

MICROWAVE AND BIOCHAR SOIL TREATMENT ALLEVIATES ARSENIC PHYTOTOXICITY IN WHEAT AND RICE

Presented by

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Declaration

I declare that this thesis comprises only my original work towards the degree of Doctor of Philosophy. Due acknowledgement has been made in the text to all other material used. This thesis does not exceed **100,000 words** and complies with the stipulations set out for the degree of Doctor of Philosophy by The University of Melbourne.

Md Humayun Kabir

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Abstract

Arsenic (As) is a toxic metalloid, which is carcinogenic i.e. cancerous to humans. Besides the drinking water, accumulation of As in food grains through plant uptake, when cultivated in As contaminated soils, is a potential route of human dietary As exposure. This has inspired research into alleviating grain As accumulation, despite there being already existing strategies with major disadvantages such as low efficiency, high costs, and usage being restricted to smaller-scale operations. Therefore, pre-sowing microwave (MW) soil heating and sawdust biochar were used to investigate if they can reduce As concentration in wheat and rice.

Microwave is a form of electromagnetic radiation, which can produce heat in the soil by inducing the rotation of the dipoles of polar molecules (e.g. water). Microwave heating depends on the dielectric properties of the soil. Therefore, a study was conducted to determine the dielectric properties of different types of soils with different moisture content. The results showed that the soil moisture was the major contributor to the dielectric behavior of soil since dielectric properties increase as soil moisture increases. Soil types also had an influence as the dielectric properties of sandy soil were much lower than the other soils such as clay and loam soil.

To investigate the effect of MW and biochar on wheat and rice grain As concentration, both the wheat and rice soils were spiked with five As concentrations (As-0, As-20, As-40, As-60, and As-80 mg kg⁻¹ soil). In addition to MW, biochar was used to reduce rice grain As accumulation since biochar has been gaining attention for its heavy metal immobilization capacity. After As application, three levels of biochar (BC-0, BC-10, and BC-20 t ha⁻¹ soil) were added only in the rice-growing soil. Then, soils were treated for 0, 3, and 6 minutes (MW-0, MW-3, and MW-6) in an MW chamber to achieve the soil temperature of around room temperature, 60 °C, and 90 °C respectively. The crops were grown in a completely randomized design with four replications in a glasshouse during 2017 (wheat) and 2018 (rice). The results demonstrated that, in both the wheat and rice, MW soil treatments, especially the MW-6, alleviated As phytotoxicity and facilitated less grain total As concentration compared with the MW-0 treatment across all the soil As concentrations. Also, MW treatment significantly reduced the concentration of arsenite [As(III)], the most toxic form of As. Decreased grain As concentration in rice was recorded at BC-10 in lower levels of soil As concentrations (As-20 and As-40) while, a negative impact was observed at BC-20 across all the soil As concentration, compared with BC-0 treatment. Furthermore, rice grain As(III) concentration increased

significantly in BC-20 treatment. Thus, MW-6 treatment could be used for the alleviation of grain As concentration in wheat and rice grain, whereas more study is needed for the best biochar application rate.

However, understanding the residual effect of MW and biochar is crucial for the sustainability of the treatment. Therefore, the same varieties of wheat and rice were grown in the following year, using the same pots, without the addition of further MW or biochar treatment. The results revealed that, 360 days after MW soil treatments there was still the potential to alleviate grain As concentration in both wheat and rice. A similar result was observed for biochar treatment in the residual year with a positive effect at BC-10 and a negative effect at BC-20 treatment. Furthermore, it is unclear whether MW soil treatment is just a heating effect or if there is some other effect of the electromagnetic wave involved. Therefore, a glasshouse pot study was designed to investigate the effect of MW and conventional electric oven (EO) soil heating on As phytotoxicity alleviation in rice. The soil was spiked with three levels of soil As concentration (As-0, As-40, and As-80 mg kg⁻¹) prior to applying MW and EO heat treatments, to achieve the soil temperature of around 80 – 90 °C. The results showed that, there was no statistically significant difference between MW and EO treatments regarding As phytotoxicity alleviation. However, the positive effect was more in MW treatment than the EO treatment. Significantly less total energy required in the MW to treat the soil than the EO.

Besides the As phytotoxicity alleviation, the effect of MW soil heating on soil microorganisms, particularly bacteria, was a topmost concern and investigation was needed to ascertain that MW soil heating does not affect it drastically. Therefore, an experiment was designed to investigate the effect of MW heating (80 – 90 °C) on the soil bacterial community in As contaminated (As-0, As-40, and As-80 mg kg⁻¹ soil) soils. The 16S rRNA bacterial gene copy numbers decreased significantly after MW soil heating but recovered back to its previous number 42 days post treatment. The bacterial diversity also decreased significantly in MW treated soils but did not recover even after 56 days from MW heating. However, there was no noticeable effects of soil As concentration on bacterial community were observed. Furthermore, relative abundance of some beneficial bacteria such as *Bacillus* and *Symbiobacterium* were significantly higher in the MW treated soils. Thus, MW soil heating at 80 – 90 °C can potentially be applied for As phytotoxicity alleviation without significantly destroying the ecologically important taxa.

Overall, pre-sowing MW soil heating could be applied as a novel technique to alleviate As phytotoxicity in wheat and rice with lower As accumulation in the grain. Thus, application of the MW technology in the As contaminated area like Bangladesh could add another feather in the crown of the As remediation techniques and help to reduce the human health risk through As contaminated food grain. However, further research needed before adopting the MW soil heating technique where different aspects should be explored such as response of MW technology in the field condition, scaling up the MW equipment for field application, cost of MW application in the field at farmers level, long-term effect of MW treatment on soil nutrient dynamics, soil organic matter and soil biota and sustainability of the MW technology in field condition. Also, sawdust biochar could be used in combination with MW soil heating for As phytotoxicity alleviation; however, more study needed to set the appropriate rate of biochar application.

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List of abbreviation

AB	Arsenobetaine
ABC	ATP Binding Cassette
AC	Arsenocholine
ADP	Adenosine diphosphate
AFS	Atomic fluorescence spectrometry
Al	Aluminium
ANOVA	Analysis of variance
AR	Arsenate reductase
ArsM	As(III) S-adenosylmethionine methyltransferase
As	Arsenic
As(III)	Arsenite
As(V)	Arsenate
ATP	Adenosine try phosphate
ATQ	Arsenate tolerance QTL
Au	Gold
AUD	Australian Dollar
B	Boron
BC	Biochar
BEC	Background equivalent concentration
BET	Specific surface area
C	Carbon
Ca	Calcium
CAT	Catalase
CCA	Copper-chromium-arsenate
Cd	Cadmium
CEC	Cation exchange capacity
Co	Cobalt
Cr	Chromium
CRD	Completely randomized design
CRM	Certified reference material
Cu	Copper

DAP	Diammonium phosphate
DAS	Days after sowing
DMA	Dimethyl arsinic acid
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DPHE	Department of Public Health Engineering
DTPA	Diethylenetriamine pentaacetic acid
EC	Electrical conductivity
Eh	Redox potential
EO	Electric oven
EPA	Environmental Protection Agency
eV	Electronvolt
FAO	Food and Agriculture Organization
Fe	Iron
GHz	Gigahertz
GPX	Guaiacol peroxidase
GR	Glutathione reductase
GSH	Glutathione
H	Hydrogen
HAC	High arsenic content
HPLC	High performance liquid chromatography
HTW	Hand tube well
ICP-MS	Inductively coupled plasma mass spectrometry
INT	Inositol transporters
ISM	Industrial scientific and medical
K	Potassium
LSD	Least significant difference
MAP	Mono ammonium phosphate
Mg	Magnesium
MHz	Megahertz
MJ	Megajoule
MMA	Monomethyl arsonic acid
Mn	Manganese

mV	Millivolt
MW	Microwave
N	Nitrogen
Na	Sodium
NATA	National Association of Testing Authorities
Ni	Nickel
NIP	Nodulin 26-like intrinsic protein
NIST	National Institute of Standard and Technology
NRAMP	Natural resistance-associated with the macrophage protein
O	Oxygen
OM	Organic matter
OTUs	Operational taxonomic units
OVT	Ovular vascular trace
P	Phosphorus
PAHs	Polycyclic aromatic hydrocarbons
Pb	Lead
PC	Phytochelatin
PCBs	Polychlorinated biphenyls
PCD	Programmed cell death
PCoA	Principal coordinate analysis
PCPs	Pentachlorophenols
PERMANOVA	Permutational multivariate analysis of variance
PHR	Phosphate starvation response
PHTs	Phosphate transporters
PIP	Plasma membrane intrinsic proteins
qPCR	Quantitative polymerase chain reaction
QTL	Quantitative trait locus
RAPD	Random amplified polymorphic DNA
RNA	Ribonucleic acid
ROL	Radial oxygen loss
ROS	Reactive oxygen species
RSD	Relative standard deviation
RTA	Real time analysis

S	Sulphur
SAM	S-adenosyl-L-methionine
Sb	Antimony
SEM	Scanning electron microscopic
Si	Silicon
SOD	Superoxide dismutase
SOM	Soil organic matter
SPAD	Soil-Plant Analysis Development
SRM	Standard reference material
STW	Shallow tube well
TCE	Trichloroethylene
Th	Thorium
TMA	Trimethylarsine oxide
UK	United Kingdom
USA	United States of America
WHO	World Health Organization
XANES	X-ray absorption near-edge structure
Zn	Zinc

Preface and dissemination of research findings

Scientific papers published or submitted by the author in collaboration with supervisors and other scientists are listed in the following:

Peer-reviewed papers published in international scientific journals:

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Kabir, H., Gupta, D., Pang, A. Graham, B. 2019. The Influence of Microwave Soil Treatment and Biochar Application on the Toxicity of Arsenic in Rice (*Oryza sativa* L.). IMPI's 53rd Annual Microwave Power Symposium, June 18-20, 2019 at Caesars Palace, Las Vegas, Nevada, USA.

Kabir, H., Gupta, D., Pang, A. Graham, B. 2018. The Influence of Microwave Soil Treatment on the Toxicity of Arsenic in Wheat (*Triticum aestivum* L.). IMPI's 52nd Annual Microwave Power Symposium, Long Beach, California, USA, June 2018. Pp: 106-108.

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Chapter 1 – General Introduction

Arsenic (As) is considered to be the most devastating toxic metalloid, which is raising global concerns due to its toxic effect and very high aptitude to bio-accumulate and persistence in the ecosystem and human body (Naidu et al., 2006). The Environmental Protection Agency (EPA) of the USA classifies inorganic As as a group I carcinogen. There are considerable evidences that long-term exposure to elevated As can increase the risk of different types of cancer including skin, liver, kidney, bladder, lung, and prostate cancer. Adverse effects of As on cardiovascular, neurological, hematological, renal, and respiratory systems are also reported (Abdul et al., 2015). It is appraised that, approximately 220 million people, globally, are exposed to elevated As through drinking water, which has a concentration above the World Health Organization (WHO) standard limit of 10 mg l^{-1} (Podgorski and Berg, 2020). For instance, in Bangladesh, which is reported to be one of the worst As polluted zone with a large number of affected people (Singh et al., 2015b), As contamination groundwater, with As concentration exceeding the national standard ($50 \text{ } \mu\text{g l}^{-1}$), is reported in 50 out of the 64 districts, and 59 districts have concentrations above the WHO standard (Huq et al., 2020).

Weathering of natural minerals (geogenic) is the major source of groundwater As pollution in this region (Chakraborti et al., 2010). Anthropogenic activities, such as metal mining and smelting, combustion of fossil fuels, use of arsenic-containing pesticides and herbicides, wood preservatives, food additives, etc. (Mitra et al., 2017), also contribute As to the environment. Although As contaminated groundwater, which is used for drinking purpose, is the major pathway of human As exposure (Gupta et al., 2017), the long-term excessive use of this As polluted groundwater for crop irrigation gives rise to high deposition of As in the topsoil and ultimately results in uptake by crops (Williams et al., 2006). Irrigation in agricultural soil, with As contaminated groundwater, can leads to high soil As deposition of up to 83 mg kg^{-1} soil, compared with the non-contaminated areas (4.0 to 8.0 mg kg^{-1} soil) (Ullah, 1998). Excessively high As pollution in water, soil and crops have already been identified in many countries like Bangladesh, India (West Bengal), China, Korea, Taiwan, Thailand, Mexico, USA, Argentina and Vietnam (Shankar and Shanker, 2014; Singh et al., 2015b; Upadhyay et al., 2019).

Although it is reported that rice is a good accumulator and the main dietary source of As, especially in South East Asia, concerns are mounting about the amount of As being found

in other major cereals (e.g. wheat) (Chandiramani et al., 2007). A considerable amount of As has already been reported in rice throughout the world. Examples include: $< 0.01 - 2.05 \mu\text{g g}^{-1}$ for Bangladesh, $0.31 - 0.70 \mu\text{g g}^{-1}$ for China, $0.03 - 0.044 \mu\text{g g}^{-1}$ for India, and $0.11 - 0.66 \mu\text{g g}^{-1}$ for US (Zavala and Duxbury, 2008) when cultivated in As contaminated ($9.00 - 105.00 \text{ mg kg}^{-1}$) soils (Ullah, 1998; Hua et al., 2005; Patel et al., 2005). In wheat, $0.005 - 0.285 \mu\text{g g}^{-1}$ (Wiersma et al., 1986), $0.004 - 0.362 \mu\text{g g}^{-1}$ (Roychowdhury et al., 2002), and $0.001 - 0.500 \mu\text{g g}^{-1}$ of As have been reported when cultivated in As contaminated ($3.00 - 201.00 \text{ mg kg}^{-1}$) soils (Williams et al., 2007). Although there is no worldwide standard safe limit of As in food grains, the European Commission recently (January 2016) set a maximum limit of as $0.200 \mu\text{g g}^{-1}$ for As in milled rice (polished or white rice) (Union, 2015). Thus, besides the drinking water, rice and wheat also could be a potential pathway of As intake and accumulation in the human body because these cereal crops are dietary staples for a large portion of world's population (González et al., 2020; Suman et al., 2020).

In the soil and water environment, As is mainly present in two forms, inorganic and organic. Arsenate [As(V)] and arsenite [As(III)] are the inorganic forms while monomethyl arsonic acid (MMA), dimethyl arsinic acid (DMA), trimethylarsine oxide (TMA), arsenobetaine (AB), arsenocholine (AC), and arsenosugars are the most common forms of organic As (Jiménez et al., 2012). The inorganic forms are more toxic to plants, animals, and humans than the organic forms (Khalid et al., 2017) and among the inorganic forms As(III) is approximately 60 times more toxic, soluble, and mobile than the As(V) (Sun et al., 2014; Wang et al., 2020a). Arsenate is a predominant form present in aerobic conditions and As(III) is mostly present in anaerobic conditions (Wu et al., 2017). Methylation of inorganic As species in the soil are mediated by microorganisms to form organic species (MMA, DMA, and TMA) (Huang et al., 2011b). For example, the microbial methylation process of As(III) is the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to As(III), which is catalyzed by As(III) S-adenosylmethionine methyltransferase (ArsM). Those soil microbes (e.g. bacteria, archaea, and fungi), which possess the *arsM* gene, can produce this enzyme to methylate the inorganic As species (Lomax et al., 2012).

Since increased As levels have contaminated water and soil, therefore, one of the strategies to minimize risk of As toxicity is As remediation of water and soil. Different techniques are being used for As remediation in drinking water such as precipitation, adsorption and ion exchange, coagulation-filtration, membrane filtration, nanofiltration, reverse osmosis, etc. (Ghosh et al., 2019). Treatment of irrigation water, in a similar way to

drinking water, is one option of As removal; however, it is a big challenge to treat the huge quantity of irrigation water required for crop cultivation. There are several physical, chemical, and biological methods for soil As remediation (Lim et al., 2014). In the physical approaches, soil replacement and soil washing or flushing with different concentration of chemicals such as sulfuric acid, nitric acid, phosphoric acid, and hydrogen bromide are most common methods. However, choice of chemicals, lower efficiency and high cost of application have often restricted these physical methods into a smaller-scale operation (Lim et al., 2014). Furthermore, the current chemical remediation techniques are adsorption by using specific media, immobilization, modified coagulation along with filtration, precipitations, immobilizations, and complexation reactions. The coagulation along with filtration method for removing arsenic from contaminated sources is quite economic but often displayed lower efficiencies. Furthermore, the use of selective stabilizing amendments is a challenging task as most polluted sites are contaminated with multiple metal(loid)s. It is reported that chemical remediation gained popularity because of its high success rate, but it could be expensive when someone would like to remediate a large area (Lim et al., 2014). Moreover, biological remediation or bioremediation have been gaining interest for As remediation due to their cost effectiveness. Basically, degradation of As by naturally occurring microorganisms and optimizing the environment conditions to promote the proliferation and activity of microorganisms that lived in that area are main area of bioremediation, but this method is more suitable for remediation of soil with a low level of contaminants (Lim et al., 2014). Phytoremediation by using hyper accumulative plants is another technique, which is probably the most environment-friendly and cheapest method (Vandana et al., 2020), but the selectivity of plants for different heavy metals, depth of remediation, time taken to do an adequate job and disposal of the biomass after remediation have been noted as major drawbacks of this method (Lim et al., 2014; Farraji et al., 2016). As well, complete soil As remediation is very difficult because of the vast scale of application. Thus, alternative options or combinations of technologies, for alleviating soil As, are required.

Pre-sowing microwave (MW) soil heating, at a certain level of temperature, has been reported to increase crop growth and grain yield of wheat and rice (Khan et al., 2016; 2019a), in addition of controlling weed infestation by destroying the soil weed seed bank through a non-chemical means, especially for herbicide resistance weed (Brodie et al., 2017). Microwave is a form of electromagnetic radiation, with 1 m to 1 mm wavelengths and 300 MHz to 300 GHz frequencies (Banik et al., 2003), which can produce heat by rotation of polar molecules

(e.g. water) (Taylor et al., 2005) when applied into the soil, due to the oscillating electromagnetic field, intermolecular friction and collision (Falciglia et al., 2016). Hence, MW soil heating depends on the dielectric properties of soil. Also, the water holding capacity of different soils are not the same. Thus, dielectric properties of different soils with different moisture content needs to be explored before the application of MW heating. However, due to the heating mechanism of MW, it produces a fast heating rate, since soil moisture is considered to be an efficient absorber of MW radiation. Thus, MW heating have some major advantages, compared with other heating processes, like short start-up, selective heating, precise control, no direct contact with the heated materials, and volumetric heating (Ayappa et al., 1991). Because of these advantages MW heating has been used in diverse applications including removal of organic contaminants (Falciglia and Vagliasindi, 2014) and immobilization of some toxic metals such as Copper (Cu), Manganese (Mn), Thorium (Th), Zinc (Zn), Nickel (Ni), cadmium (Cd), Chromium (Cr), and Lead (Pb) in soil (Abramovitch et al., 2003) and solid sediments (Chen et al., 2005a). Arsenic contaminated soil could be treated with MW equipment on-site in the field without further pre and post processing of soil which could be a unique advantage of MW compared with other remediation techniques. However, no study has been found in terms of soil As remediation using MW heating. Therefore, MW soil heating could be a novel method to lessen the As phytotoxicity and grain As accumulation.

Furthermore, to immobilize soil organic and inorganic pollutants and heavy metals, biochar has been progressively gaining more attention because of its high carbon content, micropores, and large surface area. Biochar is a carbonaceous solid material obtained from thermochemical decomposition of residual biomass at relatively high temperature (500 – 1500 °C) through a process called pyrolysis (heating in oxygen-depleted conditions). It is porous and rich in stabile and resistant carbon, with different surface functional groups (Lehmann and Joseph, 2015). Due to these beneficial physicochemical properties of biochar, different research suggest it to be an effective sorbent, which can immobilize and reduce the bioavailability of heavy metals (Méndez et al., 2012). Electrostatic attraction, surface complexation, ion-exchange, and precipitation are the predominant phenomena for the adsorption of metals including As by biochar, due to the presence of different surface functional groups (Ahmad et al., 2014). However, increased mobility and bioavailability of As is also reported after the application of some biochar (Beesley et al., 2014; Gregory et al., 2015; Zheng et al., 2015). Hence, this advocates that all types of biochar are not suitable for the remediation

of As contaminated soils. The remediation efficiency depends on the biochar properties such as feedstock type, pyrolysis temperature, heating rate and application rate (Hartley et al., 2009).

Microwave assisted pyrolysis is a unique method and possibly offers a good alternative to conventional pyrolysis owing to its volumetric energy transfer rather than conductive heat transfer, non-contact and rapid heating. Thus, compared with conventional pyrolysis, more uniform heating in MW pyrolysis through internal uniform temperature distribution can be achieved. Also, undesired secondary reactions may be avoided in MW pyrolysis. As a result, better control of the process and more desired products will be obtained. Several pieces of research reported, higher char yield, quick rise of temperature, higher heating rate, and less or no pre-processing of feedstock in MW assisted pyrolysis compared with conventional pyrolysis (Zhao et al., 2010b; Li et al., 2016a).

Although, several studies reported As immobilization using different types of biochar, a combination of MW soil heating and biochar produced from MW assist pyrolysis is a new technique to alleviate As phytotoxicity and accumulation in wheat and rice grain.

1.1 Research objectives

The main aim of this thesis is to explore the effect of MW soil heating and MW generated biochar treatment on the phytotoxicity and uptake of As in wheat and rice. Additionally, soil dielectric properties of soil must be determined to understand MW soil heating and its application. The persistence of these treatments also needs to be understood; therefore, in the following year, using the same soils without further MW and biochar treatment, the crops need to be grown to assess the residual effect of treatments. Also, it is important to understand whether any effects of MW soil heating are purely thermal or if there is some unique quality of MW radiation, so a study need to be conducted to compare MW soil heating with conventional heating. Because the form of As in the environment affects its toxicity, a speciation analysis needs to be performed to know which As species accumulated in the grain, and 16S rRNA amplicon sequencing and 16S rRNA qPCR analysis need to be conducted to observe the impact of MW heating on soil bacteria.

Therefore, the specific objectives of this thesis are:

1. To determine the dielectric properties of different types of soils (e.g. Sandy, loamy, clay etc.), with different moisture content (e.g. Oven dry and different field capacity levels), over a wide range of frequencies (e.g. 700–7000 MHz);
2. To investigate the potential impacts of MW soil heating and MW generated biochar on the phytotoxicity and accumulation of As in wheat and rice;
3. To compare the effect of MW soil heating and conventional electric oven heating on plant growth, grain yield, and the grain As accumulation in rice;
4. To examine the effect of MW soil heating and biochar on the As speciation in wheat and rice and their relationship with As toxicity alleviation; and
5. To observe the changes in bacterial community composition and richness in response to MW soil heating and their subsequent recovery after heating.

1.2 Research hypothesis

1. The dielectric properties of soil increase as soil moisture increases and the dielectric properties of sandy soil is much lower than that of other soils.
2. Microwave soil treatments and biochar application alleviate As phytotoxicity by increasing plant growth and grain yield and decreasing grain As accumulation. Microwave soil heating increase some soil nutrients which increase plant growth, reduce the uptake of As, and reduce the grain As concentration. Adsorption of the As on the biochar surface reduce the plant As uptake.
3. Without further application, MW soil heating and biochar treatment alleviate the As phytotoxicity in wheat and rice in the following season as well.
4. Microwave soil heating is more time, cost, and energy efficient than conventional heating.
5. Compared with the control treatment, MW and biochar treatment reduce the accumulation of more toxic forms of As in wheat and rice grain.
6. The soil bacterial community reduces immediately after MW soil heating but recovers over time.

1.3 Thesis overview

In this thesis, following this introduction section, Chapter 2 highlights the status of As pollution in groundwater, agricultural soils, and grains of wheat and rice. It outlines the possibilities of MW and biochar as new As phytotoxicity alleviation techniques, reviews diverse literature on the MW soil heating technology and biochar application for better plant growth, grain yield and As immobilization. Therefore, Chapter 3 (published in the Journal of Microwave Power and Electromagnetic Energy) determines the dielectric properties of different soils with different moisture content over a wide range of frequencies and provides an understanding the relationship of soil dielectric properties with moisture. The data obtained from this chapter is considered for further experimental soil heating with the MW system. Consequently, Chapter 4 explores the application of MW soil heating to alleviate the As phytotoxicity and subsequent grain accumulation in wheat. Accordingly, Chapter 5 investigates the effect of MW soil heating and biochar application on the As phytotoxicity alleviation and grain accumulation in rice. In addition, Chapter 6 inspects the residual effect of MW and biochar soil treatments in response to wheat and rice plant growth, grain yield, and grain As accumulation in subsequent seasons. To find out whether the better plant growth is only a heating effect or is due to any unique MW effect, Chapter 7 compares the effect of MW and electric oven soil heating on plant growth, grain yield, and the grain As accumulation of rice. The phytotoxicity of As not only depends on its concentration but also on its chemical speciation. Thus, Chapter 8 estimates the different As species concentrations in wheat and rice grain. In relation to the impact of MW heating on soil bacteria, Chapter 9 evaluates the soil bacterial community composition, richness, relative abundance, and shift, based on the 16S rRNA amplicon sequencing and 16S rRNA gene copy number. Finally, Chapter 10 appraises the potential application of biochar and MW soil heating technology, based on the results of experiments presented in all the chapters, and gives some directions for further research needed in this area.

Chapter 2 – Literature review

2.1 Arsenic

2.1.1 History of arsenic

Arsenic (As) is a steel-gray, brittle, crystalline metalloid. In the periodic table As belongs to the group V-A. The atomic number of As is 33 with $74.9216 \text{ g mol}^{-1}$ atomic mass, 817°C melting point, and 613°C boiling point (Mandal and Suzuki, 2002). When heated, As undergoes a phase change directly from solid to gas. Thus, the boiling point is lower than the melting point. The name ‘Arsenic’ originated from a Greek term *arsenikon* which means strong (Choong et al., 2007). In the 8th century, an Arabian, Geber discovered As while heating orpiment (As_2S_3) (Mellor, 1960) and in 1250 A.D. As was isolated by Albertus Magnus (Mandal and Suzuki, 2002). Arsine gas (AsH_3) was discovered by Scheele, a famous Swedish chemist, in 1775. The deadly nature of AsH_3 was identified in 1815, when a chemistry professor in Munich, died from inhaling AsH_3 . Between 1850 to 1950 human beings have been affected by As in medicine, food, and the environment (air and water) and the world production of white As (As trioxide; As_2O_3) increased from 5,000 to 60,000 tons year⁻¹. Different As minerals (e.g. realgar and orpiment) are used in the leather industry, wall painting, and for pigment formation (Nriagu, 2002). For the manufacturing of dyes, wood preservatives, ceramics, herbicides, pesticides, and cotton desiccants, various As compound, such as lead arsenate (PbAsO_4), calcium arsenate (CaAsO_4), and sodium arsenite (NaAsO_2), have been used. However, despite its importance, the use of As has been banned or restricted due to its toxic effect on human health. For example, in the production of the agricultural chemicals As is still used, although the amounts produced vary between countries, depending on the restrictions on this use that are in force (it is banned in the United States) (Koren, 1991). Furthermore, in countries like the United States, inorganic arsenic was used as a wood preservative in the form of copper chromate arsenic (CCA), which became a predominant source of As contamination of soil. As of 2003, CCA is banned from any form of residential use in the United States (Gerstein and Zaccaria, 2004).

2.1.2 Why arsenic pollution is our concern?

It is estimated that a substantial number of people (approximately 220 million), worldwide, are at risk of As poisoning because their drinking water has As levels which are above the WHO (World Health Organization) recommended maximum allowable limit of 10 mg l^{-1} (Podgorski and Berg, 2020). According to EPA (Environmental Protection Agency) of the USA, inorganic As is a group 1 carcinogenic agent, which increases the risk of different types of cancer. Chronic As poisoning also has detrimental effects on respiratory, neurological, renal, cardiovascular, and hematological systems (Abdul et al., 2015). It is evident from the literature that a considerable number of people throughout the world are already affected by different diseases due to long term exposure of As (Mandal and Suzuki, 2002). Excessively high As pollution in groundwater, soil and crops has already been identified in many countries such as Bangladesh, India (West Bengal), China, Pakistan, Cambodia, the USA, Thailand, Mexico, Argentina, and Viet Nam (Figure 2.1a) (Shankar and Shanker, 2014; Singh et al., 2015b; Upadhyay et al., 2019). In Bangladesh, which is considered to be the worst polluted area, with a huge affected population (Singh et al., 2015b), As pollution is so common that out of the 64 districts the groundwater of 50 districts contains As levels which exceed the safe limit of Bangladeshi standard ($50 \text{ } \mu\text{g l}^{-1}$) (Figure 2.1b) and 59 districts are above the standard limit recommended by the WHO ($10 \text{ } \mu\text{g l}^{-1}$) (Huq et al., 2020), with 12.40 % and 24.80 % of the population's drinking water often not meeting the WHO or Bangladeshi provisional guidelines, respectively (BBS, 2014; Mihajlov et al., 2020). Thus, drinking water is considered to be the main source of human As intake. In addition, using As contaminated water for long periods of time to irrigate crops has allowed As to build up in soil. As a consequence, As is accumulating into the edible part of crops through plant uptake and is resulting in dietary As exposure (Abedin et al., 2002b; Naidu et al., 2006). Different pathways of As exposure to human are illustrated in Figure 2.2. Although rice grain is reported as the main dietary source of As, especially in South East Asia, concerns are mounting about the amount of As being found in other major cereals (e.g. wheat) (Chandiramani et al., 2007).

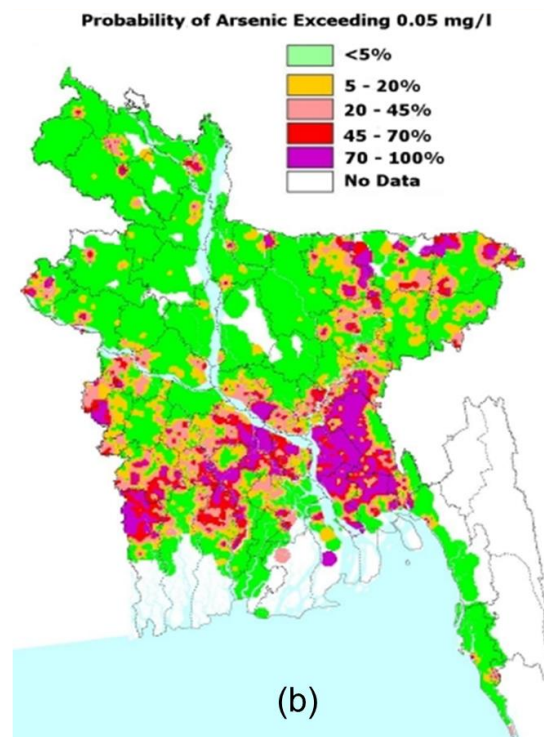
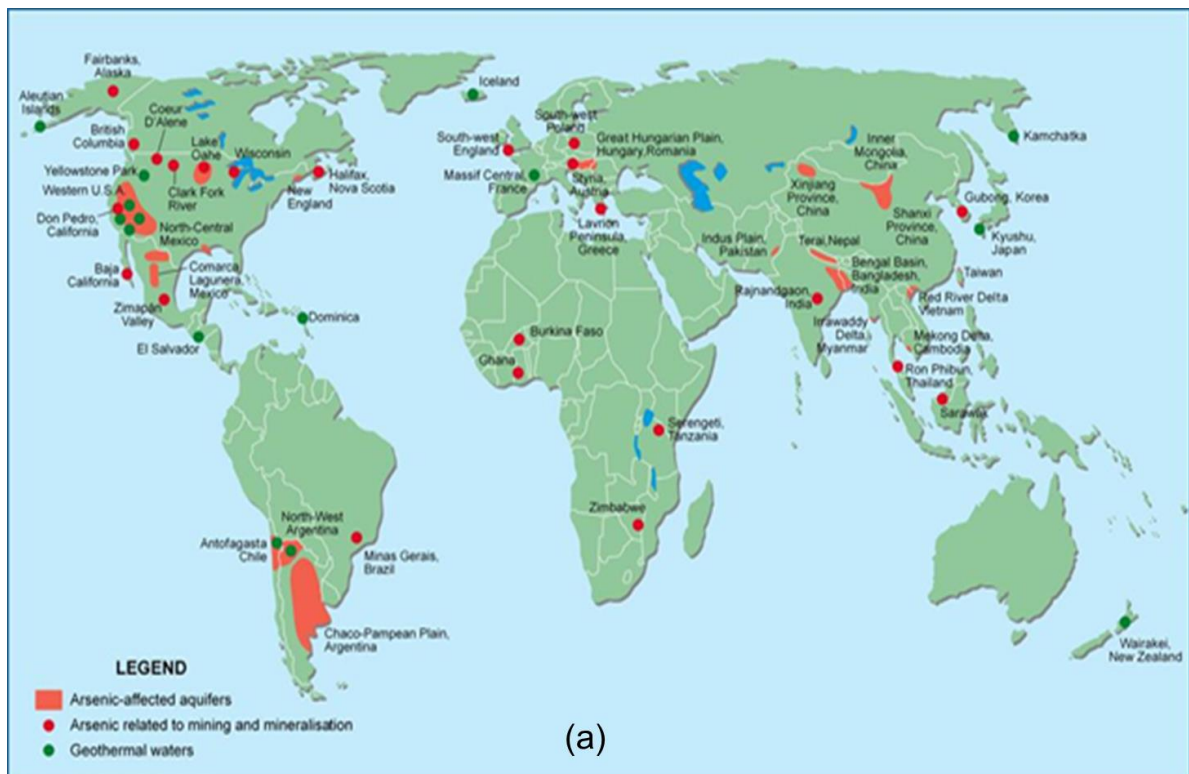


Figure 2.1. (a) Arsenic affected aquifers throughout the world [Adopted from (Smedley and Kinniburgh, 2002)] and (b) groundwater arsenic pollution in Bangladesh [Adopted from (Ravenscroft et al., 2011)]

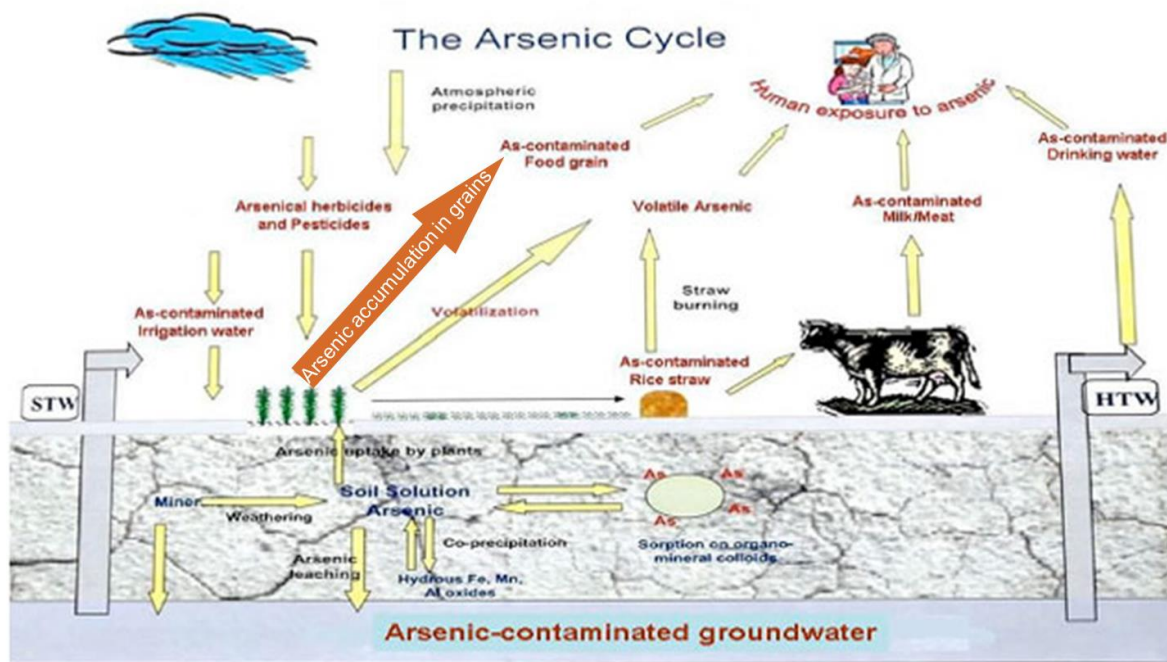


Figure 2.2. The arsenic cycle showing the probable contamination source and human exposure pathways. HTW: hand tube well, STW: shallow tube well [Adopted from (DPHE, 2000)]

2.1.3 Arsenic distribution in the environment

Arsenic is the 20th most abundant element, which is ubiquitous and broadly spread in the environment. It is present in more than 200 minerals (Smedley and Kinniburgh, 2002) of which, arsenates cover approximately 60 % along with sulfides and sulfosalts (20 %) and elemental As (20 %). Some oxide, arsenite, and silicate minerals are also reported (Wedepohl et al., 1969). Numerous natural processes, such as volcanic eruptions, pedogenesis, forest fires, geothermal/hydrothermal activity, and dust storms, contribute to environmental background As concentration (Garelick et al., 2009). As concentration in soils primarily depends on the parent material. Soils, which are developed from igneous rocks, have a lower As concentration (1.50 – 3.00 mg kg⁻¹) than sedimentary rocks (1.70 – 400.00 mg kg⁻¹) (Nriagu and Pacyna, 1988). Soils originating from granites mostly form sandy soil with lower As concentrations while, alluvial and organic soils contain higher As concentrations (Mandal and Suzuki, 2002). Arsenopyrite (FeAsS), orpiment (As₂S₃), and realgar (AsS) are considered to be the main As bearing minerals. These naturally occurring As sulfide minerals contain relatively higher amount of As compared with other potential sources. Various minerals of As, occurring in soil, are presented in Table 2.1.

