, first used for As removal from water by (Zhang et al., 2013a), could be used to reduce As uptake from soil and improve growth of plants. When magnetic biochar was added to contaminated soil, dry weight of roots and shoots, number of leaves, and mycorrhizal colonization of maize were increased. Although total uptake of As was the same, concentration of arsenic in tissues was reduced while the ratio of phosphorus to arsenic, and phosphorus uptake, were increased. This supported the hypothesis that magnetic biochar can be used for remediation of As contamination in soil.

5.4.1 Arsenic concentration and uptake

Most recent studies have focused on using magnetic and modified biochar in solution systems to remove pollutants from water, but none have used magnetic biochar in soil. In this study magnetic biochar reduced As concentration in maize shoots significantly more than biochar and to a similar level as iron. This may be because when the biochar is combined with iron it creates magnetic biochar with properties different from each one separately. The magnetic biochar used in this experiment was made according the method of (Sun et al., 2015). They showed that, with this method, particles of biochar became coated with nanoparticles of Fe_3O_4 . In the electron micrographs of magnetic biochar presented by (Chen et al., 2011; Sun et al., 2015) most of the surface of the biochar was coated with iron oxide. The main physical structure (surface area) would therefore depend on the biochar, but the chemical interactions would mainly be due to the iron oxide. With this combination the exchange surface area is increased relative to iron oxide aggregates, and the adsorption capacity for anions like arsenate would be increased relative to untreated biochar. This was confirmed in the sorption experiment. This result was similar to the behaviour of modified biochar in removing pollutants from solution observed by (Agrafioti et al., 2014). They found that biochar modified with Ca and Fe had much greater capacity to remove As (V) from aqueous solutions, compared to the non-impregnated biochars.

On other hand, our study showed that high levels of biochar increased As uptake and concentration inside the maize shoots. The reactions of biochar in soil depend on many factors include pH, presence of toxins and the porosity (Atkinson & Fitzgerald, 2010; Singh et al., 2010). The pH around biochar particles initially increases and then decreases as acidic functional groups are formed on biochar surface (Beesley et al., 2010; Zheng et al., 2013). The result from this study that biochar increased As uptake is similar to the finding of (Zheng et al., 2012; Zheng et al., 2013) who studied the effect of adding biochar on the transfer and accumulation of Cd, Zn, Pb and As in rice and wheat. The biochar significantly increased As concentration in the shoots by up to 199% of both cereals, but it decreased concentration of Cd, Zn, and Pb in the shoots. Biochar increased NH_4NO_3 extractable concentration of As soil by 64 to 2650%. The reason for increased As concentration by biochar explained by (Zheng et al., 2013) was attributed to the increase in soil pH after addition of biochar, and the change in sorption capacity of the soil to negatively charged oxy-anions decreased with increase of soil pH. On the other hand, (Namgay et al., 2010b) in a pot experiment studied the influence of activated wood biochar on availability of As, Cd, Cu, Pb and Zn to maize, using biochar with 3 rates (0, 5, 10 g kg^{-1}) in sandy soil. They found that the addition of wood biochar to the soil did not have any significant effect on dry matter of maize, but the highest rate of biochar decreased the concentration of As in maize shoots especially at high soil concentrations of As. As well, the level of extractable As in soil increased with biochar application, which is contrary to what would be expected from the tissue concentrations in the experiment of (Namgay et al., 2010b) but is consistent with the higher tissue As concentrations found in this experiment. Biochar usually acts as a reducing agent in soil (Choppala et al., 2012). (Joseph et al., 2010) found that biochar addition increased the rate of reduction of As (V)

to As (III), which is more soluble and more toxic. This process could explain the increased uptake of As in shoots when biochar was added to soil in contaminated soil.

The effect of magnetic biochar on As was mostly due to the iron bound to it. Iron precipitate adsorbed much more of the arsenate in the sorption experiment than magnetic biochar. However among parameters measured in the pot trial it only showed a significant difference from magnetic biochar in As uptake, which was lower with iron. This difference may mostly be due to the smaller dry weight of plants in the iron treatment.