Table 2.1. Arsenic containing minerals in the soil environment (Mandal and Suzuki, 2002)

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

2.1.4 Natural sources of arsenic

Different As containing minerals undergo diverse natural processes like weathering reactions, biological activities, and volcanic emission to release their As. These sources are often called geogenic sources (Smedley and Kinniburgh, 2002). It is reported that geogenic sources are responsible for most of the world's As pollution. For example, weathering of As containing minerals (mostly sulfide) in the Himalayas region and their subsequent transportation and downstream deposition in the Gangetic plains is the primary source of As pollution in West Bengal and Bangladesh (Nickson et al., 1998). These deposited minerals undergo transformation to form iron (Fe) hydroxides, oxyhydroxides, and oxides, via oxidation, when exposed to the atmosphere. For instance, the groundwater As contamination in Bangladesh is attributed to the combination of As in aquifer sediments as a source, mobilization to groundwater and transportation through natural groundwater circulation (Hossain, 2006).

2.1.5 Anthropogenic sources of arsenic

The major anthropogenic sources of As are refining of metalliferous ore including by-products such as slag, mining and smelting operations, emissions from industrial manufacturing processes including electroplating, energy and fuel production, copper chromium arsenate treatment as a preservative of wood timber, and agricultural practice such as the application of different As containing pesticides, fertilizers, and municipal waste to the land (Mandal and Suzuki, 2002). A brief overview of major anthropogenic sources of soil As contamination are given below:

2.1.5.1 Mining activities

Mining and smelting of Zn, Cu, Au, and Pb from their ores, which contain As as an impurity, release As with the waste materials. Subsequent exposure of these waste materials, tailings and slag by erosion potentially creates contamination of nearby soils. Transportation of fine particles from this mining waste to surface water can cause secondary contamination of further sites (Karczewska et al., 2007). In Australia, gold mining activities has been reported as a source of soil As contamination. A high quantity of As (280 – 15,000 mg kg⁻¹) was reported in gold mining waste disposal areas (McLaren et al., 2006). Mining activities in other countries including Brazil, Iran, Northern Chile, England, Thailand, Ghana, USA, Greece,

Mexico, South Africa, and Columbia were also reported as a source of soil As pollution (Garelick et al., 2009).

2.1.5.2 Industrialization

Copper-chromium-arsenate (CCA) mixture is used in many countries, including New Zealand and Australia, as a wood preservative in timber industries. Spilling, leaking and run-off of As from the treated timber contaminates the surrounding soils. A high proportion of soil As (500 – 10000 mg kg⁻¹) in these sites have been reported. As-trioxide is considered to be a major compound for many industrial uses such as cosmetics production, ceramic manufacturing, glass manufacturing, Cu-based alloys to increase resistance against corrosion, pigments and antifouling agent production, electronics, and fireworks preparation (McLaren et al., 2006).

2.1.5.3 Agriculture activities

Use of As based pesticides can pollute agricultural soils. Paris green [Cu(CH₃COO)₂.3Cu(AsO₂)₂] was a famous pesticide, which was widely used in agriculture (Garelick et al., 2009). Other pesticides include magnesium arsenate [Mg₃(AsO₄)₂], zinc arsenite [Zn₃(AsO₃)₂], calcium arsenate [Ca₃(AsO₄)₂], zinc arsenate [Zn₃(AsO₄)₂], and lead arsenate [Pb₃(AsO₄)₂] (Smith et al., 1998; Garelick et al., 2009). In the US, Paris green was used to control the Colorado potato beetle, calcium arsenate was used to control boll weevil in cotton and in other vegetable crops as a broad-spectrum insecticide, and lead arsenates was used as insecticides, especially in apple orchards. However, in the late 1980s, the application of several As based pesticides was barred by the Environmental Protection Agency (EPA) of the USA (Garelick et al., 2009). Different fertilizers are manufactured from their respective rocks, where As was also present as an impurity. Application of these fertilizers released As into the agricultural soil. For example, phosphate fertilizers, manufactured from phosphate rocks, contain As (Smith et al., 1998). Crop irrigation with As contaminated waste effluent is a potential source of soil As accumulation especially in agricultural soils surrounding industrial sites. Besides, As contaminated ground water also deposits As in soil while irrigating agricultural crops (Dittmar et al., 2007; Roberts et al., 2007). Bangladesh is a notable example of this incidence, where farmers often lift water from As affected aquifers, through deep and shallow pumps, for crop irrigation.

2.1.6 Fate of arsenic in soil

In the soil environment, As is mainly present in two forms, inorganic and organic. Arsenate [As(V)] and arsenite [As(III)] are the inorganic forms while monomethyl arsonic acid (MMA), dimethyl arsinic acid (DMA), trimethylarsine oxide (TMA), arsenobetaine (AB), arsenocholine (AC), and arsenosugars being the most common forms of organic As. The inorganic forms are more toxic to plants, animals, and humans than the organic forms (arsine > As(III) > organic arsenite > As(V) > MMA > DMA) (Jiménez et al., 2012; Khalid et al., 2017) and among the inorganic forms As(III) is about 60 times more toxic, soluble, and mobile than the As(V) (Jain and Ali, 2000; Sun et al., 2014).

As concentration in the soil is the sum of the As fractions of the following pools (Wenzel et al., 2001): (i) exchangeable or weakly adsorbed (As present as free ions or in soluble forms), (ii) sorbed to the mineral surface also called phosphate extractable As, (iii) Fe and Al hydrous oxides bound (inner-sphere complexed), (iv) associated with crystalline Fe/Al oxides, and (v) residual. The equilibrium condition between the soil solution and solid phase forms largely determine the As solubility. Equilibrium condition may be affected by numerous reactions such as ion-exchange, redox reactions, complexation with organic and inorganic ligands, precipitation-dissolution, and adsorption (Morel, 1996). The plant available As is not dependent on the total soil As but soil solution As. Among these pools, the first three are considered as bioavailable forms for plant uptake. Thus, high soil As concentration may not necessarily result in high plant uptake (Devesa et al., 2008; Ko et al., 2008). Different physical, chemical, and biological processes within the soil environment also control the As availability. Physical processes are largely dependent on soil type, soil texture, soil structure, soil aggregates, compaction, resistance, organic matter and water storage capacity (Smith and Naidu, 2009). Soil pH, redox condition, and speciation are chemical properties which influence the As availability, mobility and subsequent plant uptake (Mahimairaja et al., 2005). Soil bacteria and fungi present in soil rhizosphere in association with higher plants act as biological factor which may significantly change the physical and chemical conditions of soil and become determining factor of As phytoavailability (Al-Agely et al., 2005). A brief description of some of these factors is presented below.

2.1.6.1 Soil pH and redox condition (Eh)

Soil pH and the redox condition play an important role in the availability of As especially inorganic As species. As(III) occurs mainly in reducing (anaerobic) conditions,

whereas As(V) is more available in oxidizing (aerobic) conditions. In a soil-water system the most common oxidation states of As are As^{3-} [As(–III)], 0 (uncharged), As^{3+} [As(III)], and As^{5+} [As(V)] (Jiménez et al., 2012). Arsine and metal arsine are examples of the As^{3-} state, which are formed under very strong reducing conditions and are very unstable in oxidized condition (Bissen and Frimmel, 2003). In aerobic conditions, As^{5+} is a predominant form, which can form As acid (H_3AsO_4) at very low pH (2 – 3) while it dissociates to H_2AsO_4^- and HAsO_4^{2-} in the pH range 3 – 11 and AsO_4^{3-} in pH more than 11 (Figure 2.3). In anaerobic condition, As^{3+} (e.g H_3AsO_3) is mostly present (Smedley and Kinniburgh, 2002). Up to pH 9, H_3AsO_3 can be present in the uncharged condition whereas, at higher pH it dissociates into H_2AsO_3^- , HAsO_3^{2-} , and AsO_3^{3-} (Figure 2.3) (Bissen and Frimmel, 2003).

Redox potential (Eh) is relatively high in aerobic soil conditions and low in anaerobic soils. At high Eh arsenates (H_3AsO_4 , H_2AsO_4^- , HAsO_4^{2-} , and AsO_4^{3-}) are more stable and available while arsenites (H_3AsO_3 , H_2AsO_3^- , HAsO_3^{2-} , and AsO_3^{3-}) mainly occur at low Eh (Figure 2.3) (Garelick et al., 2009). Methylation of inorganic As species in soil are mediated by microorganisms to form organic As species (e.g. MMA and DMA) (Huang et al., 2011b). Relatively higher methylated As species are present in the reduced soil condition due to the higher rate of microbial methylation (Jia et al., 2013), which is greatly enhanced by reducing conditions (Mestrot et al., 2009).

The mobility of As species in soil also depends on the pH and Eh. As compounds can adsorb to oxides and hydroxides [Fe(III), Al(III), and Mn(III/IV)], humic substances, and clay minerals. In reduced condition, oxides and hydroxides of Mn and Fe are mainly responsible for bounding As and their mobilization due to the reduction of Fe(III) to Fe(II) and Mn(III/IV) to Mn(II). In this context, the reduction begins at a Eh of +200 mV under neutral to acidic pH whereas, at alkaline pH reduction occur at lower Eh (Bissen and Frimmel, 2003). For example, the influence of pH and Eh on As speciation and solubility was investigated where soil pH and soil As concentration were 5.6 and 555 mg kg⁻¹ respectively (Masscheleyn et al., 1991). The study showed that in the Eh range of +500 mV to +200 mV the solubility of As was low and 65 to 98 % of As was As(V). Conversely, As(III) was the major species in the Eh range of 0 to –200 mV. The solubility of As was 13 times higher at –200 mV compared to +500 mV. Additionally, they showed that at lower Eh (100 to 0 mV) in alkaline conditions the solubility of Fe oxides and hydroxides determine the solubility of As. Thus, the pH should not be in the alkaline range and Eh should be high to prevent the mobilization of As. In another rice field study it was reported that 30 % of As species was As(III) during non-flooded condition (high

Eh; around 600 mV) and up to 70 % was under flooded condition (low Eh; around 0 mV) at 0.1 – 0.2 m soil depth (Takahashi et al., 2004).

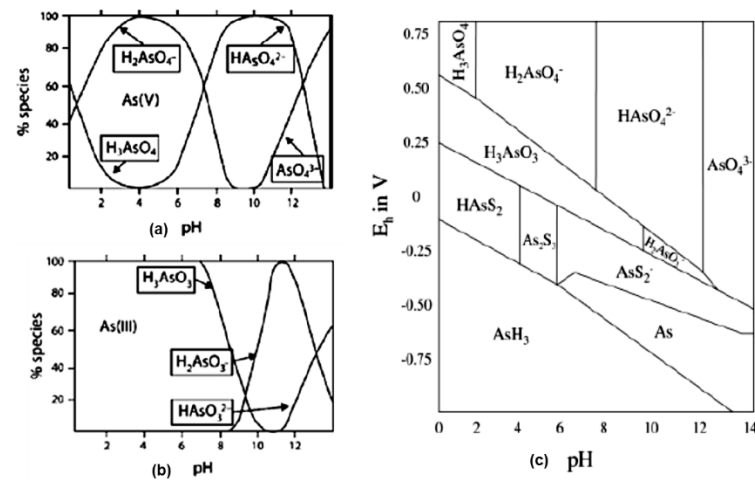


Figure 2.3. Availability of arsenate [As(V)] and arsenite [As(III)] at different soil pH and redox condition (Eh). (a) effect of pH on As(V) availability, (b) effect of pH on As(III) availability, and (c) availability of As(V) and As(III) in combination of pH and redox condition. [a and b adopted from (Garelick et al., 2009) and c adopted from (Bissen and Frimmel, 2003)]

2.1.6.2 Iron hydroxides (FeOOH)

Iron (Fe) redox chemistry plays an important role in As behavior in soil. For both As(III) and As(V), FeOOH acts as the main sorbent. FeOOH can adsorb As(V) more strongly than As(III), which explains the higher availability of As(III) (Fitz and Wenzel, 2002; Heikens et al., 2007). In clay soil, FeOOH is present in greater amounts than in other types of soils and therefore, total As content is higher in clayey soils compared with sandy soils. At the same time, clayey soils are less toxic to plant at the same As concentration because of the strongly bound As. Alongside carbonates in calcareous soil, manganese oxides in acidic soil are also important as sorption substrates (Fitz and Wenzel, 2002; Mahimairaja et al., 2005). FeOOH is readily dissolved under anaerobic conditions, which desorbs the As(V) into the soil solution and then it is reduced to As(III) (Fitz and Wenzel, 2002). Before desorption of As(V) from FeOOH, the reduction of As(V) to As(III) is also reported (Takahashi et al., 2004). Since FeOOH is relatively insoluble under aerobic condition, FeOOH acts as a sink for As. The aging process also can reduce the availability of As in soil. However, Fe and As chemistry are of a dynamic nature in rice fields where both aerobic and anaerobic soil condition oscillate, making it less likely to influence the aging (Heikens et al., 2007). The adsorption of As(V) on FeOOH decreases with increasing soil pH particularly above 8.5 and maximum adsorption lies around

pH 4. While, maximum adsorption of As(III) occurs at pH 7 – 8.5 (Fitz and Wenzel, 2002; Mahimairaja et al., 2005). Another study also reported approximately 50 % decrease in As(V) adsorption in the pH range 7 – 10, because FeOOH becomes more negatively charged above pH 7 (Williams et al., 2003).

2.1.6.3 Phosphate concentration

Phosphate (PO_4^{3-}) is an analogue of As(V), which makes it an important factor of As availability, especially in aerobic soils (Geng et al., 2006). Both the ions compete for sorption sites and plant uptake. Therefore, increasing PO_4^{3-} in the aerobic soil results in enhanced competition between PO_4^{3-} and As(V) for sorption sites on soil particle surfaces, which can increase the mobility and bioavailability of As(V). Some researchers reported that displacement of soil PO_4^{3-} by As(V) increased the availability of PO_4^{3-} to the plant, at low soil As concentration, which resulted in the increase of plant growth parameters (Gulz et al., 2005). In anaerobic soils, PO_4^{3-} has less influence over As behavior except for where aerobic condition can occur under flooded conditions (Takahashi et al., 2004)

2.1.6.4 Silicon concentration

Soil Silicon (Si) concentration has been reported to control the availability of As(III) for plant uptake. For example, in rice, the same uptake pathway is identified for the uptake of Si and As(III) (Ma et al., 2008). Both indigenous soil Si (Bogdan and Schenk, 2008) and applied Si (Guo et al., 2009; Li et al., 2009b) inhibit As(III) uptake in rice. However, contrasting results are also reported, especially at higher soil As concentration where Si increased the As(III) concentration in soil solution and facilitate more As(III) uptake by the plant (Hu et al., 2013).

2.1.6.5 Soil organic matter

Soil organic matter (SOM) consists of different organic compounds and their derivatives. Some organic compounds can compete with As for sorption sites. Macromolecular organic substances with a higher number of functional groups (Kim and Kim, 2013), organometallic and coordination compounds (Taylor et al., 2005), can adsorb soil heavy metals (Yin et al., 2002). Therefore, these fractions of SOM could reduce As bioavailability as well. However, As can mobilize due to reactions with ionizable functional groups of SOM and interactions with dissolved organic carbon (DOC). As(V) can reduce to As(III) by interacting with DOC, where DOC serves as electron donors (Yang et al., 2016), which also can enhances

As mobility (Park et al., 2011b). Humic acid can bind with As, which reduces the As sorption and DOC can form soluble complexes with As and can reduce the sorption of As to soil particle (Fitz and Wenzel, 2002; Mahimairaja et al., 2005).

2.1.6.6 Microbial activity

Microbial activity can influence the As availability via numerous mechanism such as redox reactions with Fe and As, methylation and demethylation of As species. For instance, before methylation to MMA and DMA, algae can reduced As(V) to As(III) (Fitz and Wenzel, 2002; Mahimairaja et al., 2005). In the presence of SOM, microbes are directly involved in the reductive dissolution of FeOOH, which results in reduction of As(V) and its subsequent release in the soil solution (Islam et al., 2004a). However, reduction of As(V) can happen without dissolution of its sorbent since some bacteria such as *Sulfurospirillum barnesii* can reduce both As(V) and Fe(III) (Zobrist et al., 2000). At the time of microbial mineralization of SOM, As(V) can be reduced to As(III), where As(V) acts as an electron acceptor (Heikens et al., 2007). Soil microbes can replace the hydroxyl groups of inorganics As species by methyl groups. Some soil bacteria (such as *Escherichia coli*, *Flavobacterium* sp, and *Methanobacterium* sp) and fungi (such as *Aspergillus glaucus*, and *Candida humicola*) play important roles in this context (Mandal and Suzuki, 2002).

MMA, DMA and TMA are mostly available organic forms of As in the soil as a result of the microbial biomethylation process (Mestrot et al., 2011). For example, in the methylation process of As(III), a methyl group from S-adenosyl-L-methionine (SAM) transfer to As(III), which is catalysed by As(III) S-adenosylmethionine methyltransferase (ArsM) (Yin et al., 2011). Those soil microbes (e.g. bacteria, archaea, and fungi) possess the *arsM* homologues, which are responsible for methylation of inorganic As (Yin et al., 2011). However, very few studies have been conducted to known about the abundance and diversity of the *arsM* operon gene in the soil environment (Jia et al., 2013; Afroz et al., 2019).

The rate of biomethylation is low in acidic soils compared with alkaline soils. It was evident from the previous study that biomethylation of As(V) and As(III) occur at pH 6.5 and pH 8.0 which significantly decreased at pH lower than 5.0 (Carbonell-Barrachina et al., 2000). Some soil bacteria, fungi, and yeasts can transform the methylated As species into volatile forms of As. Monomethylarsine (H_2AsCH_3), dimethylarsine [$\text{HAs}(\text{CH}_3)_2$], and trimethylarsine [$\text{As}(\text{CH}_3)_3$] are formed by biomethylation under anaerobic conditions. Contrariwise, the oxidation of methylated As species can occur under aerobic condition to form inorganic species

(Bissen and Frimmel, 2003). Some studies have shown that methylated As species (mostly DMA), which are present in the crop grain (e.g. Rice), most likely originated from the soil via the activities of methylating microorganisms and are not from *in vivo* methylation in the plants (Arao et al., 2011; Lomax et al., 2012). Thus, soil microbial communities are also a key regulator of species dependent As phytotoxicity in plants.

2.1.6.7 Influence of rhizosphere

The condition in the microenvironment around the root zone (rhizosphere) may differ substantially from the bulk soil. Plant roots have influence over the soil solution composition through the uptake of essential and nonessential elements and excretion of different substances (e.g. organic acids). Thus, rhizosphere pH may differ from the pH of the bulk soil. Cation and anion uptake ratio also influence the rhizosphere pH. For example, more uptake of NH_4^+ can reduce the rhizosphere pH while, the opposite effect can be attributed to NO_3^- uptake. The buffering capacity of soil can also influence the rhizosphere pH (Fitz and Wenzel, 2002; Heikens et al., 2007).

2.1.7 Arsenic uptake and translocation mechanism in plants

2.1.7.1 Arsenic uptake from soil to root

Plants uptake different essential nutrient elements from the soil through different transporters, present in plants' roots. Due to the imperfect selectivity of these transporters, plants also uptake nonessential and toxic elements. As(V) and As(III) differ in their mode of toxicity and transport in plants. Since As(V) is phosphate analogous, it is taken up by the high-affinity phosphate transporters (PHTs) (Figure 2.4). For instance, OsPHT1;1 (Kamiya et al., 2013), OsPHT1;8 (Wu et al., 2011), OsPT2 and OsPT8 (Wang et al., 2016), and OsPT8, OsPT4, OsPHT1;2 (Begum et al., 2016) in rice and AtPHT1;1, AtPHT1;4, AtPHT1;5, AtPHT1;7, AtPHT1;8, AtPHT1;9 in Arabidopsis (Awasthi et al., 2017).

For uptake of As(III), aquaglyceroporins the more likely nodulin 26-like intrinsic protein (NIP) class of aquaporin channels are reported (Zhao et al., 2010a; Mosa et al., 2012) (Figure 2.4). A well-known transporter for silicic acid (Si) named Lsi1 (OsNIP2;1) was found to be a major transporter of As(III) in rice (Ma et al., 2008). Other transporters, such as OsNIP1;1, OsNIP2;2 (OsLsi6), OsNIP3;1 (Ma et al., 2008), OsNIP3;2 (Bienert et al., 2008), and OsNIP3;3 (Katsuhara et al., 2014) in rice and AtNIP1;1, AtNIP1;2, AtNIP5;1, AtNIP3;1, AtNIP6;1, and AtNIP7;1 in Arabidopsis (Awasthi et al., 2017) are also reported for As(III)

uptake. A recent study found an important role of OsNIP3;2 in As(III) uptake in rice by the lateral roots (Chen et al., 2017). Aquaporins of PIP (plasma membrane intrinsic proteins) such as OsPIP2;4, OsPIP2;6, and OsPIP2;7 were also reported to play a role in As(III) uptake and transport (Mosa et al., 2012). In addition, natural resistance-associated with the macrophage protein 1 (NRAMP1) was suggested in As(III) uptake and transport (Tiwari et al., 2014). Ma et al. reported the involvement of OsLsi2 in As(III) efflux and xylem loading (Ma et al., 2008). However, the expression of transporters also depends on the plant genotype. A recent study found differential expression of two aquaporin-coding genes (Os06g12310 and Os02g51110) in six contrasting genotypes of rice which can regulate As(III) mobility differentially (Rai et al., 2015).

Due to the lower affinity of transporters for organic As, the uptake rate of MMA and DMA is much slower than the inorganic forms (Raab et al., 2007b). A NIP class transporter Lsi1 has been reported to uptake MMA and DMA in rice (Figure 2.4). Whereas, Lsi2 was not found to be permeable to DMA (Li et al., 2009a). Another study reported that the MMA uptake is partly mediated by the silicic acid transporter Lsi1, while specific transport of DMA is still unclear (Carey et al., 2011). Although, it was previously reported that methylation of As species can occur in plants (Wu et al., 2002), recent studies have cast doubt on this. In this context, rice plants appeared to lack the ability to methylate As, but instead take it up from soil (Jia et al., 2013). The As uptake magnitude of rice differs in the direction of As(III) >As(V) >DMA>MMA (Raab et al., 2007b).

2.1.7.2 Translocation of arsenic from root to shoot

Both the inorganic and organic forms of As are translocated from roots to shoots via the xylem. Once in the shoot, both the As species are taken up through aquaporins in leaf cells (Figure 2.4) (Zhao et al., 2010a). Although, organic As species, which are taken up by roots, are much smaller than inorganic species, organic As can translocate more readily in shoots than inorganic As (Raab et al., 2007b). Zhao et al. reported that, only 10 % of the taken up As(III) can be transported to shoots and 3.3 % to the rice grain (Zhao et al., 2012). Whereas, the mobility of DMA was higher than As(III) (Carey et al., 2010; Carey et al., 2011). Phytochelatin (PC) mediated complexation with As(III) and subsequent transportation to the cell vacuole is attributed to this lower As(III) translocation. While, in contrast to As(III), poor SH (sulfhydryl) coordination may contribute to the comparatively good translocation of DMA in shoots (Raab et al., 2007b). Different PHT proteins also mediate the As(V) transportation from roots to

shoots (Figure 2.4) (Wu et al., 2011). PHR2 (phosphate starvation response 2) and OsPHF1 (phosphate transporter traffic facilitator 1) are known to regulate phosphate transport and were also found to control As(V) uptake and transport (Wu et al., 2011).

2.1.7.3 Translocation of arsenic from shoot to grain

Nutrients are transported to the grain through phloem transportation, specifically via chalaza to nucellar projection to endosperm. The same route is also used by As (Awasthi et al., 2017). It was evident from a study that, phloem transport is responsible for 90 % of As(III) and 55 % of DMA transport to the rice grain. This indicates that inorganic As is mainly transported via the phloem, whereas both phloem and xylem are accountable for organic As transportation. However, DMA transport to grain is more efficient than As(III) (Carey et al., 2010). A greater portion of flag leaf DMA and MMA is translocated to rice grains; however, it was also reported that translocation of As(V) was poor and it was rapidly reduced to As(III) within flag leaves (Suriyagoda et al., 2018b). The distribution of As in the rice grain is different, based on the As species. Inorganic As is mainly localized in the ovular vascular trace (OVT) region, while organic species have been found in the endosperm. For example, in brown rice, inorganic As species were found in bran whereas, DMA was found in the endosperm (Awasthi et al., 2017). The higher mobility of DMA and lower mobility of MMA and As(III) may explain the difference in location. Thus, polishing of rice would remove some As before consumption. A recent study stated a putative peptide transporter (OsPTR7) in rice for long distance transport, which expressed significantly during the grain filling stage in roots, leaves and nodes (Tang et al., 2017). Inositol transporters (INT2 and INT4) were found to regulate the As translocation from shoot to seeds in Arabidopsis (Duan et al., 2015). In summary, the uptake, mobility, and transport of As in plants largely depend on the concentration and As species present in the soil, uptake rate of As species by roots, plant genotypes, phytochelatins (PCs) complex formation, vacuoles sequestration, xylem flow rate, and phloem unloading.

2.1.8 Arsenic metabolism in plant

Once As enters the plants, mostly it exists in its reduced form, i.e. As(III). As(V) can be reduced to As(III), where the arsenate reductase (AR) enzyme plays a vital role. In Arabidopsis, ATQ1 (arsenate tolerance QTL1) and HAC1 (High As Content1), two AR genes, have been identified (Bermejo et al., 2014; Chao et al., 2014). In rice, OsHAC1;1, OsHAC1;2, and OsHAC4 have been identified as AR genes and are expressed mainly in roots, where they

act as As(V) reductase. However, the expression of OsHAC1;1 was also identified in stems and nodes (Shi et al., 2016; Xu et al., 2017).

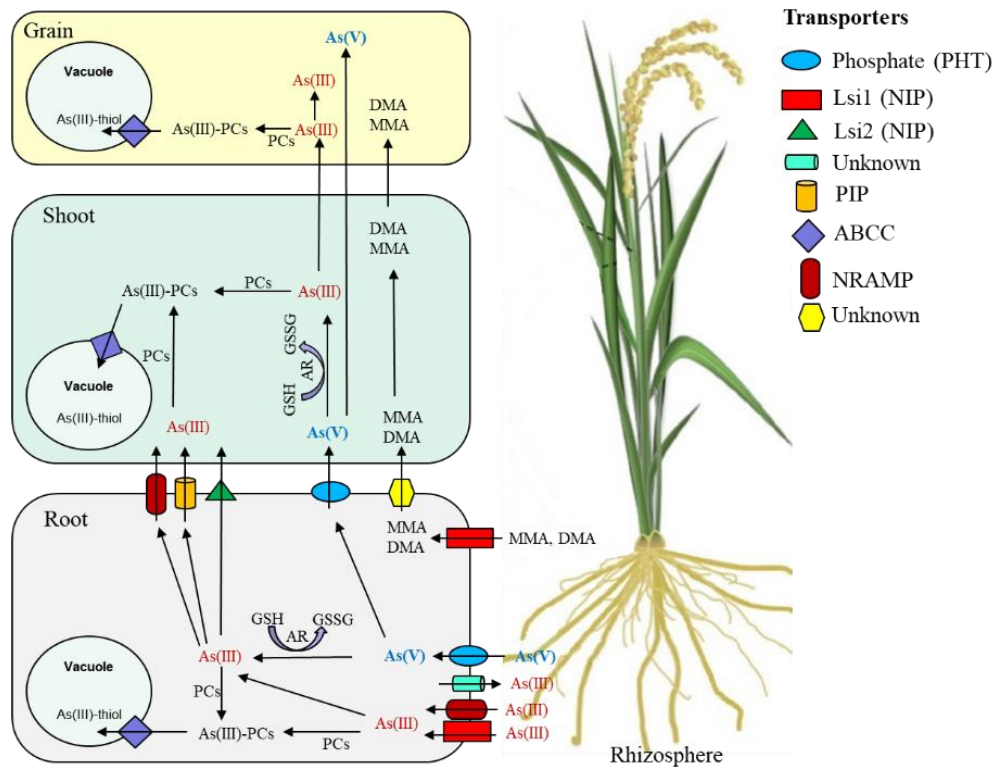


Figure 2.4. Schematic illustration of arsenic uptake and translocation in rice plant [Adopted and modified from (Zhao et al., 2009; Kumar et al., 2015; Abbas et al., 2018)]

2.1.8.1 Arsenic detoxification and tolerance mechanism in plant

The As detoxification and tolerance mechanisms in plants include a range of strategies, such as lowering assimilation, and limiting As translocation to the shoot and seed. Phytochelatins (PC) dependent detoxification is one of the most important mechanisms to combat As toxicity. Complexation of As(III) with phytochelatin forms a PC-As(III) complex, which is transported to cell vacuoles (Raab et al., 2007b). The PC-As(III) transportation is facilitated by the members of ABC (ATP Binding Cassette) transporter family (Figure 2.4). In this context, ABCC1 and ABCC2 were found as transporters of PC-As(III) in rice (Song et al., 2014) and Arabidopsis (Song et al., 2010). In *Pteris vittata* As(III) may be transported via PvACR3 (As Compounds Resistance) to vacuoles (Indriolo et al., 2010). Higher PC-As(III) complex and phytochelatins-synthase activity was reported in rice as a means of As tolerance (Tripathi et al., 2012), which reduce the translocation of As from leaves to grain (Duan et al., 2011). The sequestration of PC-As(III) complexes into vacuoles mostly happens in root cells and to a smaller extent in stems and leaf cells.

However, localization and expression regulation of the transporters is also important in As tolerance. In Arabidopsis, transcription factor WRKY6 and WRKY45 were found to regulate the AtPHT1;1 phosphate transporter to modulate As(V) uptake (Castrillo et al., 2013; Wang et al., 2014). Cytokinin signaling plays a regulatory role in As stress tolerance mechanisms including increased synthesis of PCs and GSH (Mohan et al., 2016). CPK31 (calcium-dependent protein kinase) was found to be a regulator of NIP1;1 for tolerance to As(III) (Ji et al., 2017). Such specific regulators are not yet identified in rice. Overall, As stress can down-regulate the phosphate transporters, and enhance PC-mediated As sequestration to vacuoles to limit the As translocation to shoots and grain. In Arabidopsis, HAC1 (High As Content1), an AR gene, has been identified, which facilitates the efflux of As(III) from roots to soil as a detoxification mechanism (Bermejo et al., 2014; Chao et al., 2014). Glutaredoxin (Grxs) proteins are known to control the redox state and oxidative stress of cells (Lillig et al., 2008). OsGrx_C7 and OsGrx_C2.1 two Grx gene in rice (OsGrx) were found to regulate As accumulation in seeds and shoot tissues, as a tolerance mechanism (Verma et al., 2016).

2.1.9 Additional mechanisms of arsenic tolerance in rice plant

2.1.9.1 Role of radial oxygen loss and iron plaque

Some special characteristics of rice plants, such as the presence of aerenchyma cells in roots, radial oxygen loss (ROL), and Fe plaque formation may all contribute to As resistance by reducing its uptake. Because of the semi-aquatic nature and flooded water cultivation of rice, rice roots have more aerenchyma, which allow O₂ diffusion from shoots to the roots, followed by the release of a part of the O₂ into rhizosphere (Suriyagoda et al., 2018b). This process is called radial oxygen loss (ROL), which differs from genotype to genotype and depends on the O₂ availability and waterlogging condition of the soil (Colmer et al., 2006) (Figure 2.5). Owing to the release of O₂, Fe(II) oxidizes to Fe(III) and results in the precipitation of Fe (oxides/hydroxide) onto the root surface, which is called Fe plaque (Figure 2.5). This Fe plaque can act as a major sink of As because of the strong binding affinity of Fe hydroxide to As (Liu et al., 2006). Thus, genotypes with higher ROL can induce more Fe plaque formation, resulting in more As sequestration and subsequently reduced As uptake and accumulation in rice grains (Suriyagoda et al., 2018b).

Chen et al. reported that PO₄³⁻ can inhibit As(V) uptake in the absence of Fe plaque, while the effect is reduced in the presence of Fe plaque. The probable explanation for this could be the competition between PO₄³⁻ and As(V) for the sorption site of the Fe plaque (Chen et al.,

2005b). On the other hand, no significant influence of PO_4^{3-} and Fe plaque was observed on As(III), which agrees with the findings of Abedin et al. (Abedin et al., 2002c). Different X-ray absorption near-edge structure (XANES) study showed that mainly As(V) can adsorb onto the Fe plaque (Frommer et al., 2011). As was also found in iron plaque present in the apoplast of root epidermal cells (Moore et al., 2011).

However, the role of Fe plaque in terms of plant uptake is debatable. Fe plaque was found to act as both a barrier (Liu et al., 2004) to entry and a source (Liu et al., 2006) of As(V). As concentration may also depend on the level of iron plaque on the root surface, which varies from root tips to old roots and from primary to lateral roots (Frommer et al., 2011). Some studies found a negative correlation between As in rice grain and ROL, which indicates that the cultivar with more ROL allowed more Fe plaque formation to reduce As uptake. More ROL may also enhance the oxidizing process of As(III) to As(V) and result in more adsorption onto Fe plaque (Mei et al., 2009). Meng et al. reported the strong binding affinity of Fe hydroxide to As(V) and subsequent translocation to shoots (Meng et al., 2002). Some microbes in the Fe plaque were found to be involved in As transformation. For example, As(III) oxidizing bacteria (viz., *Acidovorax* and *Hydrogenophaga*) can transform As and influence As concentration in rice tissues (Hu et al., 2015). Furthermore, it is important to consider that ROL and iron plaque formation is also influenced by the As itself (Wu et al., 2013).

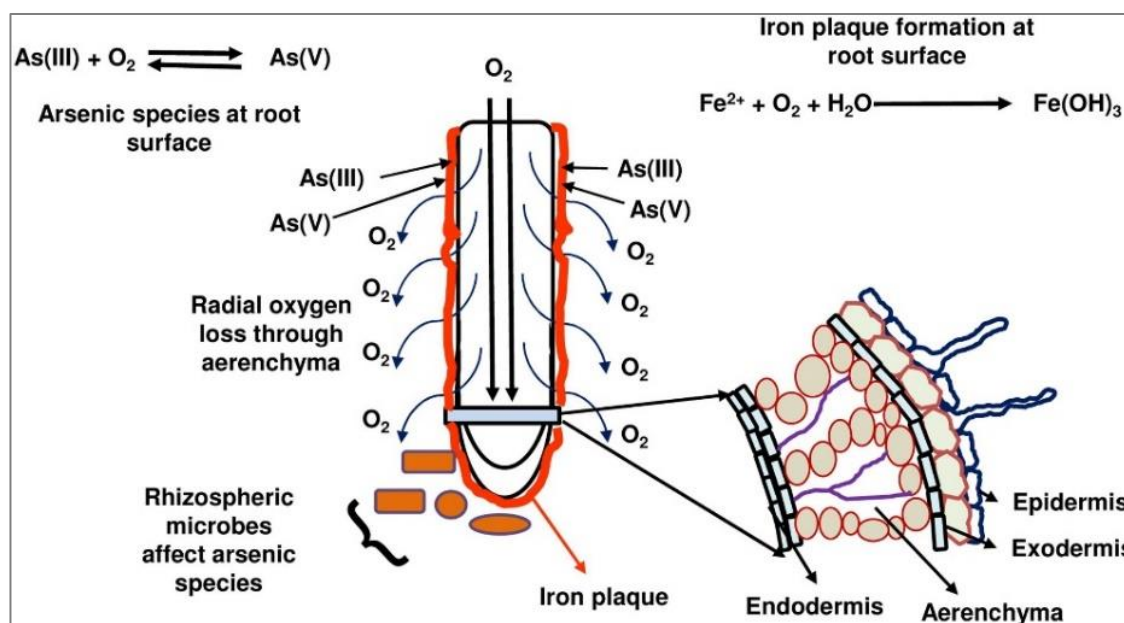


Figure 2.5. The effect of iron plaque formation and radial oxygen loss on the availability of different arsenic species to rice plant [Adopted from (Awasthi et al., 2017)]

2.1.9.2 Role of rice nodes in arsenic translocation regulation

In terms of As mobility in rice plants, nodes act as a controlling point, which regulates As storage and distribution to the rice grain (Yamaji and Ma, 2014). Nodes can store much higher As concentrations than leaves and internodes (Moore et al., 2014). In support of this findings, another study reported that, rice nodes act as a filter of As(III) and limits its translocation into the grain (Chen et al., 2015). The ABCC transporter (OsABCC1) present in the tonoplast of nodes phloem cells mediates the transportation of the PC-As(III) complex to vacuoles (Song et al., 2014). The presence of OsABCC1 in companion cells of the vascular bundle may enhance the inhibition of the translocation of As(III) into grains (Moore et al., 2014).

2.1.9.3 Role of Sulphur

Sulfur (S) plays an important role in As tolerance through complexation of As by thiol ligands, glutathione, and phytochelatins (Dixit et al., 2015) and subsequent transportation to vacuoles as an As detoxification mechanism (Song et al., 2010). Several studies opined that the application of S into the growth medium enhanced the rate of As(III)-thiol complexation in the roots and restricted As mobility to rice shoots and grain (Hu et al., 2007). High S supply was found to down-regulate the phosphate transporter (OsPT23) and the aquaporin gene (OsTIP4;2), and upregulate the phytochelatin synthase genes (OsPCS1, OsPCS3 and OsPCS13) and ABC transporter genes (OsABCG5, OsABCI7_2 and OsABC6) (Zhang et al., 2016). Thus, plants synthesize more PCs and sequester more As into vacuoles and therefore reduce the uptake of As(V) and As(III).

2.1.9.4 Role of silicon

The application of Si to the soil has been found not only to reduce As(III) uptake by the rice plant (Bogdan and Schenk, 2008), but also evident that Si interacts with As(III) once taken up by the plant. Sanglard et al. reported the alleviation of negative impacts of As(III) on photosynthesis and carbohydrate formation in rice, when treated with Si in a hydroponic culture (Sanglard et al., 2016). However, there are more chances of As(III) uptake by plants, which are genetically more efficient at Si assimilation because of the imperfect selection of Si transporter present in the root. This increases As loading into rice grains (Suriyagoda et al., 2018b). Thus, genus, species, and varietal variation needs to be considered in Si mediated As remediation.

2.1.9.5 Biochemical adaptation

Glutathione (GSH) plays a vital role in maintaining the redox condition of plant cells (Dubey et al., 2016). Arsenate reductase (AR) uses the GSH system as a reductant to reduce As(V) to As(III). This process leads to As toxicity and excess production of ROS, which creates oxidative stress in the plant. To protect against oxidative stress, plants increase the production of numerous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), and some other peroxidases, which act as a scavenger of excess ROS (Suriyagoda et al., 2018b).

In response to As exposure, several metabolites are also synthesized in plants. Increased abundance of some specific amino acids, such as proline, histidine, cysteine, glycine, glutamic acid and methionine, and peptides, such as glutathione and phytochelatins, reduce As stress (Dave et al., 2013). Previous studies revealed the differential expression of several genes in response to As stress in rice. For instance, the differential expression of transcripts encoding glutathione-S-transferases, glutathione synthase, superoxide dismutase, sulfur metabolism, heat-shock proteins, and metal transporters, were observed (Chakrabarty et al., 2009; Rai et al., 2011). In addition, the expression of one glutaredoxin gene (Os01g26912), specifically in the shoot of As(III) treated plants, was reported. This indicate the presence of As(III) and As(V) specific gene responses in rice (Chakrabarty et al., 2009).

2.1.10 Toxicity of arsenic in plant

Phytotoxicity of As can be explained mainly by means of two processes. One is disfunction of some key enzymes, either by displacing the obligatory ions from their active site or through interaction with sulfhydryl groups. Another is indirectly through the eruption of ROS, which results in a cataract effect of irreversible injuries in plants (Finnegan and Chen, 2012). The toxicity of As(V) in the plant mainly occurs due to an antagonistic relationship with PO_4^{3-} , which is a consequence of a reaction with PO_4^{3-} or a PO_4^{3-} -ester as a substrate, thereby disrupting vital biochemical processes including phospholipid metabolism, protein phosphorylation/dephosphorylation, RNA/DNA metabolism, oxidative phosphorylation, and glycolysis (Gresser, 1981; Finnegan and Chen, 2012). For example, As(V) can replace the PO_4^{3-} in the process of ATP synthesis by forming ADP-arsenate, ultimately inhibiting the oxidative phosphorylation and increasing As(V) toxicity (Rahman et al., 2014b; Suriyagoda et al., 2018b).

As(III) has a higher affinity to bind with sulfhydryl groups (–SH) of numerous enzymes and proteins, which leads to the disruption of the protein/enzyme structure and function (Finnegan and Chen, 2012), deterioration of membranes, including functioning of aquaporins (Wan et al., 2004), reduction in water uptake (Hachez and Chaumont, 2010), inhibition of cellular functions, and subsequently cell death (Suriyagoda et al., 2018b). Recent research demonstrated that As(III) can bind with the reduced cysteine residues in proteins, which affects catalytic functions (Xu et al., 2017). Translocation of DMA into grain's parts, such as embryo, endosperm, and aleurone, can significantly reduce the seed setting rate, which results the grain sterility and ultimately yield loss (Wang et al., 2015). There is substantial evidence that generating excess ROS in the plant, due to As toxicity (Mishra et al., 2008; Kumar et al., 2015), results in progressive oxidative damage by causing protein oxidation, nucleic acid damage, lipid peroxidation, enzyme inhibition, damage of cell membranes, programmed cell death (PCD) pathway activation, and ultimately cell death (Sharma et al., 2012). Although ROS is well known for its destructive activity, it also acts as a signal for several cellular processes, including tolerance to environmental stresses (Yan et al., 2007), while excess ROS, induced by the As toxicity, will create oxidative stress in the plant.

Furthermore, plants, which are exposed to As toxicity, can synthesize PCs, which are responsible for glutathiones depilation, since glutathiones are the precursors of PC (Whitaker et al., 2001). Therefore, plants experience more stress conditions through As toxicity, which ultimately reduces plant growth. In addition, As(V) was found to up-regulate the genes involve in encoding anti-oxidative enzymes (Suriyagoda et al., 2018b). Therefore, As can interfere with several metabolic processes, including photosynthesis and respiration, which can lead to plant death at higher As concentration. Ahmed et al. reported that As can significantly affect genomic template stability (measured as changes in RAPD profile), which indicate the possible alteration in numerous physiological and biochemical processes of the rice plant, due to As exposure (Ahmad et al., 2012b).

The presence of As in leaf tissues can affect photosynthetic processes. Previous studies revealed a significant decrease of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) under As stress condition. Furthermore, reduction in chlorophyll concentration, yields of photosystem II, electron transport rate, and chloroplast 29 kDa ribonucleoproteins, were reported. Along with the above-mentioned effects, reduced water uptake and conductance at stomatal and mesophyll levels, may have jointly countersigned to a diminution in photosynthetic rate under As stress condition (Suriyagoda et al., 2018b). Despite the decreases

of photosynthesis, increases in the concentration of reducing sugar in rice seedlings (Choudhury et al., 2010), and total and reducing sugar in grain (Bhattacharya et al., 2013), were also observed. In agreement to this contention, increases of sucrose degrading enzymes (acid invertase and sucrose synthase) activity and decreases in sucrose synthesizing enzyme (sucrose phosphate synthase) activity, leading to the accumulation of soluble sugars, were reported (Choudhury et al., 2010). This may also cause a remarkable reduction in plant growth and interrupt numerous other biochemical processes.

2.1.11 Rice grain arsenic concentration

Rice grains contains both organic and inorganic forms of As. In general, As is present in rice grains in the order of inorganic As > organic As, As(III) > As(V), and DMA > MMA. Thus As(III) and DMA are the major As species in rice grain, with some As(V) and MMA (Suriyagoda et al., 2018b). Huang et al. reported that in the case of As contaminated grain, around 90 % of inorganic As in Asian rice grain was As(III), regardless of rice cultivar, origin, cultural practice and polishing treatment (Huang et al., 2012). In contrast, in the UK and the USA rice grain, a higher percentage of organic As species than the inorganic species was reported (Suriyagoda et al., 2018b). In support of this, Smith et al. found 85 – 94 % DMA, and Syu et al. found 40 – 60 % organic As in rice grain (Smith et al., 2008; Syu et al., 2015). Suriyagoda et al. also reported a relatively high inorganic As level in Asian rice (e.g. India), compare with UK and USA (Table 2.2).

2.1.12 Why rice can uptake more arsenic than other crops?

Rice is considered to be a good accumulator of As. It has been demonstrated that, rice can accumulate 10 time more As than other cereal crops (e.g. Wheat and barley) (Williams et al., 2007; Su et al., 2010). Rice is mainly grown as a low land crop in flooded soil under reducing condition. In flooded conditions, the mobility and availability of As(III) is higher than the As(V) because of the reductive dissolution of Fe oxide or hydroxides (Takahashi et al., 2004). This condition enhances the As bioavailability and results in higher plant uptake and ultimately excessive As accumulation in rice grain. In addition, rice is one of the most efficient accumulators of Si and rice plants uptake As(III) through Si transporters, which facilitate greater As(III) accumulation (Ma et al., 2008; Norton et al., 2010). Specific localization of influx and efflux of As transporter, in exodermis and endodermis cells, also facilitate efficient As uptake and xylem transportation in rice plants (Su et al., 2010). These factors contribute the higher As accumulation in rice grains, which is sometimes greater than the safe human

exposure limit. Since rice is the main diet of many people around the world, it appears to be a major source of dietary exposure of As for humans (Zhao et al., 2010a).

Table 2.2. Rice grain arsenic concentration and inorganic fraction from different countries. [Adopted from (Suriyagoda et al., 2018b)]

Country	Mean ($\mu\text{g kg}^{-1}$) (sample size)	Min–Max ($\mu\text{g kg}^{-1}$)	Inorganic As fraction (sample size)
Bangladesh	200 (316)	80 – 700	0.64 (92)
Cambodia	201 (11)	–	0.83 (5)
Canada	65 (2)	20 – 110	0.61 (4)
China	133 (2033)	54 – 1160	0.61 (197)
France	283 (39)	183 – 535	0.86 (4)
India	146 (374)	41 – 995	0.71 (165)
Italy	188 (161)	81 – 283	0.52 (19)
Japan	264 (79)	117 – 445	0.76 (19)
Pakistan	122 (15)	85 – 147	–
Philippines	70 (1)	–	–
Sri Lanka	122 (154)	12 – 540	–
Thailand	146 (81)	100 – 241	0.77 (13)
UK	238 (22)	120 – 470	0.53 (27)
USA	153 (375)	66 – 816	0.46 (36)

2.1.13 Wheat grain arsenic concentration

High concentrations of As are not only reported in rice but also in wheat (Chandiramani et al., 2007). Wheat field experiments, with a 12 mg kg^{-1} soil As, reported $2.00 - 17.00 \mu\text{g kg}^{-1}$ of As in grain samples (Zhao et al., 2007). While, $5.00 - 285.00 \mu\text{g kg}^{-1}$ (Wiersma et al., 1986), $4.00 - 362.00 \mu\text{g kg}^{-1}$ (Roychowdhury et al., 2002), and $1.00 - 500.00 \mu\text{g kg}^{-1}$ of As were also reported in wheat grain, collected from an As contaminated site, where soil As concentration ranged from $3.00 - 201.00 \text{ mg kg}^{-1}$ (Williams et al., 2007). Although there is no worldwide standard safe limit of As in food grains, the European Commission recently (2016) set the maximum limits for As in milled rice (polished or white rice) as $200 \mu\text{g kg}^{-1}$ (Union, 2015). The same concentration is recommended as the maximum allowable limit by the WHO, according to a recent report (WHO, 2018). Thus, a considerable amount of As in wheat grain could be another potential dietary As exposure beside rice grain.

2.1.14 Strategies for safe grains

Different strategies are being used for reducing As accumulation in food grains. In this context, different physical, chemical, and biological techniques have been reported for soil As remediation (Table 2.3). Hitherto, these methods have been commonly determined as ineffective, costly, or too lengthy, with usage restricted to smaller-scale operations with lower efficiency (Lim et al., 2014). Thus, contrasting responses of these strategies demand designing an integrated approach to safeguard food grains and humans from the As hazard.

Recent research has revealed that pre-sowing microwave (MW) soil heating has potential to increase crop growth and grain yield in addition to non-chemical weed control (Khan et al., 2016; Brodie et al., 2017; Khan and Brodie, 2018). Removal of organic contaminants (Falciglia and Vagliasindi, 2014) and immobilization of some toxic metals (Cu, Mn, Th, Zn, Ni, Cd, Cr, and Pb) in soil (Abramovitch et al., 2003) and solid sediments (Chen et al., 2005a; Hsieh et al., 2007) using MW heating, has also been reported. However, so far, to best of our knowledge, no published study has investigated soil As remediation using MW heating, which could be a novel method to lessen the As phytotoxicity and grain As accumulation.

Furthermore, to immobilize soil organic and inorganic pollutants and heavy metals, including As, biochar has been progressively gaining more attention because of its high carbon content, micropores, and large surface area (Rajapaksha et al., 2016). However, increased mobility and bioavailability of As is also reported after the application of some biochar (Beesley et al., 2014). The mobility and bioavailability of As after biochar application depends on the biochar types and its characteristics. Different physical and chemical characteristics of biochar mainly depends on feedstock types and pyrolysis conditions i.e., residence time, temperature, heating rate, and reactor type. For example, different biomass properties and composition such as particle size, moisture content, and contents of cellulose, lignin and ash influences the properties of biochar. Also, biochar produced at high temperatures (600–700 °C) shows highly aromatic nature with well-organized C layers but has fewer H and O functional groups due to dehydration and deoxygenation of the biomass, possibly with lesser ion exchange capacities (Uchimiya et al., 2011b; Ahmad et al., 2014; Rajapaksha et al., 2016). In contrast, biochar produced at lower temperatures (300–400 °C) has more diversified organic characters, including aliphatic and cellulose type structures and contain more C=O and C–H functional

groups (Rajapaksha et al., 2016). Because of these complex and heterogenous properties, all biochar is not equally efficient in immobilising soil As.

Conventional carbonization (i.e. slow pyrolysis), fast pyrolysis, flash carbonization, and gasification are the main thermochemical processes that are usually employed for biochar production (Manyà, 2012). In this context, MW assist pyrolysis could be a better choice because of higher char yield, quick rising of temperature, faster heating rate, volumetric and uniform heating thereby accelerating reaction rates and increasing energy and cost efficiency, and less or no pre-processing of the feedstock in MW assist pyrolysis compared with conventional pyrolysis (Li et al., 2016a). Moreover, MW pyrolysis delivers affluence of operation by instantaneous on/off control and improves the yield and quality of the char. Also, it minimizes release contaminants and decreases harmful product formation, making the technology environmentally friendly (Rajapaksha et al., 2016). The main difference between MW and conventional heating can be attributed to unlike heating mechanisms (dielectric heating vs. convective and conductive heating) and to the occurrence of the micro-plasma hot spot caused by MW heating (Menéndez et al., 2010). This different heating mechanism of MW results production of biochar with different compositions and high-quality product compared with conventional pyrolysis. For example, significant cracks and fissures produced in MW pyrolysis which lead to a more fragile biochar with higher surface area and pore volume than that from conventional pyrolysis. Moreover, based on the previous research, the micropores of MW pyrolyzed biochar are uniform and quite clean, and its surface area is highly improved (Yagmur et al., 2008). However, no published study has so far been reported on a combination of MW soil heating and biochar treatment for soil As remediation, which could be a possible technique to lessen As phytotoxicity and grain As accumulation. The detailed about the MW and biochar are described below.

Table 2.3. Physical, chemical, and biological strategies of soil arsenic remediation

Strategies	Method	Disadvantages	References
Soil washing	Soil washing by different acids, bases, chelant, surfactants and organic reagents.	Destructive impact on soil properties, diffusion of leachate, transfer of As from soil to water, high cost, chance of other chemical contamination and <i>in situ</i> application difficulties with lower efficiency.	(Wan et al., 2020)
Immobilization by Fe-based nanoparticles, Fe oxides, and solid waste	Oxidation of As(III) to As(V), decreasing the mobility of As(V) by forming complexes and precipitation	Needs selective stabilizer for nanoparticles to prevent aggregation, other heavy metals deposition in soil from solid waste, immobilization efficiency monitoring, returning of immobilized As to an active state, expensive when applied at large scale, and soil type-based application	(Wan et al., 2020)
Soil replacement, soil cover, and turnover	Replacement, cover, and turnover the contaminated soil with clean soil	High cost and energy, required large amount of clean soil, limited to small scale application, secondary pollution, and may affect crop growth.	(Wan et al., 2020)
Electrokinetic remediation	Creation of electric field gradient.	Low efficiency, soil pH control required, only removes mobile fractions of As, not suitable for high permeable soil, and costly for large scale operation.	(Wan et al., 2020)
Phytoremediation	Phytoextraction of soil As with hyperaccumulator (e.g. <i>Pteris vittata</i>)	Long remediation period with long nursery cycle of hyperaccumulator plant, space occupation during remediation time, reduction of removal efficiency with time, extensive labor requirement with monitoring cost, challenges of controlling pollution during harvest, and safe disposal of As rich biomass.	(Wan et al., 2020)

Mycorrhizal	Inoculation with mycorrhizae as a biological agent.	Vulnerable to temperature, oxygen, and pH, limited in application, may incur secondary pollution.	(Yao et al., 2012; Poonam et al., 2017)
Microbial	Biomethylation, oxidation immobilization by converting As(III) to As(V), increasing the Fe oxidizing, and Sulphate reducing microbes, volatilization of As from soil.	Chance to mobilize As by reducing As(V) to As(III) or dissolve minerals, release of As into atmosphere may be toxic to relevant working people, low efficiency in field condition despite the high efficiency of laboratory, need specific environmental condition, suitable for low level contamination, and less stable.	(Wan et al., 2020)
Water management	Alternate wetting and drying or intermittent flooding, aerobic cultivation etc.	Possible reduction in yield, increase risk of other metal toxicity, and may alter soil microbial activity.	(Suriyagoda et al., 2018a)
Fertilizer amendments	Application of P, S, Fe, and Si	Si can increase soil solution As(III) due to desorption of As(III) from soil surface while competing for same adsorption site and increase As(III) uptake by the plant, at low pH Fe can create toxicity, and S can induce soil acidity.	(Suriyagoda et al., 2018a)
Genetic engineering: Altering the expression of transporters	Transporters of As(V) and As(III) uptake, vacuolar sequestration, chelators synthesis.	Homeostasis of other essential elements, enhanced Zn complexation with phytochelatin, increase synthesis of GSH and PCs exert stress to S metabolism, and may yield contrasting result while transfer to field from the laboratory due to environmental influences.	(Tripathi et al., 2007; Song et al., 2014; Rodríguez et al., 2019)

2.2 Microwave

2.2.1 Application of microwave

Besides the telecommunications and sensing applications of MW, it is widely used in industrial processing as a remarkably effective and multipurpose energy source. For example, food industry is an exclusively big sector of MW energy application using for different food processing (Chandrasekaran et al., 2013). In chemical industries, in polymerization, organic synthesis, ceramics processing, plasma chemistry (Falciglia et al., 2016), and in solvent-free chemistry (Arrieta et al., 2007) MW energy has been using. MW heating also has been using in other industries such as in textiles and leather processing, oil extraction from tar sands, cross-linking of polymers, metal casting (Ayappa et al., 1991), paper and cardboard drying (Hasna, 2009), drying of wood (Antti and Perre, 1999) and modifying the structure of wood (Torgovnikov and Vinden, 2004) where MW treatment letting it dry much faster and be infused with resins to make it stouter (Brodie, 2007), and glass industry (Jerby et al., 2002). Another big sector of MW energy is medical application (Bond et al., 2003). Furthermore, MW energy also used in laboratory and field scale for pest control (Nelson, 1996), enhancing seed germination (Nelson and Stetson, 1985), treatment of various animal fodders to increase their digestibility (Sadeghi and Shawrang, 2006; Brodie et al., 2010), reducing weed emergence in agricultural farming system by killing the soil weed seed bank (Khan et al., 2016; Brodie et al., 2017; Khan et al., 2019a).

2.2.2 Removal of organic and inorganic contaminants by microwave

Microwave treatment is also used for removal of organic contaminants through heating. Different studies have investigated the application of MW energy for remediation of contaminants from soil. Several researchers have proposed removal of hazardous waste (Dauerman et al., 1992), PCBs (Liu et al., 2014a), Crude oil (Huang et al., 2011a), hexachlorobenzene (Yuan et al., 2006), TCE (Kawala and Atamańczuk, 1998), diesel-contamination (Falciglia and Vagliasindi, 2014), and PAHs and PCPs (Di and Chang, 2001; Lin et al., 2010), from contaminated soil with an efficiency of 80 – 100 %. Organic contaminant removal through heating mainly occurs by thermal desorption. Extraction and volatilization of some organic contaminants (e.g. PCBs) after the application of MW heating are also mentioned as the mechanisms of contaminant remediation (Abramovitch et al., 2003). Furthermore, like

organic pollutants, remediation of some metals (e.g. Cd, Mn, Th, Cr, and Cr) of contaminated soils through immobilization with MW irradiation also reported (Abramovitch et al., 2003).

2.2.3 Basic concept and principle of microwave

The use of radio frequency for heating applications dates to the early 19th century, but there have been major advances in the use of MW for heating since the 1950s. From that time, it was realized that MW had the potential to provide energy-efficient and rapid heating of materials. MW energy is a form of electromagnetic radiation, with wavelengths ranging from 1 m to 1 mm and frequencies between 300 MHz to 300 GHz (Figure 2.6) (Banik et al., 2003). Like other electromagnetic waves, MW travels at the speed of light ($300,000 \text{ km sec}^{-1}$), propagates through space, and carries electromagnetic radiant energy. MW photon energy is relatively low ($0.03 - 0.00003 \text{ kcal mol}^{-1}$), affecting only kinetic molecular excitation. MW is self-propagating transverse oscillating wave of electric and magnetic fields (Figure 2.7). A transverse wave is a moving wave that consists of oscillations occurring perpendicular to the direction of energy transfer (CEM, 2020).

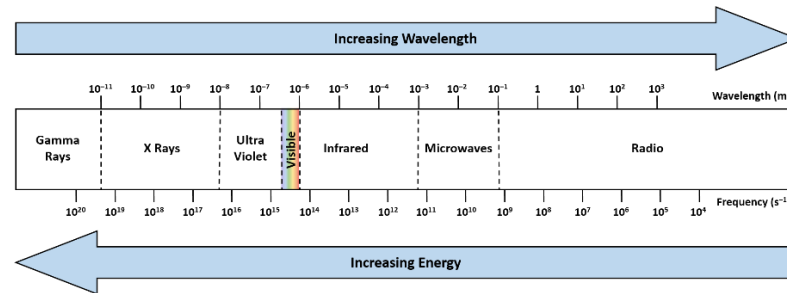


Figure 2.6. Spectrum of electromagnetic radiation [Adopted from (CEM, 2020)]

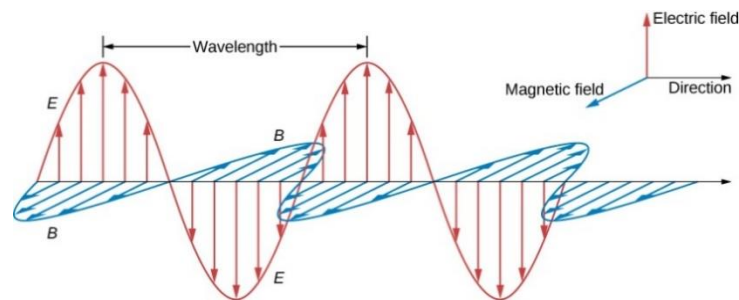


Figure 2.7. Transvers oscillating waves of electric and magnetic fields [Adopted from (Tang, 2015)]

The MW wavelength is related to the frequency by following Equation (2.1) and (2.2):

$$\lambda_0 = \frac{c_0}{\nu} \quad (2.1)$$

$$c_0 = (\epsilon_0 \mu_0)^{-\frac{1}{2}} \quad (2.2)$$

Where, λ_0 = free space wavelength, c_0 is the speed of light at free space, ν is the frequency, ϵ_0 = dielectric permittivity of free space = 8.854×10^{-12} F/m, and μ_0 = 1.257×10^{-16} H/m. Microwave radiation is also subdivided, according to its different wavelengths, into the bands of decimeter waves (0.3 – 3 GHz), centimeter waves (3 – 30 GHz), and millimeter waves (30 – 300 GHz) (Kaatz, 1995). Microwave ovens, which are generally used for domestic and industrial purposes, function at a frequency of 2.45 GHz consistent with a free space wavelength of 12.2 cm and photon energy of 1.02×10^{-5} eV (Jacob et al., 1995).

2.2.4 Mechanism of microwave heating

Microwave can produce heat by absorption of the energy in the treated materials. Microwave heating depends on the dielectric heating of a material. Dielectric heating depends on dipolar and interfacial polarization effects and ionic conduction (Figure 2.8) (Metaxas and Meredith, 1983). When MW radiation is applied, it causes rotation of the dipoles of polar (e.g. water) molecules because of the oscillating electromagnetic field (dipolar polarization) and produces heat by intermolecular friction and collision (Falciglia et al., 2016). Kinetic energy, associated with this dipolar rotation, is responsible for the generation of thermal energy and is dissipated in the form of heat. Interfacial polarization effects are also known as the Maxwell-Wagner effect (Komarov, 2012). In ionic conduction, a free ion or ionic species moves translationally through space, struggling to align with the shifting electric field. Similar in dipolar rotation, the friction among these moving species consequences in heat generation, and the higher the temperature of the reaction mixture, the more efficient the transfer of energy becomes. In both cases, the more polar and/or ionic a species, the more efficient the rate of heat generation (CEM, 2020).

The dielectric properties, or dielectric permittivity, of materials is important in terms of interaction of matter with electromagnetic energy. Dielectric materials are poor conductors of electricity but good supporters of electric fields, with some having electric dipoles, which also absorb electromagnetic energy. The dielectric permittivity is a measure of how an electric field effects and is affected by a dielectric medium. The permittivity of the material can be expressed as a combination of the capability of the material to store electric energy and dissipation of electric energy in the material by conversion of electric energy into heat energy. The permittivity, in relation to free space, can be express as $\epsilon^* = \epsilon' - j\epsilon''$, where ϵ' is called the real

part of dielectric property, known as dielectric constant, which implies the material's ability to store energy; j symbolizes the complex operator, $\sqrt{-1}$; and ϵ'' is the imaginary part of dielectric property, known as the dielectric loss, which indicates the ability to convert energy into heat (Metaxas and Meredith, 1983; Nelson, 1996; Menéndez et al., 2010). The loss tangent, $\tan \delta$, also frequently used as an index of energy dissipation or loss in a material exposed to MW electric fields, is defined as $\tan \delta = \epsilon'' / \epsilon'$. The ac electrical conductivity, associated with the dielectric loss in the material, is $\sigma = \omega \epsilon_0 \epsilon''$ Siemens/m (S/m), where ω is the angular frequency, $\omega = 2\pi f$; where f is the frequency of the applied electric field; and ϵ_0 is the permittivity of free space, 8.854×10^{-12} farads/m (F/m) (Nelson, 1996).

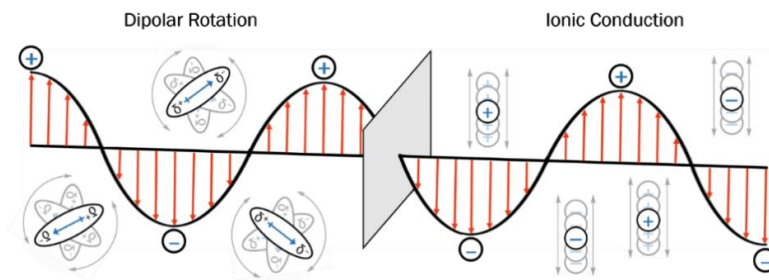


Figure 2.8. Dipolar rotation and ionic conduction mechanism of microwave heating [Adopted from (CEM, 2020)]

The power dissipated per unit volume in a nonmagnetic, uniform material, exposed to MW fields, can be expressed as (Nelson, 1996):

$$P = E^2 \sigma = 55.63 \times 10^{-12} f E^2 \epsilon'' \quad (2.3)$$

Where, P is in watts per cubic meter (W/m^3) when f is the frequency (Hz) and E is the rms electric field intensity in volts/m (V/m). The power density equation ($P = E^2 \sigma = 55.63 \times 10^{-12} f E^2 \epsilon'' \text{ W m}^{-3}$) describes the power per unit volume in the material that is dissipated in the material when irradiated with microwave energy. If we know the thermal capacity and density of the material it is possible to determine the heating rate. The heating rate is given by: $dT/dt = P/\rho C = (55.63 \times 10^{-12} f E^2 \epsilon'')/\rho C$. Where, ρ is the density of the moist soil (kg m^{-3}) and C is the thermal capacity of the moist soil ($\text{J kg}^{-1} \text{ }^\circ\text{C}^{-1}$). The absorption of MW energy, propagating through a material, depends upon the variables of Equation (2.3). Thus, the dielectric loss factor of the material is significant. The frequency of the wave is also a factor, and the power absorption also depends on the square of the electric field intensity. For a plane wave, the electric field intensity E , which has a $e^{j\omega t}$ dependence, can be given as (Von Hippel, 1954):

$$E(z) = E_0 e^{j\omega t - \gamma z} \quad (2.4)$$

Where, E_o = rms electric field intensity at a point of reference, t = time, γ = propagation constant for the medium in which the wave is traveling, z = distance in the direction of travel. The propagation constant is a complex quantity:

$$\gamma = \alpha + j\beta = j \frac{2\pi}{\lambda_0} \sqrt{\epsilon^*} \quad (2.5)$$

Where, α = attenuation constant, β = phase constant, λ_o = free space wavelength. The attenuation constant α and phase constant β are related to the dielectric properties of the medium as follows (Von Hippel, 1954):

$$\alpha = \frac{2\pi}{\lambda_o} \sqrt{\frac{\epsilon'}{2} [\sqrt{1 + \tan^2 \delta} - 1]} \text{ nepers/m} \quad (2.6)$$

$$\beta = \frac{2\pi}{\lambda_o} \sqrt{\frac{\epsilon'}{2} [\sqrt{1 + \tan^2 \delta} + 1]} \text{ radians/m} \quad (2.7)$$

Energy attenuation is another term to express the heating properties of materials. When a wave penetrates through a material with enough dielectric loss, the energy will be attenuated. Then the electric field intensity at the site of interest can be obtained by combining Equation (2.4) and (v) as follows:

$$E(z) = E_0 e^{-\alpha z} e^{j(\omega t - \beta z)} \quad (2.8)$$

Where, the first exponential term controls the magnitude of the electric field intensity at the point of interest. High attenuation (dB/m) exhibits quick taper of dielectric heating with the propagation of wave into the materials. With the power densities and electric field intensity values, attenuation can be expressed as (Von Hippel, 1954):

$$10 \log_{10} \left(\frac{P_o}{P_{(z)}} \right) = 20 \log_{10} \left(\frac{E_o}{E_{(z)}} \right) = 8.686_{\alpha z} \quad (2.9)$$

The dielectric properties of soil are very important in terms of evaluating the penetration of energy where attenuation can be express in decibels when $(\epsilon'')^2 \ll (\epsilon')^2$ as follows:

$$\alpha \approx \left(\frac{8.686\pi\epsilon''}{\lambda_o\sqrt{\epsilon'}} \right) \text{ dB/m} \quad (2.10)$$

Reflection, transmission, absorption, and refraction affect MW propagation when it encounters a material object. If the soil surface is exposed to MW, some of the energy will be reflected and the rest, P_t , will be transmitted into the soil. This can be calculated as follows:

$$P_t = P_0 (1 - |\Gamma|^2) \quad (2.11)$$

Where, P_0 is the incident power and Γ is the reflection coefficient, which can be expressed in terms of complex relative permittivity of the soil as (Stratton, 2007):

$$\Gamma = \frac{1 - \sqrt{\epsilon^*}}{1 + \sqrt{\epsilon^*}} \quad (2.12)$$

To determine the heating pattern of a geometry during the MW treatment, penetration depth and wavelength are important. The penetration depth (d_p) is the distance at which the electromagnetic power density declines to a value of $1/e$ ($e = 2.718$) or 36.8 % from its initial value at the surface and is stated as (Metaxas and Meredith, 1983; Basak et al., 2016).

$$d_p = \frac{c}{\sqrt{2}\pi f \left[\sqrt{(\epsilon')^2 + (\epsilon'')^2} - \epsilon' \right]^{1/2}} \quad (2.13)$$

Where, the dielectric properties are described by: $\epsilon = \epsilon' + j \epsilon''$ and the wavelength (λ_m) can be calculated as (Basak et al., 2016)

$$\lambda_m = \frac{c\sqrt{2}}{f \left[\sqrt{(\epsilon')^2 + (\epsilon'')^2} + \epsilon' \right]^{1/2}} \quad (2.14)$$

Where, c is the velocity of light in space ($3 \times 10^8 \text{ ms}^{-1}$) and f is the frequency (Hz).

2.2.5 Mechanism of conventional heating

Conduction and convection are the main mechanisms of conventional heating where, the surface area of a material is heated first and there is a transfer of heat into the interior of the material by thermal conduction (Figure 2.9) (Menéndez et al., 2010; Li et al., 2016a). In convection, heat is transfer through the movement of the molecules within the medium where warmer molecules above a hot surface expands, becomes less dense, and rises (Figure 2.9). As a consequence, conventional heating usually takes a longer time to achieve a certain temperature in comparison with MW heating since conventional heating needs to overcome the heat transfer barrier (Li et al., 2016a).