5.4.2 Effects on growth in contaminated soil

Magnetic biochar addition enhanced maize growth. It significantly increased the height of maize, shoot dry weight, root dry weight and number of leaves more than other treatments except the iron oxide treatments. Moreover it reduced As concentration in shoots. This may be attributed to reduced arsenic toxicity inside maize due to the increase in exchange surface area of modified biochar that led to increase in the adsorption capacity for anions like arsenate relative to untreated biochar.

All biochar additions did not led to a noticeable increase in maize growth. There were no significant difference between biochar treatments and control in height, number of leaves, shoot and root dry weight and in P concentration in shoot and roots. There were significantly higher As concentration and uptake in maize shoot by biochar 15 mg kg^{-1} than all the other treatments. This may be because the feedstock of biochar was from coconut shell which is poor in nutrients and it did not add nutrients to support the growth that was obvious with the rate 5 g kg^{-1} (Mukherjee & Zimmerman, 2013; Rajkovich et al., 2012; Zheng et al., 2013).

Iron oxide at 5mg kg^{-1} addition increased the growth of maize (shoots and root dry weight, number of leaves) and increased mycorrhizal percentage, while at rate 15 mg kg^{-1} of iron oxide addition caused the death of plants 15 days after planting. This may be attributed to the role of mycorrhiza on Fe availability and transported to maize. It may be of at changes in microbial communities can contribute to enhanced Fe accumulation in plants, or it may be related to the role of fungi which can release iron chelators and can cause increased of Fe availability to the plant (Khan et al., 2006; Lemanceau et al., 2009). Low rates of Fe concentration as a nutrient to plant while high rates cause toxicity and death to maize (Santiago et al., 2013; Tekaya et al., 2016).

5.4.3 Effects on growth in uncontaminated soil

Although magnetic biochar improved growth in As contaminated soil, it had a negative effect on the growth of plants in uncontaminated soil. Magnetic biochar and iron reduced the dry weight, the height and number of leaves of maize. This may be because the rate of application caused iron toxicity to the plant compared with the control. This would also be consistent with the early death of plants in the contaminated soil at the high rates of magnetic biochar and iron. An alternative explanation is that the magnetic biochar and iron had a negative effect on the availability or uptake of another nutrient, although there was no evidence of an effect on phosphorus.

Biochar reduced the growth of the plants in uncontaminated soils. Biochar reduced shoot dry weight, leaf number and height of maize. These results agree with (Borchard et al., 2014). They found that three different biochars did not affect maize yield at an application

rate of 15 g kg⁻¹ of soil, but increasing the rate to 100 g kg⁻¹ resulted in decreased plant biomass. Biochar is widely promoted as beneficial to plant growth. However, its effects will differ depending on crop, soil type, and type of biochar. The mechanism for the reduction in growth due to biochar was not investigated, because this was not the purpose of the experiment.

5.4.4 Mycorrhizal colonization

Magnetic biochar and iron increased the rate of mycorrhizal colonization of roots in the contaminated soil. It was found in the previous chapters that mycorrhizal colonization was reduced at high concentration As. The effect of magnetic biochar and iron is probably because they reduce As availability and therefore reduce the effect of As on colonization. Magnetic biochar and iron did not have an adverse effect on mycorrhizal colonization in uncontaminated soil.

However, biochar at high rate of application in this experiment increased mycorrhizal colonization in maize roots in contaminated soil. This is unlikely to be by the same mechanism as the effect of magnetic biochar and iron. (Warnock et al., 2010) studied the effect of biochar on soil microbial activity in As-contaminated soil in a glasshouse experiment. They used two rates of biochar (10 and 20 g biochar kg⁻¹ soil), and found that biochar increased soil microbial activity. Biochar could have different effects on plants and microbes in contaminated soil. when using different types and rates of biochars to study their effects on mycorrhizal abundance in roots of *Plantago lanceolata* found that AMF abundance was either decreased or remained unchanged across all biochar treatments.