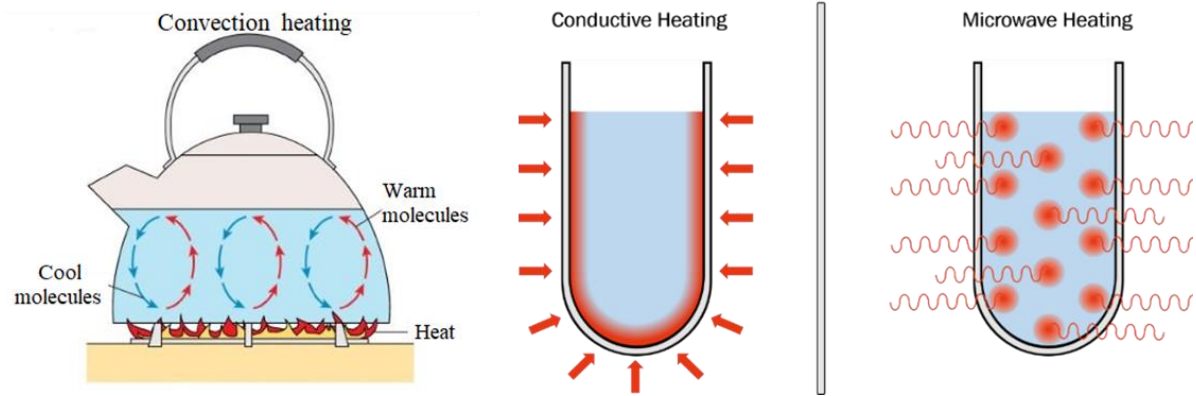


Figure 2.9. Mechanism of microwave heating and conventional heating [Adopted from (File, 2016; CEM, 2020)]

2.2.6 Temperature distribution through soil after microwave heating

The temperature distribution in the soil (Figure 2.10), depends on the dielectric properties of soil, which is controlled by the soil moisture content, textural properties, and mineral content of the soil (Von Hippel, 1954; Velazquez-Marti et al., 2005) and peak temperature is found between 2 and 5 cm depth of the soil (Brodie et al., 2007b). Higher top layer soil moisture content can increase the reflectivity (Von Hippel, 1954), reduce the MW penetration into soil, more freely absorb MW energy which helps to overcome the energy loss (Metaxas and Meredith, 1983) and act as a heat-diffusing agent which quickly transports the heat throughout the soil particles (Brodie, 2007). Clay/loam soils heat faster than the sandy soil due to presence of more bound moisture on the soil particles (Brodie et al., 2007c).

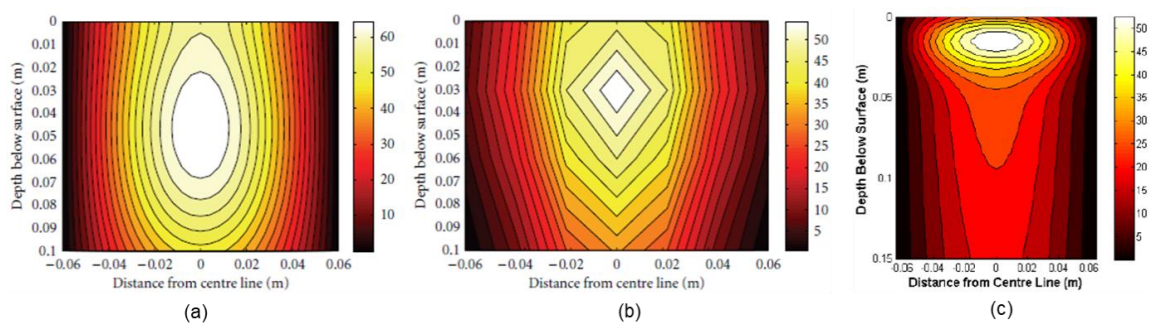


Figure 2.10. Temperature (°C) distribution through soil profile after microwave heating. (a) expected soil temperature distribution and (b) measured soil temperature profile after 150 seconds exposure with 750 W prototype microwave (Brodie et al., 2011), and (c) 130 mm wide horn antenna with 1.5 kWhm^{-2} microwave energy on dry sandy soil (Brodie et al., 2009). The images were taken after microwave irradiation of the soil and post processed in the MatLab software to predict the temperature distribution throughout the soil profile.

2.2.7 Advantages of microwave heating over conventional heating

The advantages of MW processing mainly ascend because of the heterogeneous nature of the process material. Therefore, MW energy can be applied at those components that are amenable to MW heating, so little energy is wasted in bulk-heating the whole materials. Also, due to the interaction of the electric field component, selectivity of MW heating arises as a further advantage from the volumetric heating. In conventional heating the heat transfer relies on the energy supply within the depth of a material, whereas with MW heating mediated by the electric field present within the body of the sample which allowing the energy to dissipated rapidly beyond the sample surface. Thus, MW heating does not rely on the heat transfer process which could reduce the heating time up to three orders of magnitude lower than with conventional heating (Meredith, 1998).

Because of these differential heating process than the conventional heating, MW heating has several advantages. The important advantages of MW treatment are short startup, fast heating, material selective heating, precise control, volumetric heating, ease of operation by instant on/off control, without direct contact with heating materials, and environment-friendly (Ayappa et al., 1991; Menéndez et al., 2010). However, non-uniform heating, uneven temperature distribution and rapid moisture movement are also reported in MW heating (Vriezinga et al., 2002; Brodie et al., 2007a; Brodie, 2008).

2.2.8 Effect of microwave heating on soil organic matter

Soil organic matter is the store house of nutrient in the soil and consists of organic residues in the soil at different degrees of humification (Stevenson, 1994). Humification occurs through geological SOM development processes, due to the conversion of various biopolymers to humus (fulvic acid, humic acid and humin) in the soil (Huang and Weber, 1997). About 5 – 25 % of organic compounds are present in the soil as proteins, peptides and free amino acids (Madigan et al., 2008). It has been demonstrated that MW irradiation markedly alters the physical and chemical properties of SOM (Hur et al., 2013), molecular composition (C, H, O and N), chemical structure, and enhances the humification of SOM (Hur et al., 2013; Kim and Kim, 2013).

Usually during the normal humification process alteration of the physical and chemical properties of SOM accomplished by various geophysical, chemical, and microbial reactions involving decomposition (oxidation/reduction), polymerization, and condensation. Humified geo-organic substances are increased in aliphatic or aromatic, condensed, high-carboncontent,

highly polymerized, nonpolar, macromolecular, and polydispersed organic materials. These changes in SOM took a long period (a geological-scale time frame) of time. Similar changes of SOM were reported after the MW irradiation of soil within a very short period (e.g. 10 min) (Kim and Kim, 2013). Where they mentioned couple of changes and their mechanisms of changes of SOM after MW treatment. For example, spectroscopic analysis of SOM revealed that the aromatic content and degree of humification and condensation were increased markedly by MW irradiation, evidenced by increases in SUVA (Specific ultraviolet absorbance) and decreases in E4/E6 (atomic ratios of C, H, O, and N and the ratio of spectroscopic absorptivities at wavelengths of 465 and 665 nm) values. Fourier transform infrared (FTIR) spectral analysis also revealed that, saturated or unsaturated hydrocarbon backbone or functional groups such as aliphatic C–H bonds; carboxylic, carbonyl, phenolic, ketone, amide, and quinone groups; and, more importantly, aromatic C=C bond increased evidently after MW irradiation which usually found in highly humified substances. Furthermore, notable decreases in H/C and O/C ratios and polarity index supported the hypothesis that the MW irradiation is effective in changing the elemental compositions of SOM in a manner like geological-scale humification. These type of changes in SOM were not reported in the case of conventional heating. This might be due to the differential heating mechanism of MW where rapid and very large increase in the temperature of the microscale localized area could favor these changes.

It also increased the soil organic carbon and nitrogen mineralization (Zagal, 1989), macromolecular organic substances that possess a higher number of functional groups (Kim and Kim, 2013), and synthesis of organometallic and coordination compounds (Taylor et al., 2005). These organic substances can retain, decrease mobility, reduce bioavailability and adsorb soil heavy metals (Yin et al., 2002) along with the As. Hur et al. reported that, MW soil heating can enhance the binding efficiency of hydrophobic organic containments with more humified SOM (Hur et al., 2013). Another study also implied that there is an increase in DOC in the soil after MW soil irradiation (Maynaud et al., 2019).

2.2.9 Effect of microwave heating on soil nutrient dynamics

The immediate effect of MW irradiation is raising the soil temperature to around 75 – 85°C, which might have a positive influence on some of the soil properties (Gibson et al., 1988; Brodie et al., 2017; Khan and Brodie, 2018). The previous research findings demonstrate that, MW treatment increased the availability of some soil nutrient e.g. nitrogen, sulfur, and

phosphorus. To investigate the MW heating effect on soil nitrogen availability Khan et al. (Khan et al., 2016) designed an experiment where they imply that MW soil treatment mineralized soil indigenous nitrogen, which resulted in a significant ($p < 0.001$) increase in crop biomass (175 %) and grain yield (92 %) of wheat, compared with the control. In support of this result some other studies also reported that, soil heating of 70 – 100°C can increase indigenous nitrogen supply (Glass et al., 2008; O'Brien et al., 2018). In addition, recent research also showed that, MW soil heating treatment has the potential to increase the indigenous soil N for plant uptake (Khan et al., 2019b).

Similarly, increased total nitrogen levels (106 mg N g⁻¹ soils) (Speir et al., 1986), and NH₄⁺ nitrogen concentrations for plant uptake (Zagal, 1989; Yang et al., 1990) were also reported. These results are in agreement with other experiments, where MW treatment was found to increase N (nitrite N), P and S availability in soil (Cawse and Crawford, 1967; Wainwright et al., 1980; Cooper and Brodie, 2009). In this instance, a recent study also revealed increased inorganic phosphorus (+1.2-fold compared with the control), and nitrate content in soil (Maynaud et al., 2019). However, some different results are also reported, like a declined in P concentration (Speir et al., 1986), and no significant effect on total N, P, K and S concentrations (Cooper and Brodie, 2009). Thus, further detailed investigation is needed, in controlled conditions, to understand the MW heating effect on soil physicochemical properties at different temperature levels, because the soil temperature of around 75 – 85°C neither strongly modifies the soil properties (O'Brien et al., 2018) nor totally disinfects the soil (Voort et al., 2016).

2.2.10 Effect of microwave heating on soil microorganisms

Soil is a highly complex and heterogenous body, which provides habitat for bacteria, fungi, nematodes, and arthropods (Lu et al., 2014). These microbes can adjust to diversified physio-chemical environmental changes (Huhta, 2007), including high temperatures (Ferriss, 1984), by adopting different mechanism (Vela et al., 1976). Various studies have investigated the effect of MW on soil microbial populations and found varying susceptibilities in response to MW heating (O'Brien et al., 2018). Specifically, MW soil heating could be detrimental to bacterial community by directly destroying the bacterial cell (Shamis et al., 2012) since bacteria constitute most of the soil biota by biomass (Marañón et al., 2017). Brodie et al. reported that the population of *Escherichia coli* decreased by 10⁵ in the top layer of soil treated with MW (Brodie et al., 2016). Another study showed a 78 % decrease in bacterial population

when soil was exposed to MW (Cooper and Brodie, 2009). However, the bacterial population was relatively unaffected in deeper soil (Brodie et al., 2015). This might be due to decreased temperature with the increase of soil depth (Brodie et al., 2011). Thus, dead microorganism, because of the MW heating, release more nutrients, which amplified the remaining bacterial population within a month (Brodie et al., 2015). Thus, MW soil heating can create momentary alterations in bacterial communities and may affect their composition and function (O'Brien et al., 2018).

Since bacteria are vastly diverse entities in the soil, the community might undergo a rapid regrowth and recovery, resulting in no significant long-term effect (Fierer et al., 2007). Although short term mortality of the soil bacterial population and subsequent decrease in relative abundance of some bacteria are reported, the effect of MW soil heating depends on the heat tolerance level of bacteria, soil type, soil moisture content, MW intensity, soil heating depth, treatment duration, and soil temperature (Nunes et al., 2018; Khan et al., 2019d; Kabir et al., 2020). For example, the bacterial community can be clustered separately according to MW treatment duration and intensity in a PCoA of Bray-Curtis dissimilarity analysis, which implies that structural changes occur in the bacterial community when exposed to MW heating (Khan et al., 2019d). In the same study, it was reported that the relative abundance of bacteria (Proteobacteria) increased at a soil depth of 0 – 5 cm, while it decreased at 5 – 10 cm and the opposite scenario was observed for other bacteria (Firmicutes) after MW soil heating with a 2 kW, 2.45 GHz, prototype MW system. Firmicutes is a heat tolerance spore forming bacteria and can withstand after MW soil heating. Thus, MW duration and soil treatment depth appeared as determining factors in the MW heat effect on the bacterial community. Furthermore, there was a 90 % reduction in the total bacterial community at 6.8 min of MW soil treatment, where Firmicutes showed the highest resistance to MW heating, followed by Proteobacteria. Actinobacteria was found to be susceptible to heating. Here, MW treatment duration appeared to determine the impact and reshaping influence over the surviving community (Nunes et al., 2018).

In summary, MW soil heating has been demonstrated as an effective technique of non-chemical weed control in agricultural farming system. Microwave soil heating was found to increase plant growth and grain yield. However, the potentiality of MW soil heating to alleviate As phytotoxicity and subsequently lower As accumulation in the grains of important cereal crops, such as wheat and rice, needs to be studied. Also, MW soil heating could change the availability of soil As species and their concentration in grain and soil nutrients, especially P.

In this context, the important questions will be about the residual effect of MW treatment, which will help to establish the frequency of MW treatment. Another question could be whether MW treatment is only a heating effect or if there are other non-thermal effects. In addition, the impact of MW soil heating on the soil microbial community, especially on soil bacteria, is crucial for the acceptance and sustainability of this technique. The results from different experiment described in this thesis will help to answer these questions and to understand the feasibility of MW application for As remediation.

2.3 Biochar

Biochar is a porous carbonaceous solid material obtained from thermochemical decomposition of residual biomass relatively at higher temperature (500 – 1500 °C) and under oxygen depleted conditions (pyrolysis), which has suitable physicochemical properties for safe and long-term carbon sequestration in the environment (Lehmann and Joseph, 2015).

2.3.1 Advantages of biochar use in agriculture

Biochar has been referred to as a millennium old practice in different major environmental management areas include soil improvement, waste management, climate change mitigation, and energy production (Figure 2.11). Biochar is an effective soil conditioner to improve the physicochemical and biological properties of soils due to its high carbon content, porous structure (micropore), and high internal surface area (Figure 2.12) (Lehmann and Joseph, 2015). The carbon present in biomass becomes more stabilized at the time of pyrolysis and is therefore more resistant to further chemical or biological decomposition (Ahmad et al., 2014). Hence, when applied into the soil, biochar remains for a much longer time. Therefore, biochar is drawing great attention for sustainability for agriculture. There are several benefits of biochar application in the soil Table 2.4.

2.3.2 Biochar for contaminants remediation

Carbon rich materials have been used for a very long time to decontaminate organic and inorganic pollutants in soil and water (Saeed et al., 2005; Yang et al., 2011). At present, biochar is the most commonly used carbonaceous sorbent, because it has high microporosity and surface area (Figure 2.12) (Cao et al., 2011). Biochar has multi-functional characteristics consisting of both the carbonized and non-carbonized fraction, which may interact with the contaminants. Predominantly, the presence of hydroxyl, carboxyl and phenolic surface

functional groups are responsible for the remediation of the soil pollutants (Uchimiya et al., 2011a).

2.3.2.1 Removal of organic contaminants

Biochar properties, like activated carbon, create the idea for use in the removal of organic contaminants from water and soil (Zhang et al., 2013). In most cases, biochar is used for removal of organic pollutants from water. Hence, limited research has been conducted on biochar application in soil for management of organic contaminants. Different researchers have conducted trials, with a range of biochar feedstocks like wood, dairy manure, bamboo, and sewage sludge on organic pollutants such as atrazine, chloropyrifos, carbofuran, pentachlorophenol, simazine, tylosin, phenanthrene and polycyclic aromatic hydrocarbons (PAHs). They reported that, biochar produced at higher temperature can adsorb higher amounts of organic pollutants due to its high surface area and micro or nano porosity. The electrically charged surface of biochar has attraction to ionic organic compound (anionic and cationic), which is called electrostatic attraction (Figure 2.13a). Whereas, dispersive and succeeding dispersion into the non-carbonized and carbonized portions of biochar may possibly be an operative sorption mechanism for non-ionic compounds (Ahmad et al., 2014). Surface precipitation, pore filling, and hydrogen bonding are also found to remove organic pollutant (Figure 2.13a). The detail about the removal of different organic contaminants from soil by using different biochar are described in Table 2.5.

Table 2.4. Properties of biochar and different benefits of biochar application

Benefits of biochar application	Responsible properties of biochar or causes	References
Increase soil organic carbon level	Rich in carbon	(McHenry, 2011)
High water retention capacity (18 % increase)	High porosity	(Sohi et al., 2010)
Increase cation exchange capacity (CEC)	Surface functional group	(Yuan et al., 2011)
Retention of nutrients and prevent their loss	High cation exchange capacity and high surface area	(Atkinson et al., 2010; Sohi et al., 2010)
Direct nutrient supply	Depending on the type of biochar	(Atkinson et al., 2010; Sohi et al., 2010)
Increased microbial population and microbial activity in soils	Capacity of being a habitat for microorganisms	(Verheijen et al., 2010; Lehmann et al., 2011; Awad et al., 2012)
Promote the release and uptake of nutrients by plants	Increasing microbial activity and CEC	(Atkinson et al., 2010; Sohi et al., 2010)
Improve soil structure	Organic amendment	(Verheijen et al., 2010)
Increase in soil carbon sequestration	Long-term sink for carbon in soil	(Sohi et al., 2010)
Improve soil fertility and increase crop yield	Improve physicochemical and microbial properties	(Atkinson et al., 2010; Sohi et al., 2010)
Pollution remediation	Adsorption of contaminants	(Atkinson et al., 2010; Sohi et al., 2010)
Agricultural by-product/waste recycling	Incorporation into soil as biochar for long term	(Atkinson et al., 2010; Sohi et al., 2010)

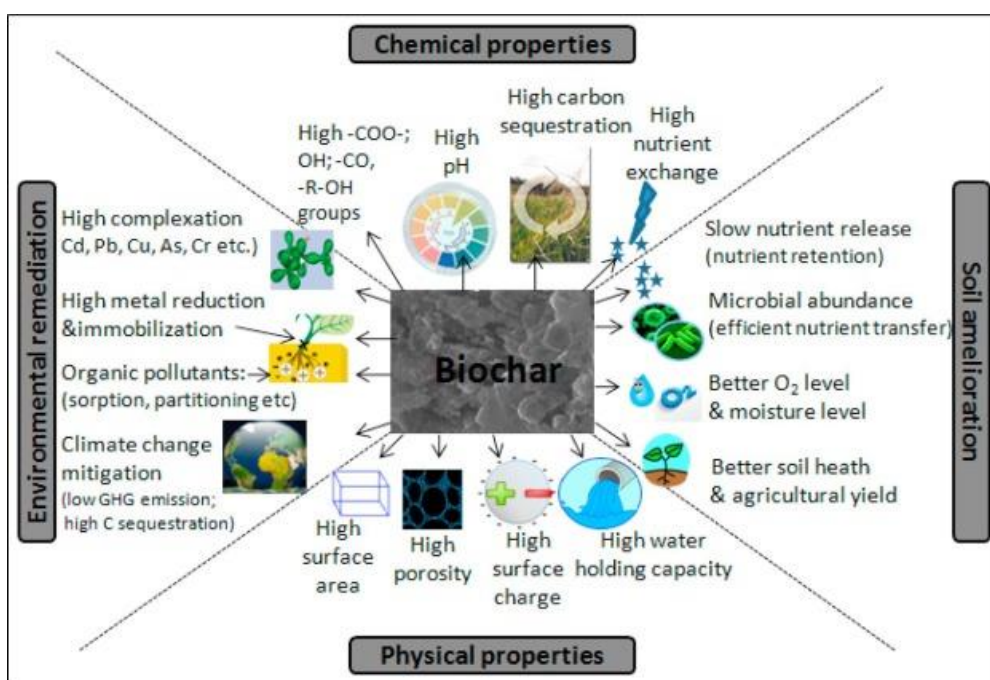


Figure 2.11. Diversified benefits of biochar [Adopted from (Oliveira et al., 2017)]

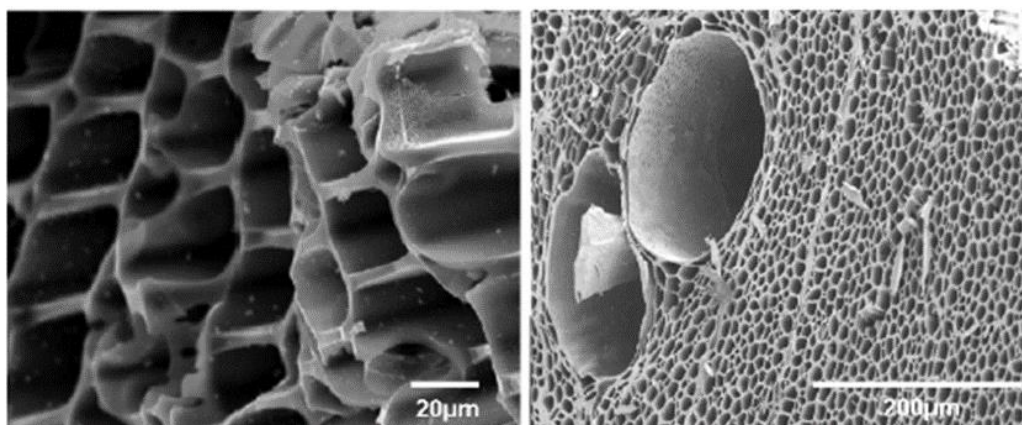


Figure 2.12. Scanning electron microscopic (SEM) study showing the porous structure and high internal surface area of wood biochar [adopted from (Thies and Rillig, 2009)]

2.3.2.2 Removal of inorganic contaminants

Although sorption of organic contaminants by biochar is more favored than that of inorganic contaminants (Kong et al., 2011), it has been used as a novel carbonaceous material with intent for *in situ* remediation of inorganic metal polluted soil (Park et al., 2011a). Several researchers revealed that both the immobilization and mobilization of metals in soil depends on the properties of amended biochar. There has been a focus on biochar application to immobilize and reduce the bioavailability of Cr, Co, Ni, Cd, Zn, Cu, Al, and Pb (Van Zwieten

et al., 2010; Méndez et al., 2012). Electrostatic attraction (inner-sphere), ion-exchange, complexation π electron-rich domain or surface functional groups, and co-precipitation to form insoluble compounds are the predominant phenomenon for the adsorption of inorganic contaminants (e.g. heavy metals) by biochar (Figure 2.13a) due to presence of higher O-containing acidic (carbonyl, lactonic, carboxylic, hydroxyl, and phenol) and basic (chromene, ketone, and pyrone) functional groups (Golber, 1985; Boehm, 1994). The detail about the removal of different inorganic contaminants from soil by using different biochar are described in Table 2.5. However, controverting results on the mobility of metals, by applying biochar, has also been reported (Beesley et al., 2010). Biochar sometimes increases the DOC, which is responsible for mobility of some metals (e.g. Cu), and increases soil pH, resulting in reduced solubility of metals (e.g. Cd and Zn) (Park et al., 2011a).

2.3.2.3 Biochar effect on soil arsenic mobility

During the last decade a significant increase of pot and field experiments have explored the effectiveness of biochar for soil As remediation. Different studies reported the immobilization of As after the biochar application (Beesley et al., 2011; Vithanage et al., 2017). Addition of the biochar in the soil could immobilize As which mainly facilitated by surface complexation of As with different functional groups present on the biochar surface, ion exchange and physical adsorption on biochar surface (Figure 2.13b). However, several studies reported the mobilization of As due to application of biochar (Hartley et al., 2009; Beesley et al., 2010). There are some mechanisms involve responsible for the mobilization of soil As after biochar application. After biochar application into the soil As(V) can be reduce to As(III) by interacting with DOC and phenolic, alcoholic and carboxylic functional group which act as electron donors to facilitate reduction reactions (Choppala et al., 2016). Thus, the mobility and bioavailability of As(III) increase. After the biochar application soil pH also plays an important role in As mobilization. During the pyrolysis process, at high temperature, cations are transformed into oxides, hydroxides or carbonates in the biochar and (Yuan et al., 2011) dissolution of these alkaline substances increase the soil pH (Houben et al., 2013). In the soil solution, As is mainly present as hydro anion and increased soil pH after biochar application can reduce As sorption capacity by decreasing the positively charged sites on soil minerals, which increases As mobilization and release from the soil (Wilson et al., 2010). Khan et al (Khan et al., 2013) reported the mobilization of As after application of sewage sludge biochar into soil due to rise in soil pH. Hartley et al. (Hartley et al., 2009) also reported the mobility of

As with biochar results from the rise in soil pH. Thus, several studies recommended some modification of biochar for soil As remediation (Yu et al., 2015; Wu et al., 2018). Some studies also suggested phytoremediation along with biochar for soil As remediation (Beesley et al., 2014; Choppala et al., 2016). However, it is possible to find similar As concentration in soils after the addition of some biochar due to changes of different characteristics in soil systems. These are most likely due to differences in soil texture, P, pH, Fe, OM, and DOC before and after biochar additions, and biochar properties such as feedstock type, pyrolysis temperature, heating rate, etc. (Hartley et al., 2009). The detail about the effect of biochar characteristics on As mobility is described in Table 2.5.

In summary, different types of biochar, prepared by different methods and from different sources, are used for As remediation. However, all biochar is not equally efficient in immobilising soil As. In this context, MW assisted pyrolysis could be a better choice because of the higher char yield, higher decomposition temperature, faster heating rate, and less or no pre-processing of feedstock in MW assisted pyrolysis, compared with conventional pyrolysis. However, no published study, so far, has been reported using MW pyrolyzed biochar, in combination with MW soil heating, for soil As remediation, which could be a possible technique to lessen As phytotoxicity and grain As accumulation. In this context, the important question is what will be the effect of biochar on As speciation, especially in grain samples, since the toxicity of As depends on its species. Also, research on the ageing of soil biochar and its effect on As remediation is not well studied. Thus, the residual effect of biochar application is important to understand the durability of treatment for As remediation. The findings of different experiments, described in this thesis, will help to answer these questions and to understand the feasibility of biochar application for As remediation.

Table 2.5. Effect of biochar application on the availability of soil organic and inorganic contaminants

Contaminants	Biochar types	Effect	References
Organic contaminants			
Atrazine	Dairy manure (450 ⁰ C)	Sorption of atrazine by biochar	(Cao et al., 2011)
Chloropyrifos and carbofuran	Woodchips (450 and 850 ⁰ C)	Adsorption because of high surface area and nano-porosity of biochar	(Yu et al., 2009)
Pentachlorophenol	Bamboo (600 ⁰ C); Rice straw	Reduced leaching due to diffusion and partition; Adsorption due to high surface area and microporosity	(Lou et al., 2011; Xu et al., 2012)
Simazine	Hardwood (450 and 600 ⁰ C)	Sorption due to abundance of micropores	(Jones et al., 2011)
Tylosin	Pulpgrade hardwood and softwood chips (850 and 900 ⁰ C)	Sorption	(Jeong et al., 2012)
Phenanthrene	Pine wood (350 and 700 ⁰ C)	Entrapment in micro- or meso-pores	(Zhang et al., 2010b)
PAHs	Sewage sludge (500 ⁰ C)	Sorption and biodegradation; Partitioning	(Beesley et al., 2010)
Inorganic contaminants			
As	Hard wood (400 ⁰ C)	Mobilization due to enhanced pH and DOC	(Hartley et al., 2009)
As and Cu	Hard wood	Mobilization due to enhanced pH and DOC	(Beesley et al., 2010)
Cd, Cu and Pb	Chicken manure and green waste (550 ⁰ C)	Immobilization due to partitioning of metals from the exchangeable phase to less bioavailable organic-bound fraction	(Park et al., 2011a)

As, Cd, Cr, Co, Cu, Ni, Pb, and Zn	Sewage sludge (500–550 ⁰ C)	Immobilization of As, chromium, cobalt, nickel and lead due to rise in soil pH; mobilization of copper, zinc and cadmium due to high available concentrations in biochar	(Khan et al., 2013)
Cd and Zn	Hard wood	Immobilization due to enhanced pH	(Beesley et al., 2010)
Cu	Broiler litter (700 ⁰ C)	Cation exchange; electrostatic interaction; sorption on mineral ash contents; complexation by surface functional groups	(Uchimiya et al., 2011b)
Cu and Pb	Oak wood	Complexation with phosphorous and organic matter	(Karami et al., 2011)
Pb	Dairy manure (450 ⁰ C)	Immobilization by hydroxypyromorphite formation	(Cao et al., 2011)
Pb	Oak wood (400 ⁰ C)	Immobilization by rise in soil pH and adsorption onto biochar	(Ahmad et al., 2012a)
Pb	Rice straw	Non-electrostatic adsorption	(Jiang et al., 2012)
Pb, Cu, Zn, and Sb	Broiler litter (350 and 600 ⁰ C)	Stabilization of Pb and Cu; desorption of Sb	(Uchimiya et al., 2012)
Ni, Cu, Pb, and Cd	Cottonseed hulls (200–800 ⁰ C)	Surface functional groups of biochars controlled metal sequestration	(Uchimiya et al., 2011a)

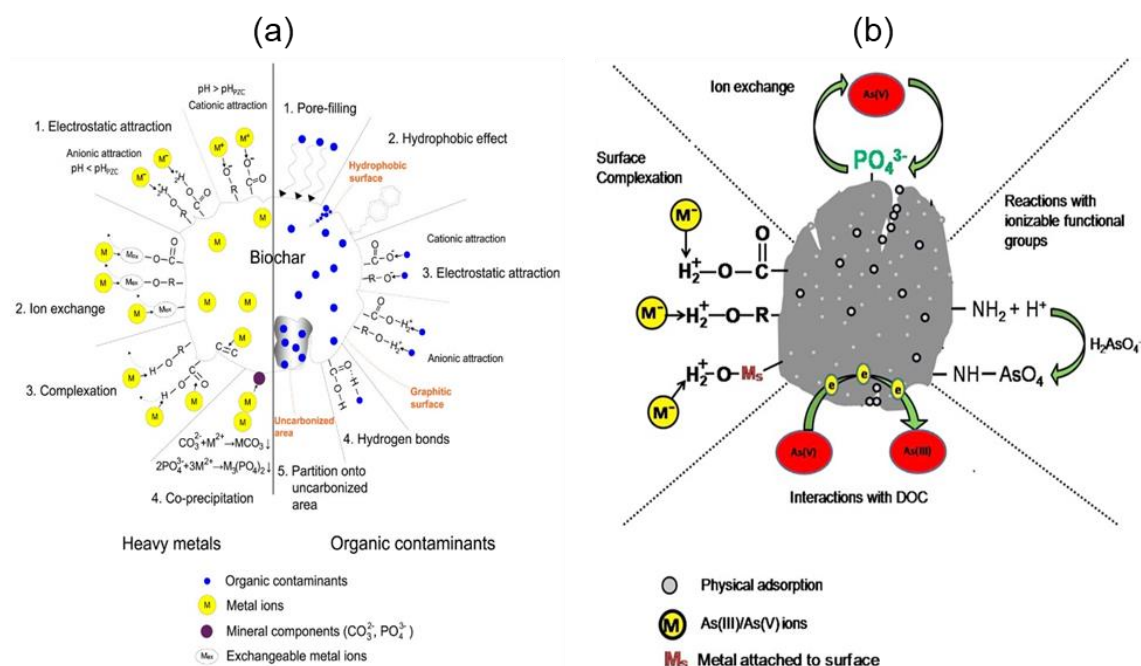


Figure 2.13. (a) Hypothesized mechanisms of the interaction of biochar with organic and inorganic contaminants [adopted from (Wang et al., 2020b) and (b) possible mechanism of arsenic adsorption on biochar surface (Vithanage et al., 2017)]

2.4 Summary

Arsenic is a naturally occurring toxic metalloid, which possess a serious threat to human health due to its adverse effect. Around the world, South East Asian countries are badly affected by As pollution. Bangladesh is reported as one of the worst As polluted areas, where geogenic As severely contaminates groundwater, which ultimately increases As exposure through drinking water and food. Arsenic contaminated drinking water has been recognized as the major pathway of human As exposure. The use of As polluted groundwater for crop irrigation gives rise to high deposition of As in topsoil and ultimately results in As uptake by crops, which expands As exposure through the food chain. Being a staple food for half the world's population, rice has become a major source of human dietary As exposure. However, considerable concentration of As in other cereals (e.g. Wheat) has become a matter of concern.

In the soil and water environment, As is mainly present in two forms, inorganic and organic. The inorganic forms are more toxic to plants and humans than the organic forms and among the inorganic forms As(III) is approximately 60 times more toxic, soluble, and mobile than the As(V). Thus, As speciation requires major attention due to its species dependent toxicity. However, toxicity may also depend on the environmental and crop genotypic variation. Development of mitigation strategies are needed to reduce plant As uptake and grain

accumulation to combat the As hazard. Although there are several techniques and management practices that have been proposed for drinking water and soil As remediation, they are either not feasible for the treatment of large amount irrigation water need for crop cultivation or ineffective, costly, or too lengthy, with usage restricted to smaller-scale operations with lower efficiency. Thus, alternative options or combination of technologies are required for soil As remediation.

Microwave energy is a form of electromagnetic radiation, with frequencies between 300 MHz to 300 GHz and wavelengths ranging from 1m to 1mm. Some major advantages like short start-up, internal volumetric heating, and precise control compared with other heating processes, makes MW heating a preferred choice and has been using in diversified field including removal of organic contaminants and immobilization of some toxic metals in soil and solid sediments. Pre-sowing MW soil heating has been revealed as a unique technique, which can significantly enhance crop growth in addition to be a means of non-chemical weed control. The increased plant growth and grain yield in MW treated soils attributed to the more availability of some soil nutrients because of the humification of SOM and dead soil microorganisms. Furthermore, humification of SOM can increase the functional group and synthesize aliphatic or aromatic, high carbon content, highly polymerized, and macromolecular organic materials. Because of these changes in SOM, there would be a possibility of As adsorption, which might reduce the bioavailability of As. However, no available study has been reported so far, the use of MW soil heating technique for soil As remediation. Thus, MW soil treatment could be a novel method for As remediation by reducing the As phytotoxicity and grain As accumulation.

Furthermore, to immobilize soil organic and inorganic pollutants and heavy metals, biochar has been progressively gaining more attention because of its high carbon content, micropores, and large surface area. Arsenic can be immobilized by precipitation and reduction, surface complexation with different functional groups, ion exchange, and electrostatic interactions on the biochar surface. However, increased mobility and bioavailability of As is also reported after the application of some biochar. Although several studies conducted to investigate the As immobilisation efficiency of different biochar, biochar produced by MW assisted pyrolysis not well studied despite having some advantages such as higher char yield, higher decomposition temperature, higher heating rate, and less or no pre-processing of feedstock. Also, no published study so far reported in a combination of biochar treatment and MW soil heating for soil As remediation.

However, the potentiality of MW soil heating and biochar application to alleviate As phytotoxicity and subsequently lower As accumulation in the grains of important cereal crops such as wheat and rice need to be studied. In this context, some key things need to be addressed such as (i) the effect of MW and biochar treatment on the availability of soil nutrients and As and their concentration in grain, (ii) the residual effect of MW and biochar treatment to investigate its application durability, (iii) in case of MW treatment, whether it is only heating effect or MW heating effect, (iv) the effect of MW and biochar on As speciation especially in grain sample, since the toxicity of As depends on its species, and (v) the impact of MW soil heating on the soil microbial community especially on soil bacteria since it is crucial for the sustainability of this technique. Therefore, experiments need to be conducted to investigate the potentiality of MW soil heating and biochar application for soil As remediation by reducing the As phytotoxicity and grain As accumulation along with addressing some key things mentioned above

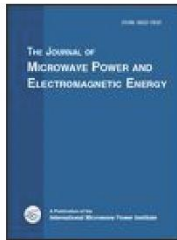
Chapter 3 – Measurement and modelling of soil dielectric properties as a function of soil class and moisture content

3.1 Introduction and aim

The application of microwave (MW) radiation into the soil induces rotation of the dipoles of polar or semi-polar molecules, like water and hydrocarbon-contaminants, due to the oscillating electromagnetic field. This results in the generation of heat by intermolecular friction (Falciglia et al., 2016). However, to develop a proper understanding of MW soil treatment and to explain soil heating, the dielectric properties of soil are required. To determine the dielectric properties of soil and its heating pattern, a wide range of soil types, moisture contents and frequencies need to be addressed.

Section 3.2. presents the published manuscript that investigate the dielectric properties of soil over a wide range of frequency (700–7000 MHz) and develop a multi-factor mixing model for predicting the dielectric properties of soil using various mathematical models, based on the dipole studies of Debye and various mixing models.

3.2 Published manuscript



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Measurement and modelling of soil dielectric properties as a function of soil class and moisture content

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Measurement and modelling of soil dielectric properties as a function of soil class and moisture content

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ABSTRACT

In this study, four textural classes of soil (Clay, Clay Loam, Loam, and Loamy Sand) were used to investigate the dielectric properties of soils, using a vector network analyser with an open-ended coaxial probe kit at room temperature ($25 \pm 2^\circ\text{C}$) in the 700–7000 MHz microwave frequency range. Four levels of soil moisture content (oven dry, 33% field capacity, 66% field capacity and 100% field capacity) were maintained to perform the experiment with three replication and three observation each. The results showed that, with increasing soil moisture, from oven dry conditions to 100% field capacity, both the real (Dielectric Constant) and imaginary (Loss Factor) components of the dielectric properties increased; however, the responses were not linear. The dielectric properties of oven dry soils were very low compared with the soils with higher moisture content. Therefore, soil moisture was the major contributor to the dielectric behaviour of soil. The dielectric properties of sandy soil were much lower than the other soils; however, the dielectric loss factor of the Dookie clay soil was higher compared with the other soils. Models were developed to explain the dielectric properties of soils as a function of frequency and moisture content. The goodness of fit (r^2) for these models varies between 0.952 for the Dookie Sandy Soil to 0.997 for the Dookie Loam Soil, suggesting that these models were adequate to describe the dielectric properties of these soils over the range of frequencies and moisture contents assessed in this study. Another model was developed to estimate the expected penetration depth of electromagnetic waves in these soils, based on the model of the dielectric properties. It was clear that penetration decreases with both frequency and moisture content. Low frequencies penetrate further into the soils than higher frequencies. Similarly, dry soils allow further penetration than moist soils.

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Soil; dielectric; moisture; soil classification

1. Introduction

Microwave energy has been applied to: food processing (Metaxas and Meredith 1983; Chandrasekaran et al. 2013; Fazaeli et al. 2013; Benlloch-Tinoco et al. 2013;

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Ruiz-Ojeda and Peñas 2013); textiles and leather processing (Ayappa et al. 1991); medical application (Bond et al. 2003); plasma (Falciglia et al. 2016) and solvent-free chemistry (Arrieta et al. 2007); drying of wood (Antti and Perre 1999; Zielonka and Dolowy 1998), paper and cardboard (Hasna 2009); pest control (Nelson 1996); enhancing seed germination (Nelson et al. 1976; Nelson and Stetson 1985); and removal of hazardous waste from contaminated soil (Dauerman et al. 1992; Liu et al. 2014; Huang et al. 2011; Kawala and Atamańczuk 1998; Falciglia and Vagliasindi 2014; Di and Chang 2001; Lin et al. 2010). Furthermore, current studies also reveal that, microwave soil treatment can kill the soil weed seed bank, which results in: the reduction of weed emergence (Jamal et al. 2017; Brodie et al. 2007; Cooper and Brodie 2009; Brodie et al. 2012; Brodie and Hollins 2015); increased carbon and nitrogen mineralization (Zagal 1989); and increased plant growth (Brodie 2016). Thus, microwave heating has extended acceptance and is gaining popularity due to its major advantages of: short start up time; precise control; and volumetric heating (Ayappa et al. 1991).

The application of microwave radiation into the soil induces rotation of the dipoles of polar or semi-polar molecules, like water and hydrocarbon-contaminants, due to the oscillating electromagnetic field. This results in the generation of heat by intermolecular friction (Falciglia et al. 2016). However, to develop a proper understanding of microwave soil treatment and to explain soil heating, the dielectric properties of soil are required.

Microwave heating depends on the ability of a material to convert the adsorbed microwave energy into heat. This is associated with the dielectric properties of the material. The dielectric properties of materials are generally illustrated by relative complex permittivity, $\epsilon = \epsilon' - j\epsilon''$, where the real part (ϵ') of the dielectric property, known as the dielectric constant, implies the material's ability to store energy and the imaginary part (ϵ'') of the dielectric property, known as the dielectric loss, indicates the ability to convert the energy to heat, and j is $\sqrt{-1}$ (Nelson 1996; Metaxas and Meredith 1983; Kraus and Fleisch 1999). The dielectric loss factor is linked to the absorbance of microwave energy by the material. Generally, the dielectric properties of a material, like soil, are controlled by the operating frequency, temperature, moisture content, density and composition of the material (Nelson 1991).

Soil is a complex solution of mineral particles, water, ions, gasses (mostly air), and micro and macro organisms. The size of the mineral particles provides one tool for soil classification, with soils being classified according to the percentage of sand, silt and clay present in the mineral portion of the solution.

There have been several studies of the dielectric properties of soil. Some of these are particularly focused on interpreting remotely sensed data (Tikhonov 1997; Boyarskii et al. 2002), especially passive microwave emission and radar response, while others have soil heating as their focus (Iben et al. 1996); however, the industrial, scientific and medical (ISM) microwave frequencies (896/915/922 MHz, 2450 MHz and 5800 MHz) seem to have been neglected in most of these studies. Thus, the dielectric behaviour of soil, at these ISM frequencies, is becoming important.

Based on other studies (Brodie et al. 2014), moisture content will probably have the greatest effect on dielectric properties. For developing fast moisture measurement

with microwave sensors, the dielectric properties have great importance due to their direct relationship with moisture content (Trabelsi et al. 1998a; Trabelsi et al. 1998b; Trabelsi et al. 2008).

Other factors that will affect the dielectric properties include: soil class (i.e., the proportions of sand, silt and clay); the organic matter content; the level of soil compaction (i.e., bulk density of the soil solution); and finally, the temperature of the soil. Because the dielectric behaviour of most materials varies profoundly with frequency, there is an imperative to study the dielectric properties of soils across the main ISM microwave frequencies.

Soil temperatures, for disinfection and seed deactivation applications, are normally in the range from 60°C to 120°C; however, Falciglia and Vagliasindi (Falciglia and Vagliasindi 2016) demonstrated that microwave soil heating can achieve temperatures well in excess of 200°C during their study of microwave treatment of hydrocarbon-polluted soil.

Organic matter will affect the water holding capacity of the soil and these organic materials have a different dielectric behaviour to soil minerals; therefore, some understanding of the effect of soil organic matter should be included in a comprehensive study of the dielectric properties of soil. According to Certini (Certini 2005), the consumption of soil organic matter during forest fires begins at about 200°C; therefore, the effect of organic matter concentration on soil heating during microwave treatment is important. Salinity will also affect the dielectric behaviour of soil, because it affects the absorption of electromagnetic energy due to ion conduction and therefore affects the loss factor of moist soil (Meissner and Wentz 2004), especially at lower frequencies.

Hence, the key parameters of interest in the study of the dielectric properties of soil, in order of their importance, are: frequency (700 MHz to 7000 MHz); soil type (texture); soil moisture content; soil temperature; soil organic matter content; and soil salinity. It is difficult to set up a single experiment to cover all these factors; however, it is suggested that a multi-factor analysis of a subset of these soil properties be initiated, to examine the impact of some of these parameters on the complex dielectric properties of soils.

The common methods for dielectric property measurements include: cavity resonator perturbation; free space analyses; lumped circuit; transmission line; and coaxial probe techniques. The cavity resonator approach is suitable for frequencies between 50 and 100 MHz and applicable for material with low loss. The free space method is applicable for large, flat, thin and parallel faced samples and is suitable for high frequencies (3 GHz to 100 GHz) as well as being used for measuring materials at high temperature, since this method is non-destructive and noncontact. The lumped circuit method is suitable for low frequencies (below 100 MHz) and not applicable for low loss materials. The transmission line method is mostly applicable for liquid and solid materials in the frequency range of 30 MHz to 100 GHz, but it is not suitable for gases due to their low permittivity (İçier and Baysal 2004; Rynänen 1995; Wee et al. 2009). The open ended coaxial probe, with a vector network analyser, has been used commonly for dielectric property measurements of food grains, such as chickpea, green pea, lentil, soybean, mung bean, black eye pea and Macadamia nut Kernel with a frequency range of 10–1800 MHz (Guo et al. 2010; Jiao et al. 2011; Wang et al.

2013; Taheri et al. 2018). This method is also suitable for measurement of soil dielectric properties (Velazquez-Marti et al. 2005; Gupta and Jangid 2011; Behari 2006).

On the basis of the soil properties, the most commonly used dielectric mixing model is proposed by Peplinski et al. (Peplinski et al. 1995). This semi-empirical model was proposed by considering soil texture, moisture content, bulk density and temperature in a wide range of frequencies (0.3-1.3 GHz). Peplinski et al. (Peplinski et al. 1995) modified another dielectric mixing model developed by Dobson et al. (1985).

To determine the dielectric properties of soil and its heating pattern, a wide range of soil types, moisture contents and frequencies need to be addressed. Therefore, this study was conducted to: (i) determine the dielectric properties of soil over a wide range of frequency (700–7000 MHz); and (ii) to develop a multi-factor mixing model for predicting the dielectric properties of soil using various mathematical models, based on the dipole studies of Debye and various mixing models.

2. Materials and methods

2.1. Soil sample collection

To investigate the dielectric properties of different soil, four types of soil were used in this study (Table 1). Clay loam soil was collected from South Australia (34° 42' S, 138° 35' E) and the other three soil types were collected from Dookie College. These were: Loam soil from the Macpoyels wheat paddock (36°23' S, 145°42' E), Clay soil from the H12 paddock (36°24' S, 145°43' E) and sandy soil from a pasture paddock (36°25' S, 145°43' E). After collection, the soil samples were air-dried for 7 days. The aggregates were broken down prior to being sieved through a 2 mm soil sieve to remove unwanted materials (viz. dry roots, grasses, stones and gravel) and to ensure uniform soil aggregates for better evaluation of the dielectric properties. Then the separate soils were thoroughly mixed by following the quartering method to ensure homogenous condition.

2.2. Analysis of soil properties

Different physicochemical properties of the experimental soils were determined by drying at 40°C for 24 hr prior to sending them to the Nutrient Advantage laboratory (NATA Accredited Laboratory # 11958, Australia) for complete analyses. The results of these analyses are highlighted in Table 1.

2.3. Maintaining of soil moisture

Four levels of soil moisture (oven dry, 33% field capacity, 66% field capacity, and 100% field capacity) were maintained. To determine the soil field capacity, 25 g of oven dry soil was placed in a funnel with filter paper (Whatman No 1). Then, 25 ml of distilled water was added to the soil. The funnel was placed in a measuring cylinder and kept for 24 hours to allow the gravitational water to drained out. Then the field capacity of that soil, on volume basis, was calculated using the following equation

Table 1. Physicochemical properties of experimental soils.

Soil properties	Analytical method	Units	Soil types			
			Dookie Clay	South Australian	Dookie Loam	Dookie Sand
Texture	Hydrometer and sieve analysis		Clay	Clay loam	Loam	Loamy Sand
Sand		%	47.0	48.7	56.2	83.8
Silt			8.6	17.1	23.8	8.7
Clay			44.4	34.2	20.0	7.5
Field capacity	Gravimetric	%	72	64	66	16.8
Organic carbon	Walkley & Black	%	0.47	0.86	1.40	0.70
Organic matter	Walkley & Black	%	0.81	1.48	2.41	1.2
Electrical conductivity	Saturated extract	dS/m	2.1	1.6	0.6	0.6
pH	1:5 CaCl ₂	N/A	7.2	7.8	5.0	5.1
Cation exchange capacity (CEC)	BaCl ₂ exchange	cmol(+)/kg	32.9	28.1	7.9	2.95
Nitrate nitrogen	Kjeldahl	mg/kg	37.0	38.0	37.0	7.4
Ammonium nitrogen	Kjeldahl	mg/kg	3.6	1.3	2.0	3.6
Available Potassium	Atomic emission	mg/kg	310	440	340	130
Sulphur	0.25M KCl at 40°C	mg/kg	9.9	9.5	7.1	3.9
Phosphorus	Colwell	mg/kg	<5.0	17	180	23
Calcium	Ammonium acetate	cmol(+)/kg	16	22	5.7	2.0
Magnesium	Ammonium acetate	cmol(+)/kg	14	4.2	1.3	0.57
Sodium	Ammonium acetate	cmol(+)/kg	1.9	0.69	0.03	0.08
Aluminium	Ammonium acetate	cmol(+)/kg	<0.10	<0.10	<0.10	<0.10
Sodium % of cations (ESP)	N/A	%	5.9	2.4	0.41	2.6
Calcium/magnesium ratio	N/A	N/A	1.1	5.2	4.4	3.5
Chloride	Soluble chloride	mg/kg	110	12	14	<10
Copper	DTPA	mg/kg	1.4	1.6	4.3	0.72
Zinc	DTPA	mg/kg	9.2	45	2.7	3.8
Manganese	DTPA	mg/kg	18	11	49	24
Iron	DTPA	mg/kg	17	13	120	67
Boron	DTPA	mg/kg	2.5	2.8	0.64	0.20

$$FC = \frac{(V_i - V_f)}{V_i} \times 100 \quad (1)$$

where, V_i is the initial water added to the soil and V_f is the water collected in the measuring cylinder, after 24 hours.

For oven dry soil, the soil samples were dried at 105°C for 24 hours. To achieve the other moisture contents, the required amount of deionised water was added into the soil and thoroughly mixed. After getting the desired moisture for further dielectric measurement, all the soil samples were stored at 4°C in plastic zip lock bags to prevent soil moisture loss.

2.4. Experimental design

Table 2 illustrates the experimental design for the simultaneous study of all the factors, to determine the dielectric properties of soils.

Table 2. Experimental design.

Soil type	Moisture content	Density	Temperature	No. of observation
Clay	Oven dry	Compact	Room temperature ($25 \pm 2^\circ\text{C}$)	9
	33% Field capacity			9
	66% Field capacity			9
	100% Field capacity			9
Clay Loam	Oven dry			9
	33% Field capacity			9
	66% Field capacity			9
	100% Field capacity			9
Loam	Oven dry			9
	33% Field capacity			9
	66% Field capacity			9
	100% Field capacity			9
Loamy Sand	Oven dry			9
	33% Field capacity			9
	66% Field capacity			9
	100% Field capacity			9

2.5. Dielectric properties measurement

To measure the complex permittivity (real part, ϵ' , and imaginary part, ϵ'') a vector network analyser (E8364B PNA network analyser) with an open-ended coaxial probe kit (Agilent 85070E Dielectric Probe kit 200 MHz to 50 GHz, Performance Probe 85070-60010) was used (Figure 1). The network analyser had the potential frequency range of 10 MHz to 50 GHz to measure the dielectric properties of materials. The main part of the analyser was connected to an external computer with corresponding Agilent analysis software with an embedded algorithm to calculate ϵ' and ϵ'' .

Before starting the measurement, the network analyser was calibrated using three standards (i.e., on open air, with a short block and in water). To get the electrical short, a metal sheet (e.g., Al or Cu) was placed under the probe. After calibration, the dielectric properties of a reference sample, for example water at room temperature or Teflon or ceramic were measured to confirm the calibration. After getting good values for the reference samples, the dielectric properties of the experimental samples were measured.

The dielectric properties of different soil samples were measured over the frequencies range of 700 MHz to 7000 MHz. This frequency range covers the most useable microwave frequencies for industries, scientific and medical applications. The samples were measured with three replications, with three observation each for each replication and the mean values were considered for further statistical analysis. The measurements were carried out in a 4.5 cm long Teflon container with a diameter of 1 cm, to ensure that the probe could enter the vial and the soil surface was wide enough to assume no influence of the container on the measurements. The whole experiment was carried out at room temperature ($25 \pm 2^\circ\text{C}$).

2.6. Penetration depth and wavelength

Dielectric constant and dielectric loss are two key factors of dielectric properties of a material, which can determine the heating pattern of a particular geometry during microwave treatment. Other important parameters, which can be derived from the

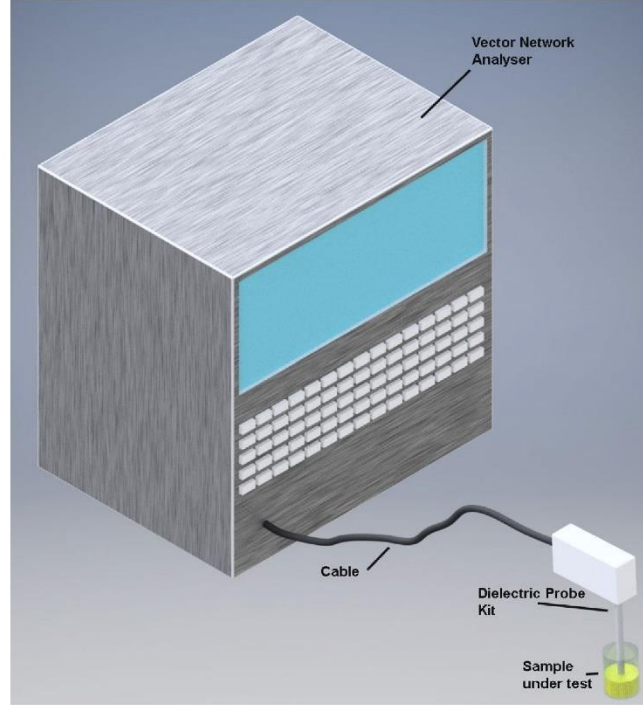


Figure 1. Schematic of vector network analyser (E8364B PNA) with open ended coaxial probe kit and sample under test.

dielectric measurements, are the penetration depth and wavelength of the electromagnetic waves in the soil.

The penetration depth (d_p) is the distance at which the electromagnetic power density decline to a value of $1/e$ ($e = 2.718$) or 36.8% from its initial value at the surface and is stated as (Metaxas and Meredith 1983; Basak et al. 2016)

$$dp = \frac{c}{\sqrt{2}\pi f \left[\sqrt{(\kappa')^2 + (\kappa'')^2} - \kappa' \right]^{1/2}} \quad (2)$$

where the dielectric properties are described by: $\kappa = \kappa' + j\kappa''$ and the wavelength (λ_m) can be calculated as (Basak et al. 2016)

$$\lambda_m = \frac{c\sqrt{2}}{f \left[\sqrt{(\kappa')^2 + (\kappa'')^2} + \kappa' \right]^{1/2}} \quad (3)$$

where c is the velocity of light in space ($3 \times 10^8 \text{ ms}^{-1}$) and f is the frequency (Hz).

2.7. Cole-Cole analysis

For further analysis of the measured complex permittivity, a curve-fitting technique is usually used based on one of the following models: Debye model; Cole-Cole model; Davidson-Cole model; Havriliak-Negami model; and KWW model. Typically, the curve-fitting study is used to inspect the dielectric relaxation time and the Debye relaxation model has been used widely for these studies. The simplest form of the Debye equation (Debye 1929) to describe the complex dielectric permittivity of a pure polar material is:

$$\varepsilon = \varepsilon_{\infty} + \frac{\varepsilon_s - \varepsilon_{\infty}}{1 + j\omega\tau} - j \frac{\sigma}{\omega\varepsilon_0} \quad (4)$$

where, ε_0 is the permittivity of free space, ε_s is the static permittivity; ε_{∞} is the permittivity at high frequency; ω is the applied angular frequency ($2\pi f$), σ is a conductivity term to account for free ion movement, and τ is the relaxation time.

Since all the materials are not purely polar, there are several relaxation times, which result in a modification to the Debye equation (Cole and Cole 1941).

$$\varepsilon = \varepsilon_{\infty} + \frac{\varepsilon_s - \varepsilon_{\infty}}{(1 + j\omega\tau)^{1-\alpha}} - j \frac{\sigma}{\omega\varepsilon_0} \quad (5)$$

where α is a power factor that accounts for a distribution of relaxation times associated with many real materials. Cole and Cole (Cole and Cole 1941) showed that this can be resolved into the real and imaginary parts of the dielectric properties:

$$\varepsilon' = \varepsilon_{\infty} + \frac{(\varepsilon_s - \varepsilon_{\infty}) \left[1 + (\omega\tau)^{1-\alpha} \sin\left(\frac{\alpha\pi}{2}\right) \right]}{\left[1 + 2(\omega\tau)^{1-\alpha} \sin\left(\frac{\alpha\pi}{2}\right) + (\omega\tau)^{2(1-\alpha)} \right]} \quad (6)$$

and

$$\varepsilon'' = \frac{(\varepsilon_s - \varepsilon_{\infty})(\omega\tau)^{1-\alpha} \cos\left(\frac{\alpha\pi}{2}\right)}{\left[1 + 2(\omega\tau)^{1-\alpha} \sin\left(\frac{\alpha\pi}{2}\right) + (\omega\tau)^{2(1-\alpha)} \right]} + \frac{\sigma}{\omega\varepsilon_0} \quad (7)$$

It is also evident that the dielectric properties of most natural materials, in the microwave frequency range, are linked to the moisture content of the material. Therefore:

$$\varepsilon(m) = \left[\varepsilon_{\infty} + \frac{\varepsilon_s - \varepsilon_{\infty}}{(1 + j\omega\tau)^{1-\alpha}} - j \frac{\sigma}{\omega\varepsilon_0} \right] \cdot F(m) \quad (8)$$

where $F(m)$ is some function of moisture content, which is to be determined from the data.

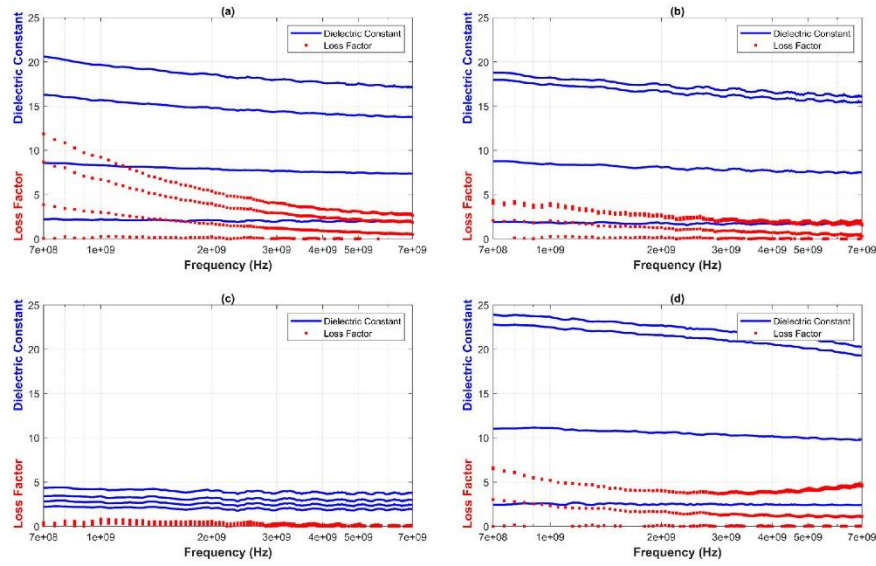


Figure 2. Measured Dielectric properties of the four soils: (a) Dookie Clay Soil, (b) Dookie Clay Loam Soil, (c) Dookie Sand Soil, and (d) South Australian Soil, as a function of frequency and moisture content (Note: both the dielectric constant and the loss increase with increasing moisture content).

2.8. Statistical and other software analysis

MatLab software (MathWorks, Inc., USA) was used to analyse and display the dielectric data. Equations (6), (7), and (8) were used in MatLab's Curve Fitting Tool as the basis for developing a semi-empirical model for the dielectric properties of the four soils.

3. Results and discussion

Figure 2 shows the dielectric properties of the four soils under study as a function of frequency and moisture content. Table 3 also shows a subset of the dielectric data as a function of soil type, frequency and soil moisture status, obtained during this study. Both the real (Dielectric Constant) and imaginary (Loss Factor) components of the dielectric properties increase as moisture increases from oven dry conditions to 100% Field Capacity, through the intermediary moisture levels (i.e., Oven dry, 33% Field Capacity, 66% Field Capacity and Field Capacity). The dielectric properties of dry soil are very low; therefore, soil moisture is the major contributor to the dielectric behaviour of soil. This increase in dielectric value with moisture was also observed by Wang (Wang 1980) and Dobson, Ulaby (Dobson et al. 1985).

Because sand has a much lower surface area per unit volume than the other mineral components of soil, the moisture holding capacity, and therefore the field capacity of soils is inversely related to their sand content. Therefore, the field capacity of the Dookie Sand Soil is much lower than that of the other three soils, because of its high sand content (83.8% - Table 1), compared with the other soils (47.0% to 56.2% -

Table 3. Subset of dielectric data as a function of soil type, frequency and soil moisture status.

Soil type	Frequency (GHz)	Moisture status							
		Oven dry soil		33 % Field capacity		66 % Field capacity		100 % Field capacity	
		ϵ'	ϵ''	ϵ'	ϵ''	ϵ'	ϵ''	ϵ'	ϵ''
Dookie Clay	0.7	2.205	0.060	8.578	3.872	16.309	8.678	20.603	11.860
	0.9	2.154	0.141	8.344	3.067	15.641	6.880	19.689	9.435
	1	2.218	0.261	8.314	3.006	15.692	6.717	19.687	9.230
	2	2.070	0.247	7.873	1.744	14.762	4.005	18.548	5.411
	2.45	1.992	0.145	7.772	1.438	14.523	3.408	18.205	4.672
	3	1.971	0.039	7.685	1.205	14.359	2.926	17.990	4.059
	4	1.996	0.007	7.546	0.918	14.119	2.403	17.643	3.366
	5	2.042	0.021	7.489	0.779	14.048	2.209	17.575	3.109
	5.8	2.018	0.117	7.432	0.629	13.890	1.987	17.324	2.827
	6	1.947	0.131	7.396	0.613	13.813	2.012	17.222	2.868
Dookie Clay Loam	7	2.005	0.196	7.384	0.507	13.723	1.882	17.076	2.652
	0.7	1.900	0.028	8.781	2.091	17.959	4.036	18.787	4.350
	0.9	1.845	0.150	8.435	1.878	17.411	3.556	18.194	3.869
	1	1.869	0.279	8.504	2.052	17.517	3.740	18.270	3.997
	2	1.772	0.200	8.071	1.300	16.639	2.598	17.404	2.748
	2.45	1.683	0.149	7.899	1.110	16.354	2.316	17.087	2.469
	3	1.668	0.056	7.759	0.919	16.083	2.076	16.801	2.227
	4	1.669	0.022	7.644	0.719	15.853	1.825	16.532	1.948
	5	1.738	0.048	7.675	0.677	15.829	1.905	16.537	2.052
	5.8	1.692	0.085	7.530	0.451	15.591	1.631	16.272	1.748
Dookie Sandy Soil	6	1.632	0.082	7.447	0.473	15.438	1.713	16.107	1.854
	7	1.686	0.158	7.451	0.297	15.364	1.560	15.999	1.668
	0.7	2.199	0.104	2.808	0.104	3.402	0.209	4.319	0.412
	0.9	2.125	0.141	2.728	0.332	3.289	0.416	4.179	0.594
	1	2.209	0.344	2.755	0.508	3.379	0.641	4.251	0.821
	2	2.036	0.259	2.577	0.396	3.162	0.449	4.006	0.576
	2.45	1.891	0.197	2.440	0.297	3.011	0.343	3.838	0.444
	3	1.857	0.067	2.373	0.147	2.925	0.185	3.731	0.275
	4	1.887	0.021	2.393	0.076	2.954	0.097	3.744	0.169
	5	1.979	0.082	2.481	0.156	3.045	0.173	3.845	0.238
South Australian Clay Loam	5.8	1.935	0.129	2.433	0.105	2.986	0.100	3.773	0.053
	6	1.829	0.132	2.308	0.109	2.859	0.103	3.638	0.051
	7	1.922	0.235	2.409	0.234	2.960	0.240	3.736	0.207
	0.7	2.460	0.001	11.029	3.010	22.805	6.601	23.884	6.470
	0.9	2.534	0.012	11.146	2.541	22.669	5.546	23.759	5.473
	1	2.559	0.031	11.114	2.336	22.512	5.219	23.648	5.193
	2	2.539	0.092	10.571	1.664	21.622	3.978	22.700	4.064
	2.45	2.404	0.044	10.388	1.499	21.246	3.807	22.265	3.870
	3	2.493	0.009	10.351	1.301	21.034	3.735	22.047	3.793
	4	2.479	0.001	10.182	1.192	20.573	3.791	21.623	3.880
	5	2.425	0.052	9.945	1.243	20.121	4.092	21.173	4.259
	5.8	2.477	0.018	9.924	1.136	19.842	4.221	20.890	4.356
	6	2.407	0.054	9.845	1.081	19.673	4.260	20.675	4.408
	7	2.434	0.039	9.783	1.129	19.283	4.535	20.292	4.677

Table 1). This is evident in the dielectric properties of this soil, which are much lower than those of the other soils (Figure 2).

The dielectric loss factor of the Dookie Clay soil rises considerably as frequency drops. This is probably due to the ionic conductivity of this soil, associated with its higher electrical conductivity of 2.1 dS m^{-1} (Table 1), compared with the other soils. Ionic conductivity, according to equation (7), is inversely proportional to angular frequency (ω), which implies that it is higher at low frequencies and lower at higher frequencies and is consistent with these data. The soil from South Australia, has the next highest soil conductivity (1.6 dS m^{-1}) and exhibit a moderate rise in the dielectric loss factor at lower frequencies, especially at higher moisture content.

Table 4. List of parameters used to model the dielectric properties of all four soils tested during this study.

Parameter	Soil type			
	Dookie Clay	Dookie Loam	Dookie Sand	South Australian Clay Loam
ϵ_∞	8.886	9.133	3.181	11.890
ϵ_s	20.570	21.920	4.775	23.260
α	0.210	0.782	0.000	0.232
Σ	0.472	0.154	0.114	0.264
C	2.512	6.110	7.622	6.129
m_o	0.277	0.245	0.117	0.234
r^2	0.996	0.997	0.952	0.996

The moisture in soil, which is at field capacity or below, is predominantly bound water because the free water component will have mostly drained away under the influence of gravity. Boyarskii et. al (Boyarskii et al. 2002). studied the dielectric behaviour of bound water in soil and derived an empirical expression to approximate the Debye relaxation time (τ) for bound water. Their equation for the relaxation time of bound water at 27°C is:

$$\tau_{bw} = \frac{1}{-4.9648 \times 10^{24} \cdot h^2 - 3.0867 \times 10^{11} \cdot \ln(h) - \frac{7.5092 \times 10^3}{h} + 3.9121 \times 10^{19} \cdot h - 5.2036 \times 10^{12}} \quad (9)$$

where h is the thickness of the bound water film (cm). They observe that as the thickness of the water film approaches the thickness of 10 water molecules (Note: a water molecule has a diameter of approximately 2.8×10^{-8} cm), τ_{bw} approaches the relaxation period of free water. Based on equation (9), and assuming a water film thickness of approximately 10 water molecules at field capacity, it was assumed that τ_{bw} is 7.5 picoseconds. This value of τ_{bw} was used, in conjunction with equations (6), (7), and (8), to develop a model for the dielectric properties of these soils, between 700 MHz and 7,000 MHz.

The best moisture variation function ($F(m)$) that would fit the measured data was:

$$F(m) = \text{erf}[-c(m-m_o)] \quad (10)$$

where $\text{erf}(x)$ is the Gaussian error function of the parameter x , c is a parameter associated with the specific soil, m is the fractional moisture content of the soil, and m_o is a threshold moisture content associated with each specific soil. Therefore, in this study, the dielectric constant and loss factors of these soils are adequately described by:

$$\epsilon' = \epsilon_\infty + \frac{(\epsilon_s - \epsilon_\infty) \left[1 + (\omega\tau)^{1-\alpha} \sin\left(\frac{\alpha\pi}{2}\right) \right]}{\left[1 + 2(\omega\tau)^{1-\alpha} \sin\left(\frac{\alpha\pi}{2}\right) + (\omega\tau)^{2(1-\alpha)} \right]} \cdot \text{erf}[-c(m-m_o)] \quad (11)$$

and

$$\epsilon'' = \left\{ \frac{(\epsilon_s - \epsilon_\infty)(\omega\tau)^{1-\alpha} \cos\left(\frac{\alpha\pi}{2}\right)}{\left[1 + 2(\omega\tau)^{1-\alpha} \sin\left(\frac{\alpha\pi}{2}\right) + (\omega\tau)^{2(1-\alpha)} \right]} + \frac{\sigma}{\omega\epsilon_o} \right\} \cdot \text{erf}[-c(m-m_o)] \quad (12)$$

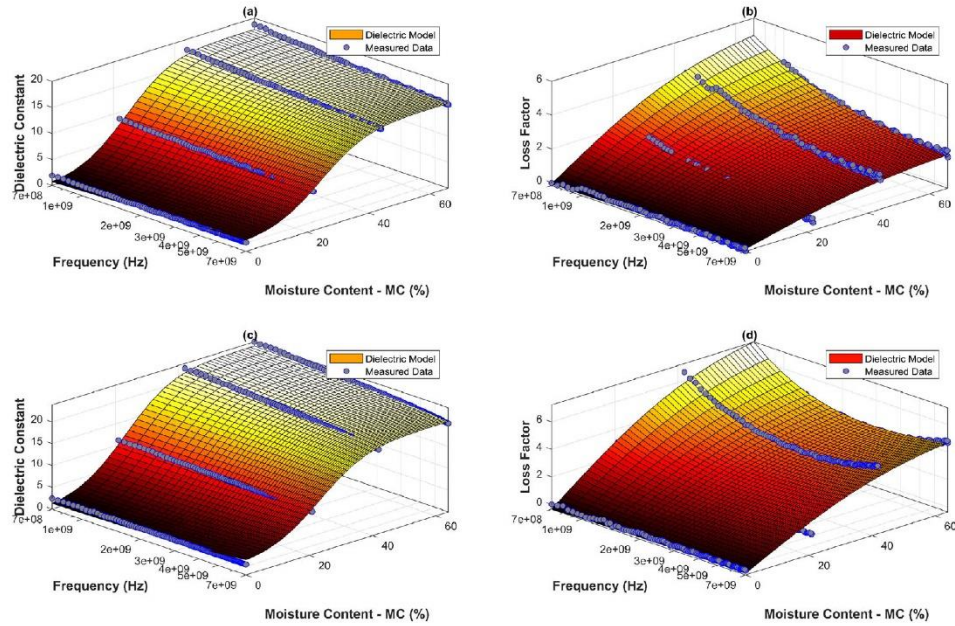


Figure 3. Comparisons between the predicted and measured dielectric properties for (a–b) Dookie Loam Soil and (c–d) South Australian Clay Loam Soil, as a function of frequency and moisture content.

For example, Figure 3 shows surface plots for the dielectric constant and loss factors for the Dookie Loam Soil and the South Australian Clay Loam soil, as a function of frequency and moisture content. Table 4 provides a complete list of parameters for the model equations for all four soils. The goodness of fit (r^2) for these models varies between 0.952 for the Dookie Sand Soil to 0.997 for the Dookie Loam Soil, suggesting that these models are adequate to describe the dielectric properties of these soils over the range of frequencies and moisture contents assessed during this study.