In uncontaminated soil, biochar at both rates of application significantly increased the percentage of mycorrhiza compared with other treatments. The probable mechanism of biochar/mycorrhizal fungi interactions hypothesised by (Warnock et al., 2007) included that biochar could be a refuge for soil fungi and bacteria, and (Hammer et al., 2014) found that AMF can penetrate and access microsites within biochar pores under artificial conditions. When biochar is produced at low temperatures are added to the soil a considerable quantity of soluble organics (Kookana et al., 2011), some of organic compounds on the surfaces of biochar can stimulate fungi and provide nutrients for microorganisms to grow faster (Kookana et al., 2011).

5.4.5 Conclusion

This experiment showed that magnetic biochar is a promising material to remediate As contaminated soil. It reduced As toxicity to plants by reducing As concentration, and promoted the growth of plants growing in As contaminated soil. However at the rates used it decreased the growth of plants in uncontaminated soil. This material needs future tests on field trials and rates of application, and to determine if it is economic to use it. On the other hand the biochar that was used was not suitable for As removal from soil and not for plants growing in contaminated soil. In uncontaminated soil biochar may be useful to enhance growth of plants but care should be taken with the rates of application.

6 General discussion

The objective of this thesis was to find a biological and natural material that reduces As concentration and toxicity in plants and the toxicity effects for humans if these plants enter the food chain. There were very few studies on origin of AMF and its effect on plant growth in contaminated soil, and on magnetic biochar, which has not yet been tested for its effect on plants growing in contaminated soil. AMF and activated biochar (biological and natural materials) were tested for their ability to reduce As uptake and concentration in maize and other vegetables within three experimental chapters. The results of this study showed that both of these techniques reduced As inside plant and in the soil and that they may have a role in As remediation.

This final chapter present a summary of the new and key findings, conclusion of the results and recommendation for further researches

6.1 Key findings

6.1.1 Chapter 3

Inoculating the plants with commercial mycorrhiza did not increase mycorrhizal colonization. However some plants (Spinach, Capsicum, and Lettuce) showed higher survival in contaminated soil when the plants were inoculated with this inoculum. Because this was seen in spinach and spinach is not a mycorrhizal plant, the reason was not because of mycorrhiza. It may be that the commercial mycorrhizal inoculum contained other promoters in the formulation (Corkidi et al., 2004).

Different plants showed different reactions towards the As in the contaminated soil. The most sensitive towards the arsenic was tomato and the most tolerant was maize. Maize showed high mycorrhizal colonization and sufficient toxicity symptoms so it was used in later experiments.

Arsenic appeared to reduce mycorrhizal colonization percentage in most of the plants, although this may have been confounded with other differences between the two soils.

Different communities of AMF were isolated from contaminated and uncontaminated soil.

6.1.2 Chapter 4

Inoculating maize with mycorrhiza (M⁺) reduced As concentration and uptake in shoots and roots, and increased P concentration in shoot and roots, compared with un-inoculated plants. At high concentrations of soil As, inoculum from uncontaminated soil resulted in lower tissue concentrations of As than inoculum from contaminated soil. On the other hand, inoculum from contaminated soil gave higher percent root colonization than inoculum from uncontaminated soil at all levels of arsenic. Increasing As concentrations in soil reduced colonization percentage for both inoculum types.

Despite reducing As uptake, inoculation with mycorrhiza reduced growth of plants in the pot system used, possibly because the benefits of increased nutrients were less than the cost of maintaining the fungus. At the highest concentrations of As (50 and 75 mg kg⁻¹) inoculation with mycorrhiza from contaminated soil (coM⁺) resulted in higher dry weight of the shoot of maize than mycorrhizal inoculum from uncontaminated soil (unM⁺).

6.1.3 Chapter 5

The sorption experiment showed that biochar removed 20% of As from solution, magnetic biochar removed 75% of As from solution and Fe removed almost 100%. Magnetic biochar reduced As toxicity to plants by reducing As concentration in the shoot, and promoted the growth of plants growing in contaminated soil. However, magnetic biochar had a negative effect on growth of plants in uncontaminated soil, possibly due to iron toxicity.