A more linear response to moisture content may have been expected during this study. During the measurement process, it was essential to apply some pressure onto the dielectric probe to make good contact between the end of the probe and the soil. Moisture acts as a lubricant within soil, sliding the particles together and increasing the bulk density of moist soil (Anonymous 2011), which will have an impact on the dielectric properties. Optimal soil compaction occurs below field capacity; therefore, the measurements at 66% of field capacity may have been higher than they should have been, due to the influence of some soil compaction of the moist soil under test.

Based on the models for dielectric properties of the different soils, as a function of both frequency and moisture content, it is possible to model the expected penetration depth of electromagnetic waves in these soils (Figure 4). Because penetration depth is a function of frequency and dielectric properties, and the dielectric properties are a function of soil moisture content, it is clear that penetration decreases with both frequency and moisture content. Low frequencies penetrate further into the soils than higher frequencies. Similarly, dry soils allow further penetration than moist soils, because their dielectric properties and therefore the amount of electromagnetic field attenuation are lower.

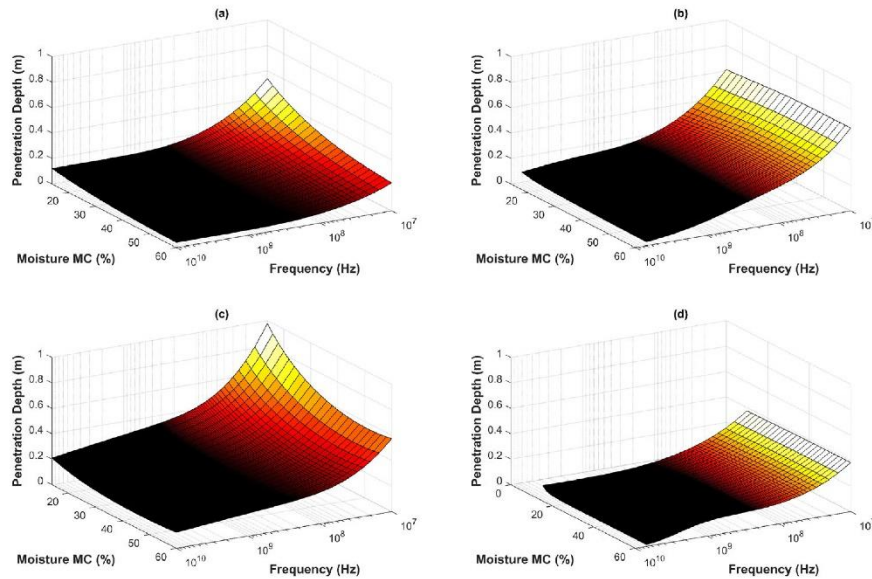


Figure 4. Comparisons between the electromagnetic penetration depths for (a) Dookie Clay Soil, (b) Dookie Loam Soil, (c) Dookie Sandy Soil and (d) South Australian Clay Loam Soil, as a function of frequency and moisture content.

It is also evident from Figure 4 that soil texture has some influence over electromagnetic penetration into these soils, because Dookie Sand Soil (Figure 4c) has a much greater electromagnetic penetration at 10^7 Hz and 14% moisture than for example the South Australian Clay/Loam Soil at the same frequency and moisture content.

4. Conclusions

The dielectric properties of four soils have been measured over a decade of frequencies, which cover three important ISM frequencies. The data clearly shows the influence of soil particle distribution (i.e., soil classification), soil electrical conductivity, and soil moisture. An adequate mathematical model has been developed for the measured data. This model can be used in future studies of these soils.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Addition of the reviewer suggestions in the published manuscript

Introduction: 5th paragraph, 6th line

There have been several studies of the dielectric properties of soil. Some of these are particularly focused on interpreting remotely sensed data (Tikhonov 1997; Boyarskii et al. 2002), especially passive microwave emission and radar response, while others have soil heating as their focus (Iben et al. 1996); however, the industrial, scientific and medical (ISM) microwave frequencies (896/915/922MHz, 2450MHz and 5800MHz) seem to have been neglected in most of these studies. Thus, the dielectric behavior of soil, at these ISM frequencies, is becoming important. However, some studies used ISM MW frequencies at 2.45 GHz for the processing of oil contaminated drill cuttings where they used MW treatment system for thermal desorption and pyrolysis of oil contaminated drill cuttings (Robinson et al., 2008b), remediation of oil-contaminated drill cuttings (Shang et al., 2006; Robinson et al., 2009a), and stripping of oil contaminated drill cuttings (Robinson et al., 2008a). Also, the same MW frequency (2.45 GHz) used for the remediation of organic pollutant (e.g PAHs) of contaminated soils (Robinson et al., 2009b; Robinson et al., 2012).

Introduction: 10th paragraph, 3rd line

The cavity resonator approach is suitable for frequencies between 50 MHz to 100 GHz and applicable for material with low loss.

Chapter 4 – Microwave soil treatment alleviates arsenic phytotoxicity and reduce wheat grain arsenic concentration

4.1 Abstract

Arsenic (As) contamination in soil and accumulation in food crops has raised much concern worldwide due to its phytotoxicity and possible risk to human health. This study was conducted to determine whether microwave (MW) soil treatment could alleviate As phytotoxicity and reduce wheat grain As concentration or not. Experimental soils were spiked to five levels of As concentration (As-0, As-20, As-40, As-60 and As-80 mg kg⁻¹) prior to apply three levels of MW treatment (MW-0, MW-3, and MW-6 minute). Plant growth and grain yield parameters and grain total As concentration were determined to evaluate the MW treatment effect. Higher plant growth and grain yield was recorded in MW treatments compared with the control treatment. For instance, significantly higher grain yield (28.95 g pot⁻¹) was recorded in MW-6 treatment compared with MW-0 (22.03 g pot⁻¹) at the same soil As concentration (As-40). Grain As concentration increased significantly with increasing of soil As concentration. However, significantly lower grain As concentration was observed in MW treatments than the control treatment. For example, the highest grain As concentration (710.45 µg kg⁻¹) was recorded in MW-0 treatment whereas, it was significantly lower in the MW-6 treatment (572.03 µg kg⁻¹). Hence, MW soil treatment has the potential to alleviate As phytotoxicity by enhancing wheat plant growth and grain yield, which also helps to reduce the grain As concentration. Ultimately, MW soil treatment will reduce As bioaccumulation in the human body even if wheat is grown in As contaminated soil. Nevertheless, further validation experiments are needed to explore the effectiveness of MW treatment on the other cereal crops (e.g. Rice) with different type of soils in field condition.

Keywords: Microwave, Soil heating, Arsenic phytotoxicity mitigation, Wheat, Grain arsenic

4.2 Introduction

Arsenic (As) is the most devastatingly toxic heavy metal and is raising global concerns for sustainable agriculture and human health, due to its ultimately toxic effect, persistence in nature, and ability to bio-accumulate in the ecosystem (Naidu et al., 2006). Inorganic As is considered to be a Group I human carcinogen and responsible for different types of cancer (Abdul et al., 2015). It is estimated that 220 million people, worldwide, are exposed to elevated concentrations of As in drinking water, which are above the World Health Organization (WHO) standard limit (10 mg l^{-1}) (Podgorski and Berg, 2020). In addition to geogenic sources of As, which mainly contaminate drinking water, it can build-up in soil because of long-term excessive use of As contaminated irrigation water and ultimately results in As uptake by crops (Williams et al., 2006). Thus, the presence of As in the food chain, through the water-soil-crop pathway is triggering concerns about human health (Naidu et al., 2006). Excessively high As pollution in water, soil and crops has already been identified in many countries (Shankar and Shanker, 2014; Singh et al., 2015b; Kabir et al., 2016; Upadhyay et al., 2019). Even though rice is a good accumulator of As, concerns are mounting about the amount of As being found in other crops, like vegetables, tubers, fruits, and even wheat (Chandiramani et al., 2007).

Although there is no worldwide standard safe limit of As in food grains, the European Commission recently (January 2016) set the maximum limits for As in milled rice (polished or white rice) as $200 \text{ } \mu\text{g kg}^{-1}$ (Union, 2015). A wheat field experiment with a 12.00 mg kg^{-1} soil As, reported $2.00 - 17.00 \text{ } \mu\text{g kg}^{-1}$ of As in grain samples (Zhao et al., 2007). While $5.00 - 285.00 \text{ } \mu\text{g kg}^{-1}$ (Wiersma et al., 1986), $4.00 - 362.00 \text{ } \mu\text{g kg}^{-1}$ (Roychowdhury et al., 2002), and $1.00 - 500.00 \text{ } \mu\text{g kg}^{-1}$ of As were also reported in wheat grain collected from an As contaminated site, where soil As concentration ranged from $3.00 - 201.00 \text{ mg kg}^{-1}$ (Williams et al., 2007). Thus, besides rice, wheat could be a major source of dietary As. Wheat is the second most-produced (771.72 million tonnes) cereal crop throughout the world, with the highest harvested area being 218.54 million ha (FAO, 2017). Therefore, to feed the raising global population, wheat will stay as a vital component of human nutrition. Hence, increasing its quality of production, free from toxic heavy metals, is an important requirement for sustainable agriculture and food security. Therefore, As remediation techniques, not only for drinking water but also for soil, are crucial to avoid food As contamination through crop uptake.

Different physical, chemical, and biological techniques are being used for remediation of As

contamination in soil. These remediation methods include vitrification, electrokinetic treatment, soil flushing and solidification and phytoremediation by hyper accumulative plants etc. (Yang et al., 2007). Hitherto, these methods have been frequently revealed to be ineffective, costly or too lengthy, with usage being restricted to smaller-scale operations with lower efficiency, selectivity, and disposal of materials after remediation (Lim et al., 2014). Thus, alternative options or combinations of technologies, for reducing soil As pollution, are required.

Recent research has revealed that pre-sowing microwave (MW) soil heating application in agricultural systems is a promising technique, which not only has potential to control weeds by deactivating the soil weed seed bank (Brodie et al., 2017; Jamal et al., 2017) but it can also significantly increase crop growth and yield of rice and wheat by increasing some soil nutrients (N, P, K and S) availability as a results of soil humification processes and nutrient recovery from dead microorganisms after expose to MW heating (O'Brien et al., 2018; Khan et al., 2019b; Maynaud et al., 2019). Microwave energy is a form of electromagnetic radiation, with wavelengths ranging from 1 m to 1 mm and frequencies between 300 MHz to 300 GHz, which can induce the rotation of the dipoles of polar molecules (e.g. water), due to the oscillating electromagnetic field, which results in the generation of heat by intermolecular friction (Falciglia et al., 2016). This produces a fast heating rate, since soil moisture is considered to be an efficient absorber of MW radiation (Kabir et al., 2020). Thus, MW heating has major advantages over other heating processes. These advantages include short start-up, selective heating, precise control, no direct contact with heated materials, and volumetric heating (Menéndez et al., 2010; Zhou et al., 2010). Because of these advantages MW has been using in diversified fields including removal of organic contaminants (Falciglia and Vagliasindi, 2014) and immobilization of some toxic metals (Cu, Mn, Th, Zn, Ni, Cd, Cr, and Pb) in soil (Abramovitch et al., 2003) and solid sediments (Chen et al., 2005a; Hsieh et al., 2007). However, no study has been found to address soil As immobilization using MW heating to alleviate As phytotoxicity. Therefore, this study was designed to explore whether MW soil treatment could alleviate As phytotoxicity and reduce wheat grain As concentration or not.

4.3 Materials and methods

4.3.1 Experimental site, soil collection and preparation

Experimental soils were collected from a wheat production paddock of the Dookie agricultural farm (36°37' S; 145°70' E) at a depth of 0 – 15 cm. The soil was a brownish grey loam and classified as a Major Clay Loam (Downes, 1949) or a red mesotrophic-haplic dermosol (Isbell, 2016). Some important soil properties are given in the Table 4.1. The collected soils were dried and sieved through a 2 mm mesh to minimize the undesired effects of stones, sticks and clods. This operation did not reflect the true field situation, where the distribution of coarse material is highly irregular; however, it was essential to ensure a uniform experimental condition for MW soil heating. After sieving, 8.5 kg of soil was thoroughly mixed and shifted into pots (diameter 27 cm and height 30 cm). Unperforated pots were used to prevent the loss of water soluble As from the pots (Rahman et al., 2007b).

4.3.2 Physicochemical properties of soil

Soil samples were sent to the Nutrient Advantage Laboratory, a NATA (National Association of Testing Authorities, Australia) accredited laboratory (Lab number: 11958, ISO/IEC 17025), for analysis of soil properties. The physicochemical properties of the soil are presented in Table 4.1.

4.3.3 Arsenic application

Five different levels of As concentration (0, 20, 40, 60 and 80 mg kg⁻¹ soil) as sodium arsenate heptahydrate (Na₂HAsO₄·7H₂O) (Bhattacharya et al., 2015) were mixed with the initial soil. Respective amounts of sodium arsenate were mixed with deionized water to prepare the As solutions. Then, the As solution was mixed with the soil by spraying and homogenizing thoroughly by hand mixing. The background As concentration in soil varies depending on the extent of As pollution in that area. For example, in highly naturally contaminated agricultural soil, the concentration of As in Bangladesh is 20 – 83 mg kg⁻¹ (Ullah, 1998), in China it is 40 – 70 mg kg⁻¹ (Hua et al., 2005), and in India it is 9 – 105 mg kg⁻¹ (Patel et al., 2005). Therefore, the As treatments in this study represented the different extent of contamination from several countries. To establish an equilibrium condition between soil and applied As, soil moisture was maintained at field capacity for two weeks prior to applying the MW treatment.

Table 4.1. Physicochemical properties of pre and post microwave (MW) treated soils before sowing

Soil properties	Analytical method	Units	Microwave treatments		
			MW-0	MW-3	MW-6
Organic carbon (OC)	Walkley & Black	%	1.41	1.35	1.34
Organic matter (OM)	Walkley & Black	%	2.43	2.32	2.30
Electrical conductivity (EC)	Saturated extract	dS/m	1.00	1.20	1.70
pH	1:5 CaCl ₂	N/A	5.60	5.60	5.60
Cation exchange capacity (CEC)	BaCl ₂ exchange	cmol(+)/kg	10.10	9.31	9.29
Nitrate nitrogen (NO ₃ ⁻ -N)	Kjeldahl	mg kg ⁻¹	49.00	45.00	43.00
Ammonium nitrogen (NH ₄ ⁺ -N)	Kjeldahl	mg kg ⁻¹	150.00	230.00	310.00
Available Potassium (K)	Atomic emission	mg kg ⁻¹	610.00	610.00	600.00
Sulphur (S)	0.25M KCl at 40°C	mg kg ⁻¹	12.00	18.00	39.00
Phosphorus (P)	Colwell	mg kg ⁻¹	120.00	170.00	190.00
Calcium (Ca)	Ammonium acetate	cmol(+)/kg	6.70	6.00	5.80
Magnesium (Mg)	Ammonium acetate	cmol(+)/kg	1.80	1.80	1.80
Potassium (K)	Ammonium acetate	cmol(+)/kg	1.60	1.60	1.50
Sodium (Na)	Ammonium acetate	cmol(+)/kg	< 0.02	0.03	0.06
Aluminium (Al)	Ammonium acetate	cmol(+)/kg	< 0.10	< 0.10	0.11
Copper (Cu)	DTPA	mg kg ⁻¹	5.10	4.70	4.80
Zinc (Zn)	DTPA	mg kg ⁻¹	4.80	4.60	5.00
Manganese (Mn)	DTPA	mg kg ⁻¹	58.00	62.00	72.00
Iron (Fe)	DTPA	mg kg ⁻¹	130.00	120.00	120.00
Boron (B)	DTPA	mg kg ⁻¹	0.78	0.77	0.85
Silicon (Si)	CaCl ₂ Soluble	mg kg ⁻¹	80.00	82.00	110.00
Arsenic	HG-AFS	µg kg ⁻¹	< 0.01	< 0.01	< 0.01

4.3.4 Microwave application

Three levels of MW energy were applied for 0, 3 and 6 minutes to attain soil temperatures of around room temperature, 60 and 90 °C, respectively. The duration of MW irradiation to heat the soil at the desire temperature was determined by following the method of previous research work (Khan et al., 2016). Soil heating at around 90 °C has been found to be effective for controlling weed infestations and destroying weed seed banks in the top soil without significantly changing the soil properties (Khan et al., 2016). Therefore, the same soil heating temperature was also used in this experiment to explore the potentiality in As phytotoxicity alleviation. Soil heating at around 60

°C was included as an intermediate treatment between 90 °C and control. A MW chamber, consisting of six magnetrons (1 kW each), operating at a frequency of 2.45 GHz, was used for soil treatment (Figure 4.1 and Appendix 4.1). Energy dissipated in the soil sample after MW treatment (Figure 4.1b), and chamber electric field (Figure 4.1c) was modeled. The modelling was done using XFDTD software version 7.9 produced by Remcom (USA). Figure 4.1 (b) illustrates the microwave's electric field distribution in the soil sample only, which Figure 4.1 (c) illustrates the microwave's electric field distribution throughout the whole chamber. Both illustrations are on a vertical plane that passes through the center of the soil sample and the soil sample was in the center of the chamber, resting on the floor of the chamber. The soil temperature was measured for each MW treatments at a depth of 10 – 15 cm, immediately after MW energy exposure, by using liquid-in-glass thermometers (Cooper and Brodie, 2009). An infrared camera was also used for taking thermal images to show the energy dissipated and temperature distribution across the MW treated soil. Due to the very high dependence of the dielectric properties on moisture content (Kabir et al., 2020), the moisture content in the soil will greatly affect the heating effect of MW energy on the soil. In this experiment, the moisture content was maintained at around 14 % (w/w) at the time of MW soil treatment.

4.3.5 Experiment setup

The experiment was conducted in a glasshouse, at Dookie campus, The University of Melbourne, Australia, by following a completely randomized design (CRD) with four replications. To describe the treatment combination more conveniently, abbreviated forms have been used for As treatments (As-0, As-20, As-40, As-60 and As-80) and MW treatments (MW-0, MW-3 and MW-6). Before the seed sowing, mono ammonium phosphate (MAP) fertilizer was applied (equivalent to 150 kg ha⁻¹) to each pot as a basal dose, as per standard practices for Australian wheat cultivation. The rest of the N requirement was calculated (based on total 150 kg N ha⁻¹) and applied, as urea, in two split doses viz. at early stem elongation (GS30-32) stage and booting (GS45-49) stage. Twelve seeds of the EGA Gregory wheat variety (*Triticum aestivum* L.) were sown per pot on the 6th of June 2017. Tap water was used for crop irrigation purposes. This water source contained As below the detection limit (< 0.01 µg l⁻¹); thus, there was no possibilities of As addition from the tap water to the pot soil. After 180 days of the growing period, at the physiological maturity stage, the crop was harvested on the 5th of December 2017.

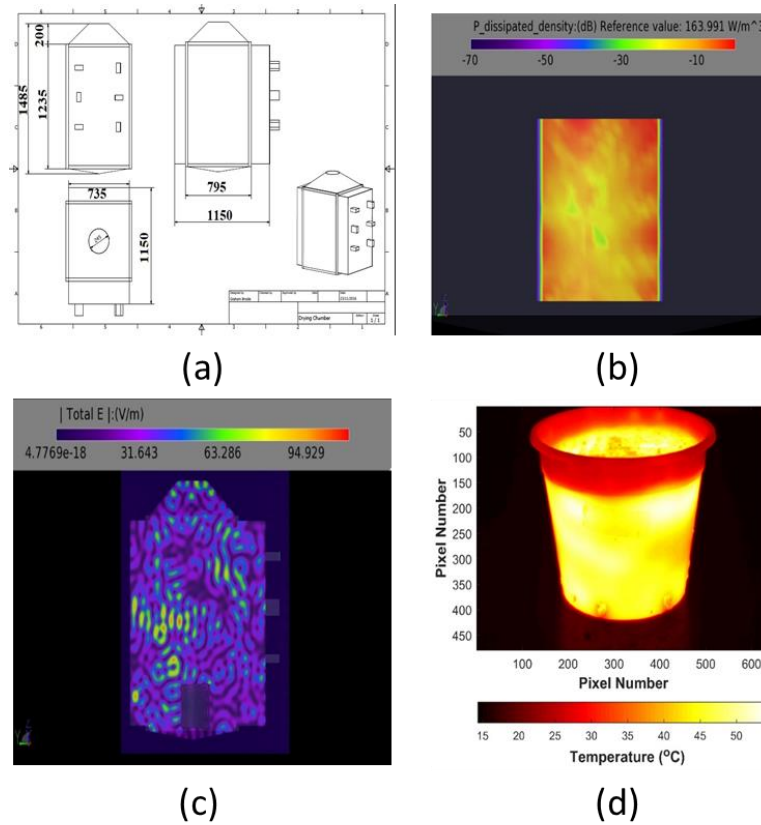


Figure 4.1. (a) Schematic diagram of 6-kW microwave (MW) chamber (internal capacity of approximately 1.0 m³) (Harris et al., 2011), (b) energy dissipated in the soil sample after MW treatment, (c) chamber electric field, and (d) thermal images captured with an infrared camera (FLIR C3) after 3 min of MW irradiation of soil showing the temperature of 60 ± 5 °C

4.3.6 Recording of crop agronomic data

The plant height was measured as distance from the soil surface to the top of a plant using a measuring scale. Plant vigor data was recorded by an ordinal scale ranging from 1 (low vigor) to 9 (high vigor). Leaf chlorophyll content was measured as SPAD (Soil-Plant Analysis Development) value using the Chlorophyll Meter-SPAD-502Plus (Yuan et al., 2016) at the tillering stage. To get the plant height, plant vigor and leaf chlorophyll content data, five plants within a pot were selected randomly and data recorded as mean value of these five plants. At the tillering stage, plant samples (3 hills per pot) were collected to determine the shoot biomass and measure the leaf area, width and length of last fully expanded leaf by using leaf area meter (LASER Leaf Area Meter, CI-202, CID Bio-Science, USA). At the physiological maturity stage, the crop was harvested and shoot biomass, total number of spikes, root biomass, and grain yield were recorded. Both the shoot and root samples were dried at 60 °C in an oven for 48 hours to determine the dry biomass.

4.3.7 Grain total arsenic analysis

Grain total As analysis was performed as per the method described in the user manual of atomic fluorescence spectrometry (AFS; PSA 10.055 Millennium Excalibur, 2009) (Analytical, 2009). Since the method is generalized for solid materials, some modifications were made for the wheat grain As analysis. The modifications were, (i) a 0.5g sample used for analysis instead of 0.25g because, generally wheat grain As concentration is lower than in soil; (ii) heating time was extended up to 90 – 100 minutes until a clear solution appeared (as an indication of good digestion) whereas 40 minutes was suggested in the original method; and (iii) digested liquid was filtered with Whatman 42 (ashless, 2.7 μm) filter paper as it is better than the 541 (ashless, 20 – 25 μm) and usually used in heavy metal analysis.

4.3.7.1 Sample preparation

The whole grain sample was oven dried at 105 °C for 24h prior to grinding and homogenizing with the ultra-centrifugal mill (RETSCH, ZM 200) (Chen et al., 2014). The powdered sample was stored in a polypropylene pot for further analysis. Sample digestion was performed by hydrochloric-nitric ($\text{HCl}:\text{HNO}_3=3:1$) di-acid (aqua regia) with a block digester (VELP Scientifica, DK-42). For pre-digestion, a 0.5 g powdered sample was taken into a 100 ml digestion tube ($\varnothing$ 26 mm). After that, 12 ml of concentrated HCl (37 %, 12 M) and 4 ml of concentrated HNO_3 (70 %, 15.8 M) was added and left overnight to allow the vigorous initial reaction to subside. Excessive foaming was reduced by adding 2 – 3 drops of n-dodecane into the mixture. The mixture was heated at 140 °C for 90 – 100 minutes until the appearance of a clear solution. After cooling the tube, the liquid was filtered with Whatman 42 filter paper.

4.3.7.2 Atomic fluorescence spectrometry

Total As analysis was performed using atomic fluorescence spectrometry (AFS; PSA 10.055 Millennium Excalibur) (Analytical, 2009). Prior to total As determination, all the samples were pre-reduced with potassium iodide (1 % m/v) and ascorbic acid (0.2 % m/v) to reduce As(V) to As(III) . For analysis standard preparation, the $1000 \pm 2 \text{ mg l}^{-1}$ CRM (certified reference material) As standard supplied in 2 % HNO_3 (Sigma-Aldrich) was used as a standard stock solution. A working standard solution of 10 mg l^{-1} was prepared weekly from the stock solution and used to prepare calibration standards (0 – 10 $\mu\text{g l}^{-1}$). The standards and samples were prepared by following the same analytical matrix of 25 % (v/v) HCl, 1 % (m/v) potassium iodide, and 0.2 %

(m/v) ascorbic acid. Sodium tetra hydroborate (0.7 % m/v in 0.1 mol l⁻¹ NaOH) was continuously added to the sample during the analysis to produce gaseous arsine (AsH₃), which was atomized using a hydrogen diffusion flame. The overall reactions are represented in the following Equations (4.1) to (4.4) (Iqbal et al., 2019). Atomic fluorescence was measured after excitation using an As boosted discharge hollow cathode lamp (Photron) (Warren, 1993). The operating states of AFS for As determination are given below in Table 4.2.



Table 4.2. Operating environment of atomic fluorescence spectrometer (AFS) for total arsenic analysis (Analytical, 2009)

Properties	Unit	Value
Carrier gas (Ar) flow rate	l min ⁻¹	0.25
Carrier gas pressure	Psi	35 – 45
Dryer gas (H ₂) flow rate	l min ⁻¹	2.50
Dryer gas pressure	Psi	35 – 45
NaBH ₄ concentration in 0.1 mol l ⁻¹ NaOH	% (m/V)	0.70
HCl concentration for hydride generation	mol l ⁻¹	3.00
NaBH ₄ flow rate (reductant)	ml min ⁻¹	4.50
HCl flow rate (reagent blank)	ml min ⁻¹	9.00
Sample flow rate	ml min ⁻¹	9.00
Lamp current	Ma	27.50 (primary), 35.00 (boost)
Lamp wavelength	Nm	197.30
Analysis period	Sec	15 (delay), 30 (analysis) and 30 (memory)
Lower limit of detection (LOD)	ng l ⁻¹	10.00

4.3.7.3 Quality assurance of arsenic analysis

For quality control, the appropriate procedures and safety measures were taken to ensure the consistency of the results by following the techniques described by Thompson and Walsh (Thompson, 2012). Samples were handled carefully to avoid cross contamination. All glassware was cleaned with the laboratory dish washing machine followed by a 10 % HNO₃ solution and rinsing with deionized water. High purity analytical grade chemicals and gases (99.99 % pure) were used for the analysis to ensure the minimal blank concentration value. Deionized water was used for all dilutions and preparation of chemicals during the analysis. To ensure good recovery of sample As, a 1568b rice flour standard reference material (SRM), from NIST (National Institute of Standard and Technology), was used at the time of digestion. Therefore, the block digestion set consisted of one blank, one SRM, one duplicate and with the remaining 39 tubes being the main samples with three replications each. Data was deemed to be acceptable if recovery of SRM As was ± 10 % and the calculated relative standard deviation (RSD) of duplicate samples was no greater than 5 %. To provide measurement clarification regarding the response of the Millennium Excalibur, the background equivalent concentration (BEC) was calculated using the following Equation (4.5) to determine the performance of the instrument.

$$BEC = \frac{\text{Background value}}{\text{Peak height}} \times \text{standard concentration} \quad (4.5)$$

The lower the BEC value the more sensitive the instrument. If the BEC value was below 0.5, the instrument was considered to be operating correctly.

4.3.8 Statistical analysis

Statistical analysis of recorded data was performed using GenStat (16th Edition, VSN International) software. Normality and homogeneity of variance of the experimental data was tested. The analysis of variance (ANOVA) test was performed to determine the significance of tested treatments on variables. The Least Significant Difference (LSD) test was performed to compare the treatments' means at 5 % level of significance. The Pearson correlation test was performed to determine the correlation coefficient among the variables. For grain As concentration data, Grubb's test was performed to identify outlier values, which were replaced by the other replicates' average values, if found. After MW soil heating, thermal images were captured with an infrared camera (FLIR C3) and post-processed in MATLAB (MathWorks, Inc., USA) software.

4.4 Results

4.4.1 Plant growth and grain yield

The results revealed that the addition of As to the soil had a significant negative impact on plant growth and grain yield, and MW soil treatments provided a beneficial effect compared with non-MW treated soils irrespective of soil As concentration. To describe the plant growth some growth parameter results are given below.

4.4.1.1 Plant height

Plant height decreased significantly ($p < 0.001$) with increasing soil As concentration. This trend was observed up to 60 days after sowing (DAS) of plant growth. After that, the effect of As on plant height was not statistically significant. Plant height increased significantly ($p < 0.001$) in MW treatments irrespective of soil As concentration throughout the growing period. Higher plant height was recorded in MW-6 compared with MW-3 and MW-0 treatment (Table 4.3).

Table 4.3. Mean plant height (cm) in response to microwave (MW) soil heating and soil arsenic (As) treatments

Soil As (mg kg ⁻¹)	30 DAS			60 DAS			90 DAS		
	MW soil treatments (minutes)								
	0	3	6	0	3	6	0	3	6
0	35.46 ^{ab}	35.87 ^a	37.65 ^a	43.25 ^{bcd}	45.85 ^{ab}	46.40 ^{ab}	68.60 ^{ab}	66.65 ^{abc}	71.20 ^a
20	34.74 ^{ab}	36.00 ^a	38.22 ^a	43.45 ^{bcd}	45.10 ^{abc}	45.45 ^{ab}	61.50 ^{bcd}	68.40 ^{ab}	71.25 ^a
40	34.47 ^{bc}	34.96 ^{ab}	37.69 ^a	44.30 ^{bcd}	45.25 ^{ab}	47.70 ^a	61.95 ^{bcd}	65.85 ^{a-d}	72.55 ^a
60	32.40 ^{cd}	33.15 ^{cd}	36.57 ^{ab}	41.95 ^d	44.35 ^{bcd}	46.20 ^{ab}	60.35 ^{cd}	67.35 ^{abc}	69.40 ^{ab}
80	28.40 ^e	30.49 ^d	35.57 ^{ab}	38.10 ^e	42.10 ^{cd}	45.50 ^{ab}	58.00 ^d	65.20 ^{a-d}	73.25 ^a
LSD _{0.05}	2.09			1.36			6.96		

Mean data with superscript same letter are not significantly different. Least Significant Difference (LSD) test performed at 5 % level of significance to determine the difference between the arsenic (As) and microwave (MW) treatments. DAS (Days after sowing) indicates the sampling time.

4.4.1.2 Plant vigor

With the increasing of soil As concentration, plant vigor decreased significantly ($p < 0.001$), while significantly ($p < 0.001$) higher plant vigor was found in the MW treatments. For instance, at

As-80 the plant vigor was lowest (4.00) in MW-0, whereas significantly higher plant vigor was observed in MW-6 (7.00) treatment (Figure 4.2a).

4.4.1.3 Leaf chlorophyll content

Leaf chlorophyll content increased significantly ($p < 0.001$) in the MW treatments compare with the control. For example, at As-80 significantly higher leaf chlorophyll content (45.88) was recorded in MW-6 treatment compared with MW-0 treatment (39.10). In the MW-6 treatment, no significant changes observed in chlorophyll content across all soil As concentration. Although the effect of soil As concentration on the leaf chlorophyll content was not significant ($p = 0.187$), a decreasing trend was observed in the MW-0 treatment (Figure 4.2b).

4.4.1.4 Tiller number

Tiller number reduced significantly ($p < 0.001$) across the treatments with increasing soil As concentration. However, a significantly ($p = 0.005$) higher tiller number was obtained in the MW treatments. This was especially so in the MW-6 treatment where the tiller number was higher than the MW-0 and the MW-3 treatment. The highest tiller number (26.25) was recorded in the MW-6 treatment at As-20 soil As concentration, while it was 21.75 and 18.75 in MW-3 and MW-0 treatment respectively (Figure 4.2c).

4.4.1.5 Plant biomass

At tillering stage, shoot biomass was reduced significantly ($p < 0.001$) with increasing soil As concentration, while in the MW treated pots, significantly ($p < 0.001$) higher biomass was recorded. In view of the MW treatments, higher biomass was harvested from the MW-6 treatment compared with the MW-3 and MW-0 treatments (Figure 4.2d). At crop harvest stage, shoot biomass was reduced significantly ($p < 0.008$) in response to increased soil As concentration, whereas significantly ($p < 0.001$) higher biomass was recorded in the MW treatments. In the MW-6 treatment, higher biomass was harvested compared with the MW-0 and MW-3 treatment (Figure 4.2e). Like shoot biomass, similar results were observed for root biomass (Figure 4.2f).

4.4.1.6 Leaf area, width and length

With increasing soil As concentration, leaf area, width and length were reduced, although the effect was not statistically significant. On the other hand, leaf area ($p < 0.001$), width ($p < 0.001$)

and length ($p = 0.048$) increased significantly in MW soil treatments. The lowest value for all leaf parameters was found at the highest As concentration with no MW treatment, while the highest value was found in the MW-6 treatment, irrespective of soil As concentration (Table 4.4).

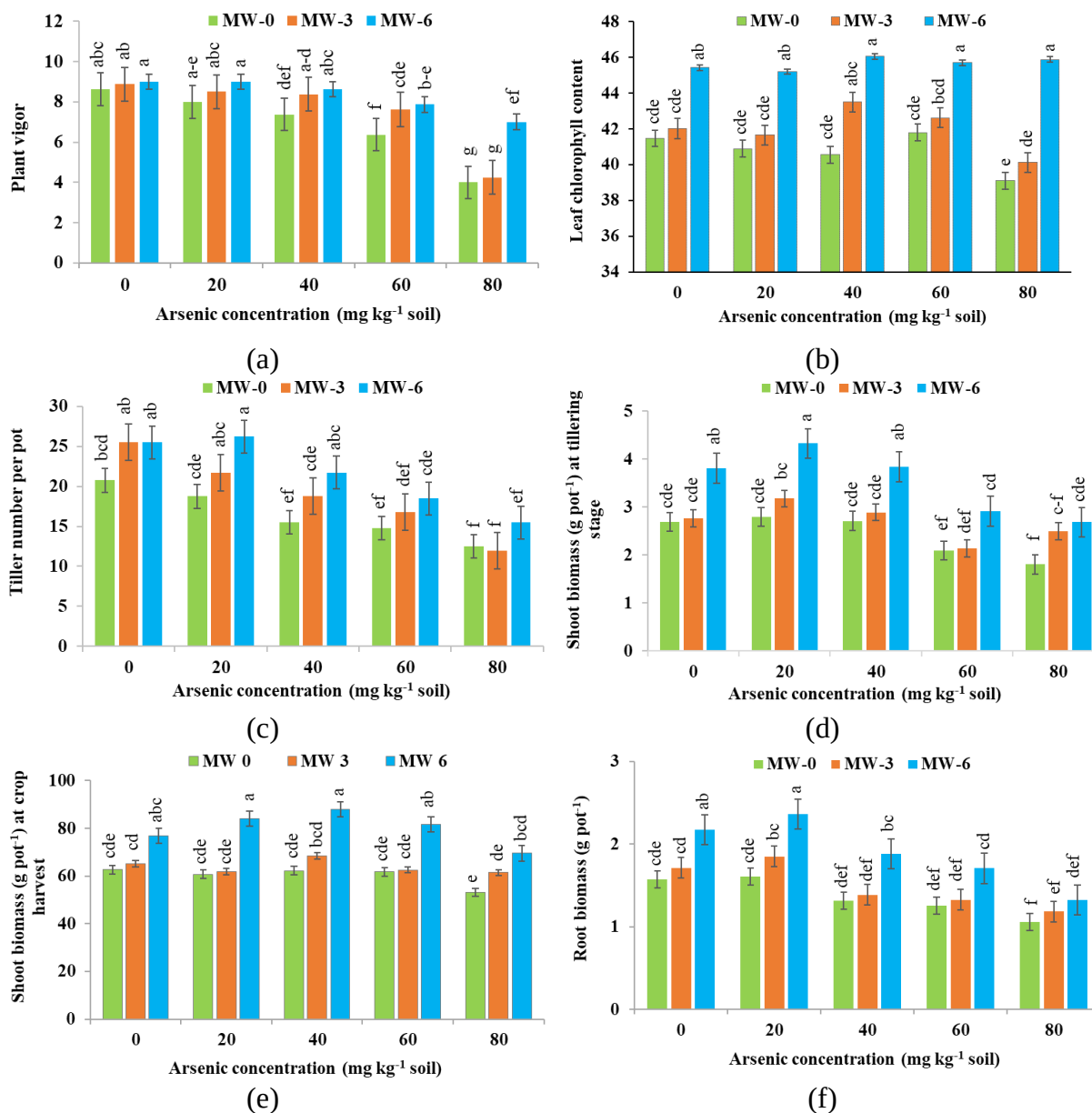


Figure 4.2. Effect of microwave (MW) soil treatment on wheat plant growth in arsenic (As) contaminated soils. (a) plant vigor, (b) leaf chlorophyll content, (c) tiller number, (d) shoot biomass at tillering stage, (e) shoot biomass at crop harvest, and (f) root biomass. Bar represent the mean value with standard error and different letter indicate the significant difference (LSD at $p = 0.05$) among the treatments

4.4.1.7 Total number of spikes

There was no significant ($p = 0.064$) effect of As on total spike number, but significantly ($p < 0.001$) higher number of spikes was found in the MW treatments. The highest spike number (17.00) was found in the MW-6 treatment, while it was 12.00 and 9.00 in MW-3 and MW-0 treatment respectively at As-20 treatment (Figure 4.3a).

4.4.1.8 Grain yield

The wheat grain yield increased significantly ($p < 0.001$) in the MW treated pots, while the effect of As on grain yield was not significant ($p = 0.210$). Higher grain yield was found in the MW-6 treatment compared with the MW-0 and MW-3 treatments. For instance, significantly higher grain yield (28.95 g pot^{-1}) was recorded in MW-6 treatment compared with the MW-3 (23.21 g pot^{-1}) and MW-0 (22.03 g pot^{-1}) treatments at As-40 treatment (Figure 4.3b).

Table 4.4. Effect of microwave (MW) treatment on leaf area, length and width at different soil arsenic (As) concentration[#]

Soil As (mg kg ⁻¹)	Leaf area (cm ²)			Leaf width (cm)			Leaf length (cm)		
	MW soil treatment (minutes)								
	0	3	6	0	3	6	0	3	6
0	21.12 ^b	20.60 ^b	24.52 ^a	1.03 ^b	1.09 ^b	1.13 ^b	25.94 ^c	25.66 ^b	27.88 ^b
20	21.12 ^b	25.04 ^a	21.99 ^b	1.10 ^b	1.12 ^b	1.11 ^b	25.12 ^b	28.91 ^b	27.26 ^b
40	20.25 ^b ^c	23.71 ^a	27.92 ^a	1.01 ^b	1.11 ^b	1.25 ^a	26.46 ^c	27.73 ^b	29.21 ^a
60	18.85 ^c ^d	22.50 ^b	27.91 ^a	0.97 ^b	1.09 ^b	1.30 ^a	25.52 ^d	26.26 ^b	29.67 ^a
80	16.51 ^e	23.62 ^a	22.24 ^b	0.82 ^c	1.14 ^a	1.03 ^b	24.98 ^e	27.16 ^b	27.10 ^c
LSD _(0.05)	4.70			0.17			4.65		

Mean data with superscript same letter are not significantly different. Least Significant Difference (LSD) test performed at 5 % level of significance to determine the difference between the arsenic (As) and microwave (MW) treatments. [#]Data recorded at tillering stage

4.4.2 Grain total arsenic concentration

Grain As concentration increased significantly ($p < 0.001$) with increasing soil As concentration while it was significantly ($p < 0.001$) lower in the MW treatments compare with the control (Figure 4.3c). At As-80 the highest grain As concentration ($710.45 \mu\text{g kg}^{-1}$) was recorded in MW-0 while, it was significantly lower ($572.03 \mu\text{g kg}^{-1}$) in the MW-6 treatment. The highest

grain As concentration reduction (37.98 %) was observed in the MW-6 treatment at As-60 followed by the MW-3 treatment (32.20 %) compared with the MW-0 treatment (Figure 4.3d).

4.4.3 Grain mineral content

Grain P ($p < 0.001$) content decreased significantly and K ($p = 0.008$) and Na ($p < 0.001$) content increased significantly with the increasing of soil As treatment, while there was no significant effect of MW treatment on grain P, K and Na content. On the other hand, Mn ($p = 0.012$) and Zn ($p < 0.001$) content decreased significantly with the increasing soil As concentration, while Mn ($p < 0.001$) and Zn ($p < 0.001$) content increased significantly in the MW treated soil compared with the control. The effect of As on grain Ca, Mg, Fe and Cu was statistically non-significant. However, grain Mg ($p = 0.012$), Fe ($p < 0.001$), and Cu ($p = 0.003$) content increased significantly while, Ca ($p < 0.001$) content decreased significantly in the MW treated soil compared with the control treatment (Table 4.5 and Table 4.6).

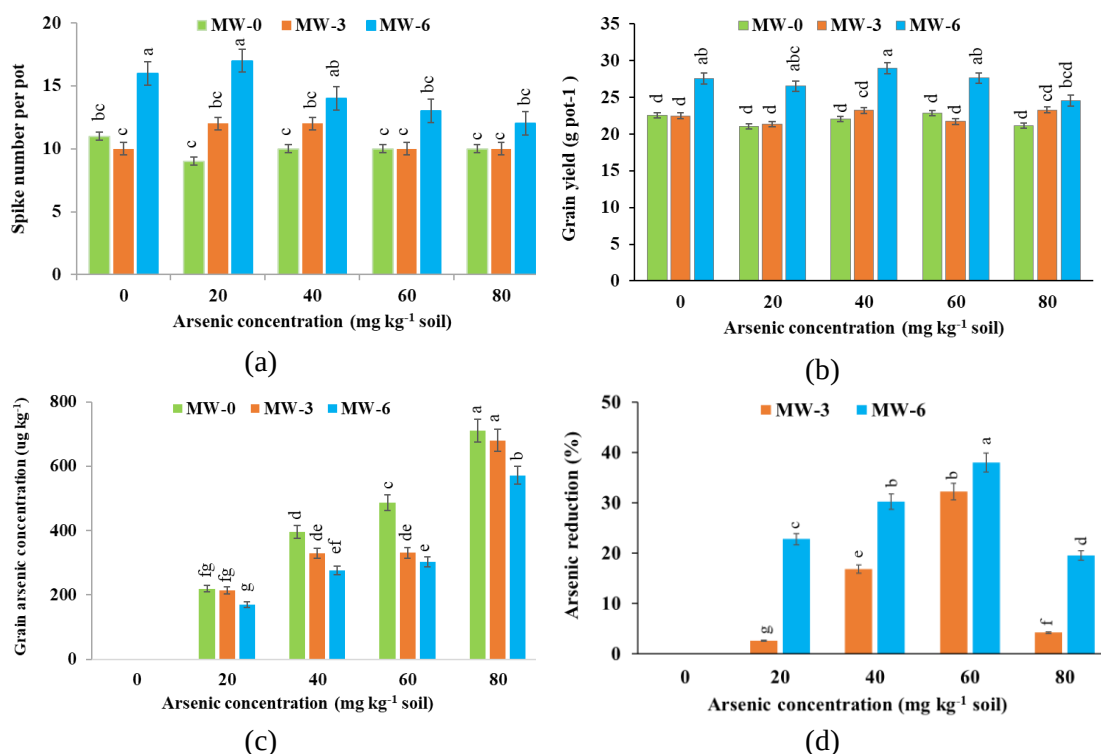


Figure 4.3. Effect of microwave (MW) soil treatment on (a) spike number, (b) grain yield, (c) grain arsenic (As) concentration in response to different soil As concentration, and (d) grain As concentration reduction in MW treatments. Bar represent the mean value with standard error and different letter indicate the significant difference (LSD at $p = 0.05$) among the treatments

4.4.4 Correlation of grain arsenic with plant growth and yield parameters and grain mineral content

Pearson's correlation coefficient (r value) showed that, all the growth parameters were positively correlated with the yield parameters and all the growth and yield parameters were negatively correlated with grain As concentration. Although the grain yield was negatively correlated with grain As concentration ($r = -0.1511$), the correlation coefficient was statistically non-significant (Table 4.7). Also, Pearson's correlation coefficient (r value) showed that, grain K ($r = 0.46^{***}$) and Na ($r = 0.48^{***}$) were positively correlated, while grain P ($r = -0.86^{***}$), S ($r = -0.34^{**}$), Cu ($r = -0.30^{*}$), Mn ($r = -0.29^{*}$) and Zn ($r = -0.62^{***}$) were negatively correlated with the grain As concentration (Table 4.8).

Table 4.5. Grain macronutrient content in response to microwave (MW) soil treatment at different soil arsenic (As) concentration

Soil As (mg kg ⁻¹)	P (mg kg ⁻¹)			K (mg kg ⁻¹)			S (mg kg ⁻¹)			Ca (mg kg ⁻¹)			Mg (mg kg ⁻¹)		
	MW soil treatment (minutes)														
	0	3	6	0	3	6	0	3	6	0	3	6	0	3	6
0	3568 ^a	3529 ^a	3571 ^a	4481 ^{bc}	4443 ^c	4543 ^{bc}	1118 ^a	1086 ^{ab}	1090 ^{ab}	341 ^a	318 ^{a-d}	309 ^{b-e}	999 ^{abc}	983 ^{bc}	1065 ^a
20	3380 ^{ab}	3355 ^{ab}	3387 ^{ab}	4497 ^{bc}	4549 ^{bc}	4490 ^{bc}	1023 ^{ab}	1098 ^{ab}	1030 ^{ab}	319 ^{a-d}	313 ^{b-e}	294 ^{def}	991 ^{abc}	1012 ^{abc}	1058 ^{ab}
40	3131 ^{bc}	3056 ^c	3016 ^c	4611 ^{abc}	4546 ^{bc}	4583 ^{bc}	1065 ^{ab}	1048 ^{ab}	992 ^b	331 ^{ab}	316 ^{a-e}	283 ^f	1029 ^{abc}	1022 ^{bc}	1019 ^{abc}
60	2881 ^c	2849 ^c	2912 ^c	4745 ^{ab}	4684 ^{abc}	4484 ^{abc}	1037 ^{ab}	1028 ^{ab}	1008 ^{ab}	321 ^{abc}	313 ^{b-e}	296 ^{c-f}	996 ^{abc}	981 ^c	1032 ^{abc}
80	2423 ^d	2431 ^d	2432 ^d	4695 ^{abc}	4819 ^a	4633 ^{abc}	1012 ^{ab}	985 ^b	1051 ^{ab}	329 ^{ab}	318 ^{a-d}	290 ^{ef}	967 ^c	969 ^{abc}	1006 ^{abc}
LSD _{0.05}	270			230			103			23			68		

Mean data with superscript same letter are not significantly different. Least significant difference (LSD) test was performed at 5 % level of significance to determine the difference between the treatments.

Table 4.6. Grain micronutrient and sodium content in response to microwave (MW) soil treatment at different soil arsenic (As) concentration

Soil As (mg kg ⁻¹)	Fe (mg kg ⁻¹)			Cu (mg kg ⁻¹)			Zn (mg kg ⁻¹)			Mn (mg kg ⁻¹)			Na (mg kg ⁻¹)		
	MW soil treatment (minutes)														
	0	3	6	0	3	6	0	3	6	0	3	6	0	3	6
0	19.00 ^{ef}	20.00 ^{def}	26.00 ^a	4.20 ^{abc}	3.90 ^{abc}	4.50 ^a	21.00 ^{cd}	22.00 ^{cd}	32.00 ^a	50.00 ^{b-e}	58.00 ^{bcd}	97.00 ^a	7.60 ^b	6.90 ^b	6.60 ^b
20	21.00 ^{b-f}	19.00 ^f	24.00 ^{ab}	3.70 ^{bc}	3.90 ^{abc}	4.10 ^{abc}	19.00 ^{def}	21.00 ^{cd}	27.00 ^b	59.00 ^{bcd}	61.00 ^{bc}	109.00 ^a	6.50 ^b	6.90 ^b	7.00 ^b
40	20.00 ^{def}	20.00 ^{c-f}	24.00 ^{a-d}	3.90 ^{abc}	3.90 ^{abc}	4.20 ^{abc}	18.00 ^{efg}	20.00 ^{cde}	23.00 ^c	43.00 ^{cde}	64.00 ^b	105.00 ^a	7.20 ^b	7.10 ^b	9.40 ^b
60	20.00 ^{def}	20.00 ^{def}	23.00 ^{a-e}	3.70 ^{bc}	3.70 ^{bc}	4.30 ^{ab}	16.00 ^{fg}	17.00 ^{efg}	22.00 ^c	42.00 ^{de}	50.00 ^{b-e}	99.00 ^a	8.60 ^b	8.20 ^b	8.20 ^b
80	23.00 ^{a-e}	23.00 ^{a-d}	24.00 ^{abc}	3.70 ^{bc}	3.50 ^c	4.00 ^{abc}	15.00 ^g	15.00 ^g	22.00 ^c	39.00 ^e	47.00 ^{b-e}	94.00 ^a	9.10 ^b	13.20 ^a	9.00 ^b
LSD _{0.05}	3.20			0.58			2.80			16.10			2.95		

Mean data with superscript same letter are not significantly different. Least significant difference (LSD) test was performed at 5 % level of significance to determine the difference between the treatments.

Table 4.7. Pearson's correlation matrix of different growth and yield parameters with grain arsenic concentration and accumulation

Variables		r-value							
Above ground biomass	1	–							
Grain As concentration	2	– 0.28*	–						
Grain yield	3	0.84***	– 0.15 ^{ns}	–					
Leaf area	4	0.48***	– 0.20 ^{ns}	0.45***	–				
Plant height	5	0.62***	– 0.55***	0.4***	0.55***	–			
Plant vigour	6	0.48***	– 0.80***	0.32*	0.25 ^{ns}	0.74***	–		
Leaf chlorophyll content	7	0.67***	– 0.23 ^{ns}	0.62***	0.52***	0.66***	0.47***	–	
Spike number	8	0.62***	– 0.30*	0.71***	0.28*	0.41**	0.45***	0.50***	–
Tiller number	9	0.52***	– 0.67***	0.43***	0.21 ^{ns}	0.57***	0.77***	0.39**	0.58***
	1	2	3	4	5	6	7	8	

*, ** and *** indicate significance at $p < 0.05$, < 0.01 and < 0.001 , respectively and ^{ns} indicate non-significant

Table 4.8. Pearson's correlation matrix of different grain minerals with grain arsenic concentration and accumulation

Variables		r-value									
Grain As concentration	1	–									
P	2	– 0.86***	–								
K	3	0.46***	0.11 ^{ns}	–							
Ca	4	0.01 ^{ns}	0.16 ^{ns}	0.16 ^{ns}	–						
Mg	5	– 0.24 ^{ns}	0.21 ^{ns}	– 0.02 ^{ns}	0.05 ^{ns}	–					
S	6	– 0.24 ^{ns}	– 0.30*	– 0.09 ^{ns}	0.38**	0.27*	–				
Fe	7	0.12 ^{ns}	0.01 ^{ns}	– 0.07 ^{ns}	– 0.17 ^{ns}	0.38**	– 0.10 ^{ns}	–			
Cu	8	– 0.30*	– 0.07 ^{ns}	– 0.41***	– 0.13 ^{ns}	0.58***	0.37**	0.32*	–		
Mn	9	– 0.29*	– 0.10 ^{ns}	– 0.27*	– 0.63***	0.42***	– 0.07 ^{ns}	0.47***	0.39**	–	
Zn	10	– 0.62***	– 0.09 ^{ns}	– 0.42***	– 0.30*	0.57***	0.34**	0.46***	0.62***	0.78***	–
Na	11	0.48***	– 0.11 ^{ns}	0.41**	– 0.04 ^{ns}	– 0.33*	– 0.26*	0.21 ^{ns}	– 0.35**	– 0.14 ^{ns}	– 0.37**
	1	2	3	4	5	6	7	8	9	10	

*, ** and *** indicate significance at $p < 0.05$, < 0.01 and < 0.001 , respectively and ^{ns} indicate non-significant

4.5 Discussion

It is well known that soil As has adverse effects on plant growth and development. Previous research revealed that plant growth traits such as plant height, tiller number, and number of grains per spike can decrease significantly with increasing soil As concentration (Asaduzzman et al., 2010). Pigna et al (Pigna et al., 2010) reported a 60 % plant biomass and 83.6 % root biomass reduction of wheat in As contaminated soil. Also, several other experiments reported that the reduction of plant growth was ultimately the result of As phytotoxicity at high soil As concentrations (Rahman et al., 2004; Liu et al., 2005; Zhang et al., 2009). This experiment also found a significant reduction in plant growth represented by plant height, plant vigor, tiller number, shoot and root biomass with increasing soil As concentration, which agrees with these other studies. Like other growth parameters, similar results were observed in leaf chlorophyll content (measured as SPAD), leaf area, leaf width and length, which also reflect the lower plant growth at higher soil As concentration. Chlorophyll consists of mainly N, as a core component, and its content in the leaf represents the chlorophyll content. Higher soil As can reduce the N content in various crops (Paivoke, 1983; Merry et al., 1986). Thus, it was anticipated that, higher soil As concentrations may also decrease N content in wheat plants, which may lead to a decrease in chlorophyll content. The results of the present experiment revealed that higher soil As concentrations decreased the chlorophyll content, therefore leading to a lower photosynthesis rate, which might have reduced plant growth and grain yield.

In relation to grain yield, a decreasing trend was observed as As concentration in the soil increased, although it was not statistically significant. From the results, it is clear that, at the early growth stage, As had more effect on the plant growth, whereas, at the mature stage As had less effect (Figure 4.2), which can be correlated with the final grain yield (Figure 4.3b). A similar result was observed in previous experiment conducted on wheat grown in As contaminated soil ($0 - 40 \text{ mg kg}^{-1}$), by Asaduzzaman et al, where less impact of As was reported on grain yield (Asaduzzman et al., 2010). Some other previous experiments also revealed a greater effect of As on the shoots but showed less effect on the yield and yield contributing characters (Zhang et al., 2009; Asaduzzman et al., 2010), which is similar to the current results of this study. Furthermore, after uptake of As by the plant it is expected that the metabolism of As, like other toxic elements, is either related to the transformation or accumulation in or excretion from the cells (Roy and Saha, 2002). In plant cells, As can induce oxidative stress and result in the formation of antioxidants. Also, arsenate [As(V)], which is

one of the forms of inorganic As, can compete with phosphate (PO_4^{3-}) by replacing PO_4^{3-} in ATP, thereby disrupting the energy flow in the cell (Meharg and Hartley, 2002). Arsenite [As(III)], the most toxic form of As, can react with sulfhydryl groups ($-\text{SH}$), groups of enzymes, and tissue proteins, which can cause inhibition of cellular function and finally cell death (Williams et al., 2005). In this way As can create toxicity in plants, which ultimately results in reduced plant growth and grain yield.

On the other hand, the results showed that the MW soil treatment, had a significantly beneficial effect on wheat plant growth and grain yield irrespective of soil As concentration (Figure 4.2 and Figure 4.3b). A previous study by Khan et al. reported that, MW soil treatment significantly increased the tiller number, plant biomass and grain yield of wheat (Khan et al., 2016). In addition, a 33.1 % increase in plant dry biomass and 39.2 % increase in grain yield in MW treated soil was also reported in another study (Khan et al., 2018). Similarly, some other studies demonstrated that increased plant growth and grain yield resulted in MW treated soil as well (Brodie et al., 2017; Jamal et al., 2017). These above findings agree with the results obtained in this experiment. One of the possible reasons for increased growth and yield of wheat is the higher availability of nutrients for the plants in MW treated soil. Increased N and S availability in soil was reported after application of MW (Wainwright et al., 1980). Speir et al. reported increased N levels in MW treated soil compared with the control (Speir et al., 1986). Additionally, another previous study showed increased indigenous soil N after MW soil heating (Khan et al., 2019c). Similar result was observed in this present study where, N, P, and S increased after MW soil heating (Table 4.1). Using SPAD as a method for leaf chlorophyll measurement, a higher value (58 – 64) was reported in MW treated soil as compared with the control (42 – 56) pots (Ling et al., 2011). In this present study leaf chlorophyll content was significantly higher in the MW treated pots which could correlate with the higher photosynthesis rate and ultimately contributed to the higher crop growth and grain yield.

Alternatively, MW also has negative impacts on the microbial community in the soil. By generating heat, MW energy would kill certain microbes. As a result, MW irradiation induced disintegration of the cell walls can release the intracellular and extracellular macromolecules, which may increase the soluble OM in the soil and release some nutrients (Zhou et al., 2010). Previous research reported three pathways of organic N (org-N) transformation: (1) microorganisms based org-N mineralization to ammonium, (2) release of org-N due to cell lysis, and (3) ammonium excreted from the bacteria grazing on soil fauna (Schimel and Bennett, 2004). Research has shown that the org-N mineralization following MW irradiation

of soil is of microbial origin (Speir et al., 1986). Furthermore, nurturing and/or shaping the soil microbial communities was reported as the result of humification and thermal denaturation of soil organic compounds after soil heating (Voort et al., 2016; O'Brien et al., 2018). These microbial communities accelerate the availability of different soil nutrients could be responsible for better plant growth and grain yield (Maynaud et al., 2019).

From Figure 4.3c it was evident that the grain As concentration increased significantly with increased soil As concentration. Several studies described higher concentration of wheat grain As when cultivated in soil containing higher As and stated that the generally expected trend of increasing soil As increased the As in roots, shoots and grain (Asaduzzman et al., 2010; Tong et al., 2014). A significantly high concentration of As in wheat grain ($220.00 - 620.00 \mu\text{g kg}^{-1}$), grown in soil containing $2.00 - 70.00 \text{ mg kg}^{-1}$ As, compared with those ($0 - 50.00 \mu\text{g kg}^{-1}$) grown in $1.00 - 13.40 \text{ mg kg}^{-1}$ soil As, has been reported (Baig et al., 2011). A similar trend was observed in this current experiment where, the grain As concentration was lower at low soil As concentrations whereas it was higher at high soil As concentrations. However, MW soil treatment significantly reduced the As concentration in wheat grain (Figure 4.3c). This reduced grain As concentration could be explained by the couple of changes after MW soil heating. One of the possible reasons could be increased plant biomass and grain yield in MW treated soil, where there was a dilution effect on As concentration in the plants grown in the MW treated soil. From the Pearson's correlation study (Table 4.7) it is evident that, the grain As concentration ($r = -0.1511^{\text{ns}}$) was negatively correlated with grain yield but non-significantly. This also explains the diminishing effect of As on grain yield. Another reason could be the increased soil P and Si concentration after MW soil heating (Table 4.1). The PO_4^{3-} level in the soil is known to control plant growth and development, and As(V) is a PO_4^{3-} analogue (Anawar et al., 2018). Therefore, increasing PO_4^{3-} in the soil results in enhanced competition between PO_4^{3-} and As(V) for sorption sites on soil particle surfaces and for plant uptake because of the similar uptake mechanism of PO_4^{3-} and As(V) through PO_4^{3-} transporter present in the plant root (Anawar et al., 2018). Furthermore, Si can compete with As(III) for plant uptake due to similar uptake mechanism through aquaglyceroporins the more likely nodulin 26-like intrinsic protein (NIP) class of aquaporin channels (Zhao et al., 2010a; Mosa et al., 2012). Thus, the increased soil P and Si concentration after MW soil heating could compete with As(V) and As(III) for plant uptake and reduce the accumulation in the grain.

However, according to the Steindorf-Rebhun-Sheintuch equation, ligand exchange theory and a share charge hypothesis, PO_4^{3-} has more probability to replace As(V) from soil

adsorption site depending on the concentration of PO_4^{3-} and As(V). Nevertheless, PO_4^{3-} might also be desorbed by As(V) due to a mass action effect of high ratio of As(V):P concentrations in the soil solution (Roy et al., 1986; McBride, 1994). Therefore, PO_4^{3-} and As(V) interaction needs to be considered for applying PO_4^{3-} amendment as As remediation technique. Some researchers reported that, at low soil As concentration, displacement of soil PO_4^{3-} by As(V) increased the availability of PO_4^{3-} to the plant, which resulted in the increase of plant growth parameters (Pigna et al., 2009; Pigna et al., 2010). In this experiment, it was also found that shoot biomass at both tillering stage and final crop harvest, root biomass, and spike number increased at low soil As concentrations (As-20 and As-40). However, all these traits decreased again at higher As concentrations (As-60 and As-80). Previous researches also reported plant growth and yield increases due to small additions of As in tomato, potato, rye, corn, and wheat (Carbonell et al., 1998; Gulz and Gupta, 2000; Gulz et al., 2005) which agrees with the findings of this present study in wheat. Although, As is not an essential element for plants, small amounts of As can stimulate plant growth and increase plant biomass by releasing some P from soil adsorption sites and make it available for plant uptake (Onken and Hossner, 1995).

Furthermore, As concentration in wheat grain also depends on the genetic differences of different varieties. Previous research reported that, different wheat varieties, grown in the same soil As concentration, can tolerate, accumulate and translocate different concentrations of As due to phytoextraction or phyto-morphological potential of the varieties (Shi et al., 2015; Kamrozzaman et al., 2016). The wheat variety used in this study could accumulate less As due to genetical constituents. However, further experiment needed with different wheat variety to explore the varietal effect on As accumulation. In addition, it has been demonstrated that, MW soil heating markedly altered the physical and chemical properties of SOM (Hur et al., 2013) and enhanced the humification of SOM (Kim and Kim, 2013). It also increased the soil organic carbon and N mineralization (Zagal, 1989), macromolecular organic substances that possess a higher number of functional groups (Kim and Kim, 2013), and syntheses of organometallic and coordination compounds (Taylor et al., 2005). These organic substances can retain, decrease mobility, reduce bioavailability and adsorb soil heavy metals (Yin et al., 2002). Therefore, more As could be adsorbed by the adsorption sites in the soil and become unavailable for plant uptake which ultimately could reduce the grain As concentration.

From the Table 4.5, it was evident that, grain P content decreased significantly while, Na content increased significantly with increasing soil As concentration. A similar result was found in another study (Shaibur et al., 2009) where, P uptake decreased in As treatments. Since,

sodium arsenate was used in this study to artificially contaminate the soil, the addition of this Na might contribute the higher plant uptake in higher As treated soil and ultimately more accumulation of Na in the grain. The result also shows that, grain Mn, Zn and Cu content decreased significantly with increasing soil As concentration, while they increased significantly in the MW treatments compared with the control. Addition of As can reduce Mn content in shoots and roots (Shaibur et al., 2009), which results in lower Mn translocation to the grain. By contrast, the opposite findings have also been reported (Yamane, 1989) where increased Mn content with increased As was observed. It is known that divalent Mn is absorbed by facilitated diffusion across the plasmalemma (Fox and Guerinot, 1998). It is possible that As phytotoxicity may hamper the activity of the root plasmalemma and reduce Mn^{2+} absorption. Similarly, decreased Zn and Cu content in shoots and roots (Shaibur et al., 2009) can facilitate the lower translocation to the grain. Similar findings were reported in another study (Liu et al., 2020) where an antagonistic relation between As and Zn was described. Another study also reported lower Zn content in rice grain where higher As was present in the soil (Duan et al., 2013). However, MW soil treatment can increase the grain Zn and Cu by reducing the As phytotoxicity. From the Pearson's correlation (Table 4.8) it was also evident that, grain K and Na content were significantly positively correlated with grain As concentration and significantly negatively correlated with grain P, Cu, Mn, and Zn content.

4.6 Conclusions

Although the elevated concentration of soil As can reduce the plant growth and grain yield of wheat due to the As phytotoxicity, MW soil treatment can mitigate this As phytotoxicity. The MW-6 treatment showed better influence than the MW-0 and MW-3 treatments. Furthermore, MW soil treatment can reduce grain As concentration. Although the soil remains contaminated after MW treatment, wheat grain As concentration was lower in the MW treated pots, which results in lower As accumulation in humans through wheat consumption. Nevertheless, further experiments are needed to explore the effectiveness of MW treatment with different types of soils in field condition.

The effect of MW soil treatment on As accumulation in rice should be considered because rice is grown in an anaerobic environment. As was pointed out in the literature review (Chapter 2), anaerobic environments affect the speciation and availability As in a different way to aerobic soil conditions, like in wheat production systems. The next chapter describes an experiment involving rice production in As contaminated soil.

Chapter 5 – Microwave soil treatment along with biochar application alleviates arsenic phytotoxicity and reduces rice grain arsenic concentration

5.1 Abstract

Rice grain arsenic (As) considered as a major pathway of human dietary As exposure and has raised global concern due to its adverse effect on crops and human health. Microwave (MW) soil treatment has been revealed as a novel technique that has shown potential to alleviate As phytotoxicity and reduce grain As concentration in wheat. This study was conducted to investigate the effect of MW soil heating along with biochar treatment on the As phytotoxicity and grain As concentration in rice. Experimental soils were spiked to five levels of As concentration (As-0, As-20, As-40, As-60 and As-80 mg kg⁻¹) prior to apply three levels of saw dust biochar (BC-0, BC-10, and BC-20 t ha⁻¹) and three levels of MW treatment (MW-0, MW-3, and MW-6 minute). The results revealed that MW soil treatment alleviates As phytotoxicity as rice plant growth and grain yield increase significantly and facilitate less grain As concentration compared with the control. For instance, significantly higher grain yield was recorded at MW-6 treatment (15.22 g pot⁻¹) than the MW-3 (10.20 g pot⁻¹) and MW-0 (4.00 g pot⁻¹) treatments at the highest soil As concentration (As-80). Highest grain As concentration (912.90 µg kg⁻¹) was recorded in MW-0 treatment while, it was significantly lower (442.40 µg kg⁻¹) in MW-6 treatment at As-80. Increased plant growth and grain yield and decreased grain As concentration was observed at BC-10 compared with BC-0 at lower soil As treatments (As-20 and As-40) while no significant effect was observed at higher soil As treatments (As-60 and As-80). Unexpectedly, BC-20 had a negative effect on plant growth and grain yield and higher grain As concentration was observed. Combination of BC-10 and MW-6 found to reduce grain As concentration (498.00 µg kg⁻¹) compared with control treatment (913.7 µg kg⁻¹). Thus, either solely MW soil treatment or combination with certain rate of saw dust biochar treatment can be used to reduce dietary As exposure through rice consumption. Nevertheless, further experiments are needed to investigate whether it is only the heating effect or the MW's that have other unique effects. Also, residual effect of MW and biochar need to be studied.

Keywords: Microwave, Soil heating, Biochar, Arsenic phytotoxicity alleviation, Rice grain arsenic

5.2 Introduction

Arsenic (As) is a toxic metalloid, which is ubiquitously present in the environment, has raised serious global concern because of its adverse effect on human health and the ecosystem (Naidu et al., 2006). Although As contaminated groundwater, which is used for drinking purpose, is probably the major pathway of human exposure (Gupta et al., 2017), the use of this As polluted groundwater for crop irrigation gives rise to high deposition of As in topsoil and ultimately results in the As uptake by crops (Williams et al., 2006), which augment As exposure through the food chain and threat to human health. Excessively high As pollution in water, soil and crops have already been identified in many countries (Shankar and Shanker, 2014; Singh et al., 2015b; Upadhyay et al., 2019).

It has been demonstrated that, rice can accumulate 10 time more As than other cereal crops (e.g. Wheat and barley) (Williams et al., 2007). As a consequence, a considerable amount of As has already been reported in rice grains throughout the world such as $<0.01 - 2.05 \mu\text{g g}^{-1}$ for Bangladesh, $0.31 - 0.70 \mu\text{g g}^{-1}$ for China, $0.03 - 0.044 \mu\text{g g}^{-1}$ for India, and $0.11 - 0.66 \mu\text{g g}^{-1}$ for US (Zavala and Duxbury, 2008). Since irrigated rice crop requires higher amount of water, compared to other irrigated and non-irrigated crops, the use of As contaminated irrigation water for rice cultivation is becoming a serious problem in several parts of the world, especially in the Bengal basin, which is not only leading to accumulation of As in rice grain but also adding to long-term soil As contamination (Heikens et al., 2007). Thus, besides the drinking water, rice among other crops has become major contributor of As intake and accumulation in the human body because it is the dietary staple of half of the world's population (Banerjee et al., 2013). China, India, Bangladesh, Vietnam, and Pakistan are not only top most rice producing countries but also a substantial number of rice consumers are living in these countries where a vast area is contaminated with As. The situation becomes of even greater concern for these region because of the high rice consumption rate ranging from 250 to 650 g of rice per day per person (Arslan et al., 2016).

There are several techniques such as precipitation, adsorption and ion exchange, coagulation-filtration, membrane filtration, nanofiltration, reverse osmosis, etc. have been used to remove As from drinking water (Ghosh et al., 2019). Soil flushing, solidification, stabilization, vitrification, washing/acid extraction, electrokinetic treatment, etc. are some techniques for soil As remediation (Ellis et al., 2002; Yang et al., 2007). Hitherto, these methods have been commonly determined to be ineffective, costly, or too lengthy, with usage restricted to smaller-scale operations with lower

efficiency (Lim et al., 2014). Phytoremediation by using hyper accumulative plants (Wang et al., 2002) is another technique, which is probably the most environment-friendly and cheapest method, but the selectivity of plants to metals, depth of remediation, time taken to do an adequate job and disposal after remediation have been noted as major drawbacks of this method (Lim et al., 2014). Thus, alternative options or combinations of technologies, for alleviating soil As, are required.

Pre-sowing microwave (MW) soil heating has been revealed as a unique technique, which can significantly enhance crop growth in addition to be a means of non-chemical weed control (Brodie et al., 2017; Jamal et al., 2017). Microwave energy is a form of electromagnetic radiation, with frequencies between 300 MHz to 300 GHz and wavelengths ranging from 1m to 1mm. The mechanism of MW heating comprises the rotation of the dipoles of the polar molecules (e.g. Water) in the materials, due to the oscillating electromagnetic field, which results in the generation of heat by intermolecular friction (Falciglia et al., 2016). Some major advantages like short start-up, selective heating, precise control, no direct contact of heated materials, and volumetric heating compared with other heating processes (Ayappa et al., 1991; Menéndez et al., 2010), makes MW heating a preferred choice and has been using in diversified field including removal of organic contaminants (Falciglia and Vagliasindi, 2014) and immobilization of some toxic metals such as Chromium (Cr), Nickel (Ni), Cadmium (Cd), Zinc (Zn), Copper (Cu), and Lead (Pb) in soil (Abramovitch et al., 2003) and solid sediments (Chen et al., 2005a; Hsieh et al., 2007). However, so far to best of our knowledge no published study has investigated soil As remediation using MW heating, which could be a novel method to lessen the As phytotoxicity and grain As accumulation.

Furthermore, to immobilize soil organic and inorganic pollutants and heavy metals, biochar has been progressively gaining more attention because of its high carbon content, micropores, and large surface area. Biochar is a carbonaceous solid material obtained from thermochemical decomposition of residual biomass at relatively high temperature (500 – 1500 °C) through a process called pyrolysis (oxygen-depleted conditions), which results biochar. It is porous and rich in stable and resistant carbon, with different surface functional groups (Ahmad et al., 2014; Lehmann and Joseph, 2015; Rajapaksha et al., 2016). These unique physicochemical properties of biochar make it effective sorbent, which can immobilize Cr, Cobalt (Co), Ni, Cd, Zn, Cu, and Pb. It also reduces Aluminium (Al) toxicity (Van Zwieten et al., 2010), and reduces the bioavailability of heavy metals (Méndez et al., 2012). Arsenic can be immobilized by precipitation and reduction, surface complexation with different functional groups, ion exchange, electrostatic interactions, and

physical adsorption on the biochar surface (Vithanage et al., 2017). For instance, biochar, prepared from hardwood and Sewage sludge, was found to be an adsorber of As and can immobilize soil As (Namgay et al., 2010; Khan et al., 2013). However, increased mobility and bioavailability of As is also reported after the application of some biochar (Namgay et al., 2010; Beesley et al., 2014). Hence, this advocates that all types of biochar are not suitable for the remediation of As contaminated soils (Beesley et al., 2013).