6.2 Mycorrhiza and arsenic

This work confirmed earlier findings that mycorrhiza can reduce As uptake by plants. (Ortowska et al., 2012). (Ortowska et al. (2012) had previously shown that an isolate of *Rhizophagus intraradices* from unpolluted soil was more effective at reducing As uptake of *Plantago* than isolates of other species from polluted soils, but it was not clear whether this was strictly due to a difference in origin or was a species effect. In the current work, whole communities from contaminated and uncontaminated soils were compared, An important finding was that inoculum from uncontaminated soil was more effective at reducing As uptake than inoculum from contaminated soil. The reason may be due to a difference in origin of mycorrhiza species between the two soils. The dominate species found in uncontaminated soil are *Acaulospora* and *Gigaspora*. This confirms that unpolluted soils can be a source of mycorrhizal fungi that are effective in bioremediation, despite not having been selected for tolerance to the pollutant. Future work could investigate the contribution of individual species to this.

Inoculum from contaminated soil doubled the concentration and uptake of As in shoots compared with inoculum from uncontaminated soil. Although the percent root length colonized was greater for inoculum from contaminated soil at all concentrations of As soil, this was probably due to differences in the intraradical growth patterns of the fungi involved and the difference was not great enough to explain the large difference in As uptake. It is possible that the adaptation of mycorrhizal fungi to contaminated soil may not be desirable, as they may be less likely to exclude As from their mycelium and from the plant.

Another finding that was different from expectations was that As reduced percent mycorrhizal colonization in both Chapter 3 and Chapter 4. According to Smith et al. (2010), most studies have shown that As does not reduce colonization. One possible mechanism is that high concentrations of As in soil increase available phosphorus, because of competition for adsorption sites. However, available phosphorus was lower in the contaminated soil in Chapter 3. Although there was a reduction in colonization, in most cases in Chapters 3 and 4 it was within what would be considered a normal range.

In this study mycorrhiza from uncontaminated soil could reduce As concentration and uptake by plant. In Chapter 4 it was found that the cobs of maize had very low As concentration compared with the roots and shoots. Combining inoculation by AMF from uncontaminated soil with the low As translocation into cobs could reduce the toxicity of sweet corn or maize grain grown in moderately contaminated soil. I recommend practically this could be done by inoculating the plant in a nursery with AMF from uncontaminated soil, then transplanting it to the contaminated site. This way may be a slow method compared with direct seeding into remediated soil because it needs preparation time for inoculum and to ensure colonization by AMF. Sometimes infection with AMF fails

(Corkidi et al., 2004). However this kind of remediation (biological remediation) is safe for the environment and it could cost less money compared with chemical remediation.

6.3 Magnetic biochar

In Chapter 5 the materials that were used in the experiment mixed natural material (carbon from coconut husk) and chemical compounds (iron chlorides). The combination of these two materials biochar: iron (1:1 w/w) produces a high anion exchange surface area with high adsorption capacity for As pollutants from soil and water (Sun et al., 2015).

Although magnetic biochar showed many advantages related to plant growth promotion and As remediation, there were a few points that should be taken care with before use of this material for remediating a contaminated site, such as the economics of use on large areas, the method of preparation and the rate of application. In this study the rate 5 g kg⁻¹ of magnetic biochar gave a high ability to enhance the growth of plants compared with 15 g kg⁻¹ of application which caused death of the plants. However, to apply magnetic biochar to As-contaminated sites at this rate would need of the order of 10 tonnes of this material for one hectare. Economically this quantity seems a very large amount in terms of the cost of the material components and of the time that is needed to prepare this amount. I suggest on this point to look for a natural material that has similar behaviour to the iron precipitate (high exchange cation capacity) which is available at cheap cost, can be mixed with activated biochar and can be used instead of iron chemicals. The rate of application also needs more study.

Further research is needed to focus on the cheapest and easiest way to prepare magnetic biochar. There are several methods for preparation of magnetic biochar (Oliveira et al.,

2002; Sun et al., 2015; Zhang et al., 2013a). Each method has its advantages and disadvantages. In this study I followed the method of (Sun et al., 2015) . The way to prepare magnetic biochar in this study was a mixture of activated biochar with mixture of iron chloride (1:1 w/w) that was precipitated as an oxide at high pH. This method was easy to prepare. It does not need a special oven with high temperature if the biochar is already available, or as long a time for preparation as other methods. However this method needs more development. The percentage of mixture needs future research to optimise the use of the surface area, to reduce the amount of available iron to avoid toxicity, and to reduce the cost.

The result of this study showed that magnetic biochar removed 75% of As from the solution, while Fe removed 100% compared with the control treatment. One of the strategies to develop magnetic biochar in future is to improve adsorption capacity by increasing the adsorption surface area and the active sites of the adsorbents (Goldberg & Johnston, 2001) . More over some works confirmed that the adsorbents have a selective ability for one species of As more than the other. (Gupta et al., 2009; Lin et al., 2012) confirmed the selective adsorption of As (V) but low adsorption efficiencies for As (III) ; other works showed high adsorption capacities toward As (III) (Deschamps et al., 2005; Yean et al., 2005; Zhang et al., 2007). The selectivity and stability of the adsorbent could also be improved by coating porous materials on the surface of the magnetic biochar. Soluble iron can increase As toxicity (Bennett et al., 2010; Cui et al., 2015; Pena et al., 2006) so the stability of the adsorbent under different pH conditions is also suggested to be test when choosing a materials.

To choose a suitable biochar for magnetic biochar many factors are important: feedstock composition, pyrolysis process conditions and particle size (Singh et al., 2010). High-

temperature pyrolysis ($> 550\text{ }^{\circ}\text{C}$) produces biochars that generally have high surface areas ($>400\text{ m}^2\text{g}^{-1}$) (Downie et al., 2009; Keiluweit et al., 2010) and are good adsorbents (Mizuta et al., 2004). Low –temperature pyrolysis ($<550\text{ }^{\circ}\text{C}$) is high in carbon and nutrients (Keiluweit et al., 2010). The nutrient content of biochars also depends on the type of feedstock (Singh et al., 2010). On other hand the pore structure, size distribution, volume and surface area depend on the feedstock and process conditions, for example wood biochar has structure different than chicken manure biochar (Downie et al., 2009; Keiluweit et al., 2010).

6.4 Integrated remediation

Other advantages for magnetic biochar were founding in this study. Magnetic biochar in contaminated soil increased mycorrhizal colonization, so both could be used in combination in contaminated sites for remediation.

In Chapter 3, 8 plants were used to test their sensitivities towards As in As-contaminated soil and similar uncontaminated soil. The most sensitive plants to As were tomato, lettuce and eggplants. The most tolerant plants to As were maize and bean. Maize had high tolerance ability towards the As. Although As affected its growth negatively it survived in contaminated soil until the end of the growth period, which is why maize was chosen for further experiments. This difference in tolerance between plants could interact with the effects of AMF and magnetic biochar. Future experiments should look at how to use relative tolerance to increase the effectiveness of environmentally friendly remediation.

6.5 Conclusions

These two simple techniques (AMF and magnetic biochar) were new and gave positive effects. Both of them may be useful to remediate As-polluted soil and inside the plant. However if mycorrhizal inoculum is used as a biological remediation its effect may be slow and needs time. However it is safe for the plant and for the environment and at the same time adds other benefits to the plants. Magnetic biochar could be used as a biological-chemical remediation for As in plant and soil because of its adsorption capacity. It is potentially faster than mycorrhizal inoculum but care should be taken with the rates of application as it may have toxicity to the plant and the soil.

The findings of all chapters supported the objectives of this study. In conclusion, I found that mycorrhizal inoculum reduced As concentration and uptake inside the plant and increased phosphorus concentration and uptake and this supports the hypothesis that mycorrhizal inoculum can alleviate arsenic inside the plant. AMF isolated from different sources had different effects on As and unexpectedly AMF from uncontaminated soil was more effective. Modifying biochar into magnetic biochar can be effective against As in soil as had previously been shown for water, but needs further development. There is scope for plenty of productive research on environmentally friendly remediation of contaminated soils.

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