Although several studies conducted to investigate the As immobilisation efficiency of different biochar, biochar produced by MW assisted pyrolysis not well studied despite having some advantages such as higher char yield, higher decomposition temperature, higher heating rate, and less or no pre-processing of feedstock (Domínguez et al., 2006; Wang et al., 2009). Furthermore, no published study so far reported a combination of MW soil heating and biochar treatment for soil As remediation, which could be a possible technique to lessen the As phytotoxicity and grain As accumulation. Therefore, this study aimed to investigate the potential impacts of MW soil heating and MW pyrolyzed biochar on the alleviation of As phytotoxicity and accumulation of As in rice grain.

5.3 Materials and Methods

5.3.1 Soil collection, and preparation

Soils were collected from a crop production paddock (Paddock H12, 36°23'51"S; 145°43'17" E) at a depth of 0–15 cm. The soil was a grey to grey-brown clay and was classified as a Congupna clay (Downes, 1949) or a Grey Vertosol (Isbell, 2016). Some important soil properties are given in Table 5.1. After collection, the soils were prepared by following the procedure described previously (Chapter 4) for further treatment.

5.3.2 Physicochemical properties of experimental soil

The physicochemical properties of the soil were analyzed to ascertain the levels of nutrients as well as other elements present, following the standard method of analysis, was undertaken. A composite soil sample was sent to the Nutrient advantage Laboratory, a NATA accredited laboratory in Australia (Lab number: 11958, ISO/IEC 17025), for analysis of the soil properties. The physicochemical properties of the pre and post-experimental soil are presented in Table 5.1.

Table 5.1. Physicochemical properties of pre and post microwave (MW) treated soils before sowing

Soil properties	Analytical method	Units	Microwave treatments		
			MW-0	MW-3	MW-6
Organic carbon (OC)	Walkley & Black	%	1.57	1.36	1.38
Organic matter (OM)	Walkley & Black	%	2.70	2.34	2.37
Electrical conductivity (EC)	Saturated extract	dS/m	0.80	0.90	1.60
pH	1:5 CaCl ₂	N/A	6.50	6.70	6.60
Cation exchange capacity (CEC)	BaCl ₂ exchange	cmol(+)/kg	9.95	10.10	10.40
Nitrate nitrogen (NO ₃ ⁻ -N)	Kjeldahl	mg kg ⁻¹	39.00	33.00	36.00
Ammonium nitrogen (NH ₄ ⁺ -N)	Kjeldahl	mg kg ⁻¹	7.50	44.00	160.00
Available Potassium (K)	Atomic emission	mg kg ⁻¹	290.00	290.00	310.00
Sulphur (S)	0.25M KCl at 40°C	mg kg ⁻¹	7.00	14.00	38.00
Phosphorus (P)	Colwell	mg kg ⁻¹	45.00	98.00	260.00
Calcium (Ca)	Ammonium acetate	cmol(+)/kg	3.70	3.70	3.80
Magnesium (Mg)	Ammonium acetate	cmol(+)/kg	4.60	4.70	5.00
Potassium (K)	Ammonium acetate	cmol(+)/kg	0.75	0.75	0.80
Sodium (Na)	Ammonium acetate	cmol(+)/kg	0.62	0.64	0.67
Aluminium (Al)	Ammonium acetate	cmol(+)/kg	0.26	0.26	0.15
Copper (Cu)	DTPA	mg kg ⁻¹	1.60	1.60	1.70
Zinc (Zn)	DTPA	mg kg ⁻¹	1.00	1.10	1.30
Manganese (Mn)	DTPA	mg kg ⁻¹	66.00	68.00	82.00
Iron (Fe)	DTPA	mg kg ⁻¹	92.00	94.00	97.00
Boron (B)	DTPA	mg kg ⁻¹	0.90	0.89	0.93
Silicon (Si)	CaCl ₂ Soluble	mg kg ⁻¹	75.00	81.00	100.00
Arsenic (As)	HG-AFS	µg kg ⁻¹	< 0.01	< 0.01	< 0.01

5.3.3 Arsenic application

The soils were spiked at five different levels of As concentration (0, 20, 40, 60, and 80 mg kg⁻¹ dry soil) as sodium arsenate heptahydrate (Na₂HAsO₄·7H₂O) (Bhattacharya et al., 2015). Arsenic was applied by following the method described previously (Chapter 4). Since the initial As concentration of the soils, prior to treatment, was < 0.01 mg kg⁻¹ (Table 5.1), there was no chance of further As being available from the soil. To establish an equilibrium condition between soil and applied As, soil moisture was maintained at field capacity for two weeks as waiting period before applying biochar and MW treatment.

5.3.4 Biochar preparation, characterization, and application

Three different levels of saw dust biochar (0, 10 and 20 t ha⁻¹) were applied in previously As spiked soils. Since several studies have been conducted on As immobilization using biochar, there are several rates of biochar application, based on the soil contamination and biochar types. In this study, the most common rate of biochar, which was used, was proposed by other researches (Zheng et al., 2015; Qiao et al., 2018). Biochar was prepared from pine sawdust by MW assist pyrolysis technique at around 650 – 700 °C temperature (Mamaeva et al., 2016). A MW chamber, consisting of six magnetrons (1 kW each), operating at a frequency of 2.45 GHz, was used to prepare the biochar (Figure 5.1a). A high temperature (1450 °C) tolerant quartz crucible (Height 30 cm, diameter 10 cm) with a lid was used to prepare the biochar. The lid was placed on the full crucible to limit oxygen availability during the pyrolysis process in the MW chamber. An infrared camera (FLIR T1050SC model) was used to measure the pyrolysis temperature by capturing thermal images immediately after the MW heating (Figure 5.1b). The yield, total ash content, and volatile matter, of biochar were calculated based on the following Equations (5.1) (Agrafioti et al., 2013), (5.2) (ASTM, 2013), and (5.3) (Agrafioti et al., 2013) respectively.

$$\text{Biochar yield (\%)} = \left(\frac{W_2}{W_1} \right) \times 100 \quad (5.1)$$

Where, W_1 is the dry weight of sawdust sample prior to pyrolysis, and W_2 is the final biochar weight.

$$\text{Total ash (\%)} = \left(\frac{W_2 - W_c}{W_1 - W_c} \right) \times 100 \quad (5.2)$$

Where, W_c is the weight of the crucible, W_1 is the weight of the sample and crucible and W_2 is the weight of the ash and the crucible.

$$\text{Volatile matter (\%)} = \left(\frac{A - C}{A - B} \right) \times 100 \quad (5.3)$$

Where, A is the weight of dried sample and crucible, B is the weight of crucible, and C is the weight of residue and crucible after ignition

The specific surface area (BET), pore size and total volume of pores were measured using a Quantachrome Autosorb iQ3 gas adsorption analyzer. Prior to gas sorption analyses, samples were

degassed overnight at room temperature and then incubated for 1 h at 250 °C. The properties of sawdust biochar are given in the Table 5.2 and the scanning electron microscopic (SEM) structure of sawdust biochar is given in the appendices (Appendix 5.1).

Table 5.2. Properties of sawdust biochar produced from microwave assist pyrolysis

Properties of biochar	Unit	Value	Method of determination
Pyrolysis temperature	°C	650 – 700	FLIR thermal camera
Residence time	min	20.00	–
Yield	wt. %	39.33	(Agrafioti et al., 2013)
Ash content	wt. %	1.34	Muffle furnace at 600 °C (ASTM, 2013)
Volatile matter	wt. %	70.32	Muffle furnace at 500 ± 50 °C (Agrafioti et al., 2013)
Dry matter	wt. %	97.84	Oven-drying at 110 °C (ASTM, 2010)
Moisture	wt. %	2.16	Oven-drying 110 °C (ASTM, 2010)
pH	N/A	8.47	1:5 water, pH meter (Singh et al., 2010)
EC	dS m ⁻¹	0.17	1:5 water, EC meter (Singh et al., 2010)
Specific surface area	m ² g ⁻¹	0.05	BET analysis (Sigmund et al., 2017)
Pore volume	mm ³ g ⁻¹	1.00	BJH adsorption-desorption (Sigmund et al., 2017)
Pore size	nm	17.39	BJH adsorption-desorption (Zhao et al., 2018)

5.3.5 Microwave soil heating

After one week of biochar application, three levels of MW energy (0, 127.06 and 254.12 kJ kg⁻¹ soil) were applied for 0, 3 and 6 minutes to treat the soil to attain soil temperatures around room temperature, 60 and 90 °C respectively (Figure 5.1). The MW treatments were applied by following the procedure described previously (Chapter 4).

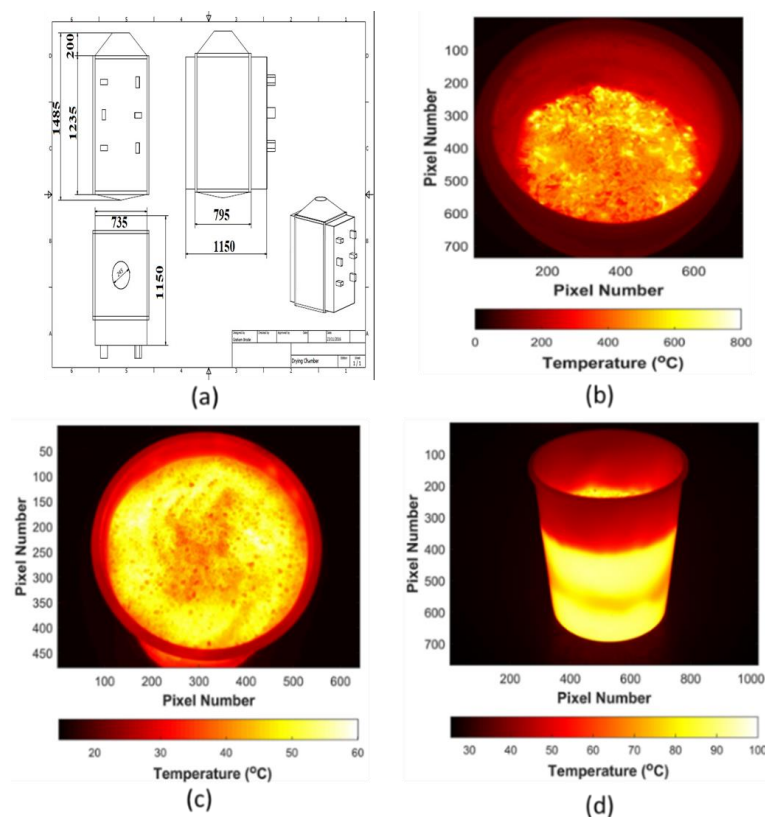


Figure 5.1. (a) Schematic diagram of a 6 kW microwave (MW) chamber (Appendix 4.1) used for soil treatment and biochar preparation, (b) a thermal image of sawdust biochar in a quartz crucible showing the pyrolysis temperature, (c) top view thermal images of soil heating after MW irradiation showing the temperature increase of $55\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$, and (d) side view thermal images of soil heating after MW irradiation showing the temperature increase of $85\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$

5.3.6 Experiment setup

After the application of As, biochar and MW treatments, along with the control treatments and each with four replicates, in a total of 180 pots were placed in the glasshouse, following completely randomized design (CRD). To describe the treatment combination more conveniently, abbreviated forms were used for As treatments (As-0, As-20, As-40, As-60 and As-80), biochar treatments (BC-0, BC-10 and BC-20) and MW treatments (MW-0, MW-3 and MW-6). To supply adequate nutrients for proper plant growth, diammonium phosphate (DAP) for N and P and Potassium Sulphate (K_2SO_4) for K and S were applied to each pot as a basal dose, as per standard practices for Australian rice cultivation (Dunn et al., 2018), prior to seed sowing. The rest of the calculated N was supplied as urea in two split doses, one at the tillering stage and another one at the panicle initiation stage of plant growth. The application rate of N, P, K and S was 200, 30, 18, and 23 kg ha^{-1} respectively. Twenty seeds of the YRM_70 variety (*Oryza sativa* L.) were sown per

pot on 15th of January 2018. At the three-leaf stage, extra seedlings were removed, leaving 12 seedlings per pot. Insects and diseases were controlled as per standard methods of rice cultivation (Dunn et al., 2018) and weeds were removed by hand when needed. Tap water was used for irrigation purposes. This water source contained As below the detection limit of AFS ($< 0.01 \mu\text{g l}^{-1}$); therefore, there were no possibilities of As addition from the tap water to the pot soil. After seed sowing, soil moisture was maintained at field capacity up to the three-leaf stage of plant growth. Flooding irrigation was started at three leaf stage and maintained at certain level in the pot throughout the growing period and stopped 10 days before harvesting the plants (Azad et al., 2009; Dunn et al., 2018). After 150 days of the growing period at physiological maturity stage the crop was harvested on the 14th of June 2018.

5.3.7 Recording of agronomic data

The plant height was measured as distance from the soil to the top of a plant using a measuring tape at 30, 60, and 90 DAS (days after sowing). Leaf chlorophyll content was measured at tillering stage as SPAD value using the Chlorophyll Meter-SPAD-502Plus (Soil-Plant Analysis Development) (Yuan et al., 2016). To get the plant height and leaf chlorophyll content, five plants within a pot were selected randomly and data was recorded as mean value of these five plants. At tillering stage, total number of tillers per pot was counted prior to collecting plant samples (3 hills per pot). From these collected samples, leaf area, width, length, and perimeter of the last fully expanded leaf were measured by using a leaf area meter (LASER Leaf Area Meter, CI – 202, CID Bio-Science, USA). At the physiological maturity stage, total number of panicles per pot was counted prior to harvest. Grains were collected from the panicle by hand threshing and yield (g pot^{-1}) was calculated as described previously (Rahman et al., 2007a). Total filled grain and sterile spikelet was counted to calculate the spikelet sterility percentage. Fresh weight of all plant samples was recorded prior to drying at 60°C in a dehydrating oven (Thermoline Scientific, TD-500F) for 48 hours to determine the dry weight.

5.3.8 Grain total As analysis

Grain total As concentration determination was performed by following the procedure described previously (Chapter 4).

5.3.9 Statistical analysis

For the statistical analysis of data R software (version: 3.6.2) (Team, 2013) was used. Normality and homogeneity of variance of the data were tested. Analysis of variance (ANOVA) test was performed to determine the significance of tested treatments on variables. Least Significant Difference (LSD) test was used to compare the treatment means at 5 % level of significance. Pearson correlation test was performed to determine the correlation coefficient among the variables. Thermal images were captured with an infrared camera (FLIR T1050SC model) and post-processed in MATLAB software (version: R2015b) (MATLAB, 2015).

5.4 Results

5.4.1 Plant growth and grain yield

The results revealed that plant growth and grain yield decreased significantly with the increasing of soil As concentration, while it increased significantly in MW-3 and MW-6 treatments compared with MW-0 treatment across all the soil As concentration. Biochar had both positive and negative effect based on the application rate and soil As concentration. In combination of MW and biochar, overall higher plant growth and grain yield was observed in MW-6 with BC-10. To describe the plant growth, plant height, leaf chlorophyll content, shoot biomass, tiller number, and leaf parameters, were recorded. Grain yield, panicle number, and spikelet sterility was recorded to represent the yield response. The detailed results are given below.

5.4.1.1 Plant height

From Table 5.3 it is evident that, plant height decreased significantly ($p < 0.001$) with increasing soil As concentration throughout the growing period, while MW increased the plant height significantly ($p < 0.001$) irrespective of soil As concentration. For example, at 90 DAS, the mean plant height at As-0 and As-80 was 70.05 and 61.25 cm respectively in MW-0, while it was 76.50 and 71.15 cm in MW-6. Significant effect ($p < 0.001$) of biochar was also observed. Plant height increased in response to biochar treatment at both 30 DAS ($p = 0.002$) and 60 DAS ($p = 0.051$) irrespective of soil As concentration. At 90 DAS, plant height increased in BC-10, however, a lower value was recorded in BC-20 compared with BC-0. Although, the combined effect of MW and biochar was not significant, the highest plant height was recorded in the combination of MW-6 and BC-10 treatment at As-0 (Appendix 5.3).

Table 5.3. Effect of soil arsenic (As) on mean plant height in microwave (MW) and biochar treated soil

Soil As (mg kg ⁻¹)	Mean plant height (cm)								
	30 DAS			60 DAS			90 DAS		
	MW treatments (minutes)								
	0	3	6	0	3	6	0	3	6
0	27.18 ^{def}	30.44 ^{a-e}	33.00 ^a	53.83 ^{de}	58.88 ^{a-d}	64.25 ^a	69.65 ^{a-f}	76.53 ^a	76.85 ^a
20	26.84 ^{ef}	31.77 ^{abc}	33.23 ^a	53.93 ^{de}	59.75 ^{abc}	62.27 ^{ab}	69.93 ^{a-f}	74.35 ^{ab}	76.67 ^a
40	27.90 ^{c-f}	32.03 ^{ab}	32.45 ^a	54.60 ^{cde}	57.88 ^{bcd}	59.63 ^{abc}	67.42 ^{b-f}	71.08 ^{a-e}	72.70 ^{abc}
60	28.05 ^{b-f}	31.11 ^{a-d}	30.65 ^{a-e}	54.82 ^{cde}	56.62 ^{cd}	59.33 ^{a-d}	63.57 ^{ef}	66.40 ^{c-f}	72.45 ^{abc}
80	24.22 ^f	28.25 ^{b-f}	29.33 ^{a-e}	50.98 ^e	54.95 ^{cde}	56.75 ^{bcd}	62.62 ^f	64.28 ^{def}	71.28 ^{a-d}
LSD _{0.05}		2.32			3.16			4.37	
Biochar treatments (t ha ⁻¹ soil)									
	0	10	20	0	10	20	0	10	20
0	28.85 ^{ab}	31.17 ^a	30.61 ^{ab}	56.70 ^{cde}	60.80 ^a	59.47 ^{abc}	75.60 ^{abc}	76.20 ^{ab}	71.23 ^{a-f}
20	29.03 ^{ab}	30.49 ^{ab}	32.32 ^a	57.18 ^{b-e}	58.60 ^{abc}	60.17 ^{ab}	73.32 ^{a-d}	77.33 ^a	70.30 ^{a-f}
40	29.8 ^{ab}	30.96 ^a	31.62 ^a	56.40 ^{cde}	58.32 ^{a-d}	57.40 ^{b-e}	71.38 ^{a-f}	71.85 ^{a-e}	67.97 ^{def}
60	29.43 ^{ab}	30.04 ^{ab}	30.34 ^{ab}	56.53 ^{cde}	57.43 ^{b-e}	56.80 ^{cde}	68.05 ^{c-f}	68.90 ^{b-f}	65.47 ^{ef}
80	26.7 ^b	26.79 ^b	28.3 ^{ab}	54.30 ^{ef}	52.97 ^f	55.42 ^{def}	66.07 ^{def}	67.90 ^{def}	64.22 ^f
LSD _{0.05}		2.32			3.16			4.37	

DAS (Days after sowing) indicates the sampling time. Values having different superscript letters indicate significant differences among the treatments. Least significant difference (LSD) test was performed at 5 % level of significance to determine the difference between the treatments.

5.4.1.2 Leaf chlorophyll content (as SPAD value)

Leaf chlorophyll content (SPAD value) decreased significantly ($p = 0.029$) with increasing soil As concentration while, it increased significantly ($p < 0.001$) in the MW treatments (MW-3 and MW-6) compare with MW-0. The lowest chlorophyll content was observed at the highest As treatment (As-80) in MW-0, whereas the value at MW-6 was significantly higher. Unexpectedly, the biochar had a significant ($p < 0.001$) negative effect on chlorophyll content, especially at BC-20 (Table 5.4). The combined effect of MW and biochar was non-significant (Appendix 5.4).

Table 5.4. Leaf chlorophyll content (as SPAD value) at different soil arsenic (As) concentration in microwave (MW) and biochar treatments

Soil As (mg kg ⁻¹)	Leaf chlorophyll content (SPAD value)					
	MW treatments (minutes)			Biochar treatments (t ha ⁻¹)		
	0	3	6	0	10	20
0	35.11 ^{bc}	36.63 ^{ab}	36.78 ^a	36.98 ^a	36.54 ^{ab}	34.99 ^{b-e}
20	34.33 ^{cd}	35.78 ^{abc}	36.42 ^{ab}	36.38 ^{ab}	35.6 ^{a-d}	34.55 ^{cde}
40	34.21 ^{cd}	35.69 ^{abc}	35.66 ^{abc}	35.54 ^{a-d}	35.28 ^{bcd}	34.73 ^{cde}
60	32.92 ^d	34.58 ^c	36.49 ^{ab}	34.12 ^{de}	35.86 ^{abc}	34.00 ^{de}
80	34.38 ^{cd}	34.39 ^{cd}	36.44 ^{ab}	35.52 ^{a-d}	36.11 ^{abc}	33.59 ^e
LSD _{0.05}		1.64			1.64	

Values having different superscript letters indicate significant differences among the treatments. Least significant difference (LSD) test was performed at 5 % level of significance to determine the difference between the treatments.

5.4.1.3 Leaf length, width, area, and perimeter

With increasing soil As concentration, leaf length, width, area, and perimeter reduced significantly ($p < 0.001$) while, all these increased significantly ($p < 0.001$) in MW-3 and MW-6 compared with MW-0 across all the soil As concentration. The effect of biochar also found to be significant ($p < 0.001$) but all the leaf parameters increased in the BC-10 treatment and decreased again in the BC-20 treatment (Table 5.5). The combined effect of MW and biochar was non-significant (Appendix 5.5).

Table 5.5. Influence of soil arsenic (As) on leaf length, width, area, and perimeter in microwave (MW) and biochar treated soil

Soil As (mg kg ⁻¹)	Leaf length (cm)			Leaf width (cm)			Leaf area (cm ²)			Leaf perimeter (cm)		
	MW treatments (minutes)											
	0	3	6	0	3	6	0	3	6	0	3	6
0	46.05 ^{b-e}	47.11 ^{abc}	49.65 ^a	1.17 ^{bcd}	1.18 ^{bc}	1.38 ^a	37.32 ^{bcd}	37.64 ^{bc}	41.64 ^a	93.12 ^{bc}	94.1 ^b	102.73 ^a
20	43.08 ^{e-h}	45.78 ^{b-e}	48.36 ^{ab}	1.07 ^{def}	1.16 ^{bcd}	1.32 ^a	35.36 ^{def}	37.21 ^{bcd}	40.31 ^a	87.28 ^d	93.02 ^{bc}	100.07 ^a
40	42.61 ^{fgh}	44.42 ^{c-f}	46.25 ^{bcd}	1.02 ^f	1.13 ^{b-e}	1.21 ^b	34.45 ^f	36.6 ^{b-e}	38.20 ^b	86.35 ^d	90.76 ^{bcd}	94.16 ^b
60	41.11 ^{gh}	42.36 ^{fgh}	45.19 ^{c-f}	0.92 ^g	1.04 ^{ef}	1.15 ^{bcd}	32.31 ^g	34.73 ^{ef}	37.06 ^{bcd}	80.77 ^e	86.59 ^d	92.65 ^{bc}
80	37.00 ⁱ	40.17 ^h	43.28 ^{d-g}	0.79 ^h	0.92 ^g	1.09 ^{c-f}	29.89 ^h	32.36 ^g	35.7 ^{c-f}	74.92 ^f	81.01 ^e	88.72 ^{cd}
LSD _{0.05}	1.51			0.10			2.00			5.30		
Biochar treatments (t ha ⁻¹)												
	0	10	20	0	10	20	0	10	20	0	10	20
0	48.34 ^{abc}	49.11 ^{ab}	45.36 ^{cd}	1.26 ^{ab}	1.31 ^a	1.16 ^{bcd}	39.17 ^{ab}	40.24 ^a	37.20 ^{bcd}	97.54 ^{abc}	99.42 ^{ab}	92.99 ^{cd}
20	45.03 ^{def}	49.83 ^a	42.36 ^{efg}	1.15 ^{cd}	1.36 ^a	1.04 ^{ef}	37.03 ^{cd}	41.11 ^a	34.74 ^{ef}	91.84 ^{de}	101.69 ^a	86.84 ^{ef}
40	44.97 ^{def}	46.61 ^{bcd}	41.69 ^g	1.13 ^{cde}	1.21 ^{bc}	1.02 ^f	36.70 ^{cde}	38.18 ^{bc}	34.37 ^{ef}	91.63 ^{de}	94.56 ^{bcd}	85.09 ^{fg}
60	42.11 ^{fg}	45.19 ^{de}	41.36 ^{gh}	1.02 ^{fg}	1.10 ^{def}	1.00 ^{fg}	34.31 ^{fg}	35.87 ^{def}	33.92 ^{fg}	85.77 ^{fg}	89.44 ^{def}	84.79 ^{fg}
80	40.53 ^{gh}	41.47 ^g	38.44 ^h	0.92 ^{gh}	1.01 ^{fg}	0.88 ^h	32.32 ^{gh}	34.12 ^{fg}	31.52 ^h	80.82 ^{gh}	85.02 ^{fg}	78.81 ^h
LSD _{0.05}	1.51			0.10			2.00			5.30		

Values having different superscript letters indicate significant differences among the treatments. Least significant difference (LSD) test was performed at 5 % level of significance to determine the difference between the treatments.

5.4.1.4 Tiller number

Tiller number reduced significantly ($p < 0.001$) across the treatments with increasing of soil As concentration. However, significantly ($p < 0.001$) higher tillers were found in the MW treated pots. The MW-6 had higher tiller number than the MW-0 and the MW-3 treatments (Figure 5.2a). Significantly ($p = 0.039$) higher tiller numbers were also observed in the biochar treatments (BC-10 and BC-20) compared with BC-0 (Figure 5.2b). The combined effect of MW and biochar was non-significant (Appendix 5.6).

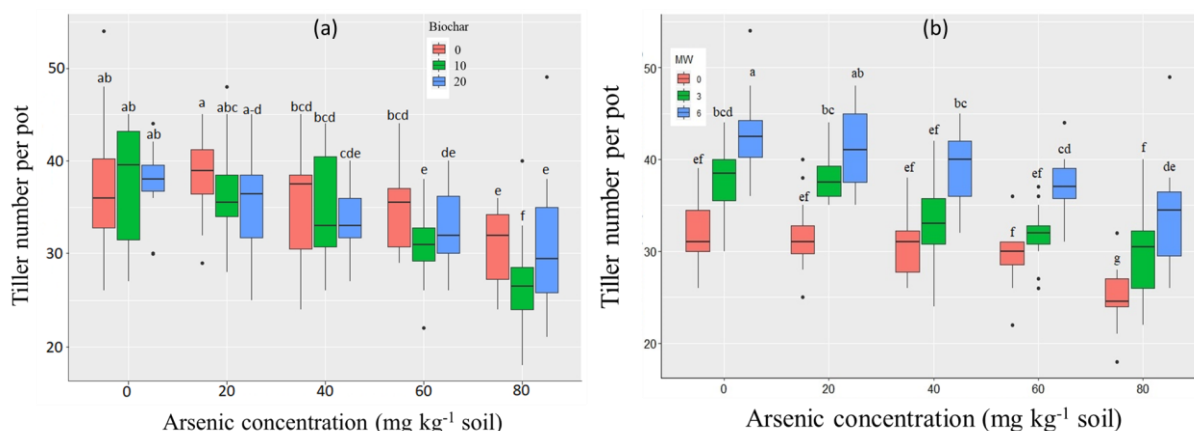


Figure 5.2. Effect of soil arsenic (As) concentration on tiller number in (a) biochar and (b) microwave (MW) treated pot. Least Significant Difference Test (LSD) was performed at 5 % level of significance. In boxplot, values having different superscript letters indicate significant differences among the treatments

5.4.1.5 Shoot biomass

Shoot biomass was collected at two different growth stages, at tillering stage and at physiological crop maturity. At tillering stage, shoot biomass reduced significantly ($p < 0.001$) with increasing soil As concentration, while in MW treated pots, significantly ($p < 0.001$) higher biomass was recorded. In view of the MW treatments, higher biomass was harvested at MW-6 compare with the MW-3 and MW-0 treatments. The highest biomass was observed in MW-6 at As-0 and lowest in the MW-0 at As-80 (Figure 5.3a). The effect of biochar and the combined effect of MW and biochar was non-significant. At physiological maturity, shoot biomass reduced significantly ($p < 0.001$) in response of soil As concentration, although significantly ($p < 0.001$) higher biomass was recorded in MW treatments (MW-3 and MW-6) irrespective of soil As concentration compared with MW-0. Especially higher biomass was harvested in the MW-6 treatment compared with the MW-0 and MW-3 treatments (Figure 5.3b). The effect of biochar application showed strong

decreasing trends ($p = 0.089$) with increasing biochar rates. However, combination of biochar and MW treatment (MW-6 and BC-10 treatment) had the highest shoot biomass at As-0.

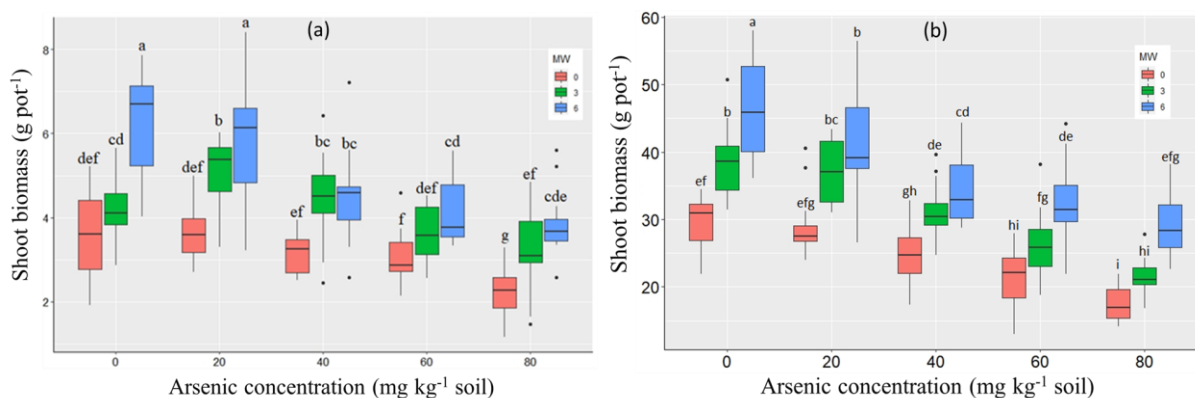


Figure 5.3. Mean shoot biomass yield at (a) tillering stage and (b) physiological maturity stage in response to arsenic (As) and microwave (MW) soil treatments. In boxplot, values having different letter indicates the significant difference (LSD test at 5 % level of significance) among the treatments

5.4.1.6 Number of panicles per pot

The number of panicles per pot decreased significantly ($p < 0.001$) with the increasing of soil As concentration while, a significantly ($p < 0.001$) higher panicle number was recorded in MW treated pots compared with the MW-0 across all the soil As concentration. The lowest panicle number was observed at As-80 in MW-0, whereas the highest number was observed in the MW-6 at As-0 (Figure 5.4a). However, the effect of biochar alone, and combined MW and biochar was non-significant ($p = 0.411$ and 0.059).

5.4.1.7 Spikelet sterility (%)

Rice spikelet sterility increased significantly ($p < 0.001$) with increasing soil As concentration while, MW soil treatment significantly ($p < 0.001$) reduced the spikelet sterility across all the soil As concentration. For example, the highest spikelet sterility was observed at As-80 in MW-0 while, the lowest value was recorded at As-0 in MW-6 (Figure 5.5a). In response to biochar application, spikelet sterility reduced significantly ($p < 0.001$) in biochar treatments compared with BC-0 (Figure 5.5b). The highest sterility was observed at As-80 with BC-0 while, the lowest sterility was observed at As-0 in BC-20. The combined effect of MW and biochar was found to be non-significant.

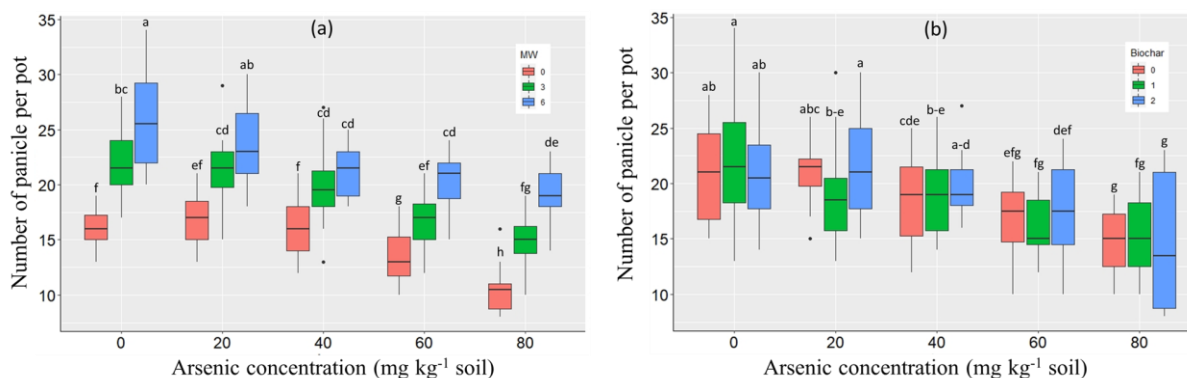


Figure 5.4. Effect of (a) microwave (MW) and (b) biochar treatments on mean panicle number in arsenic (As) contaminated soils. Replicated mean values are shown in boxplot. Different letter indicates the significant difference (LSD test at 5 % level of significance) among the treatments

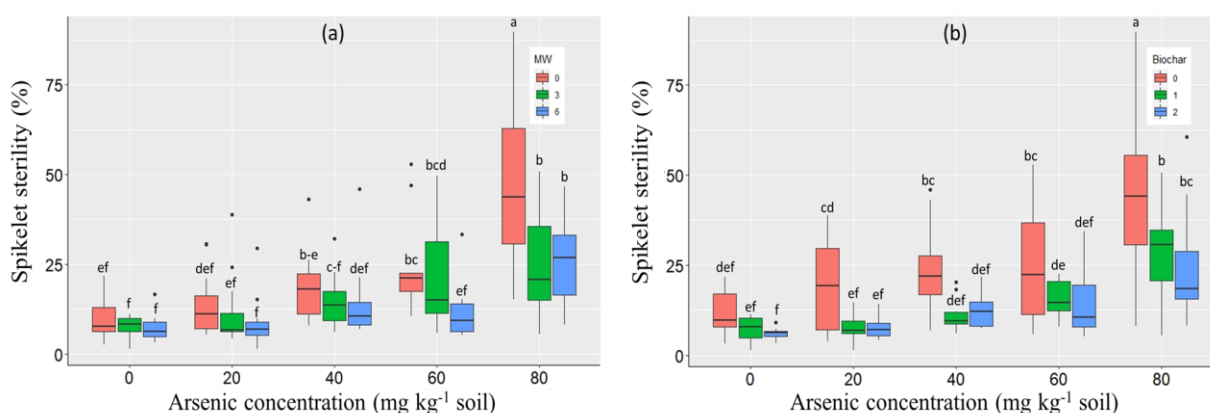


Figure 5.5. Effect of soil arsenic (As) on spikelet sterility in response to (a) microwave (MW) and (b) biochar treatments. Replicated mean values are shown in boxplot. Different letter indicates the significant difference (LSD test at 5 % level of significance) among the treatments

5.4.1.8 Grain yield

Rice grain yield per pot reduced significantly ($p < 0.001$) in response of soil As concentration increases while, significantly ($p < 0.001$) higher grain yield was recorded in MW treatments compared with MW-0 irrespective of soil As concentration. Especially, significantly higher grain yield was harvested in the MW-6 treatment compared with the MW-0 and MW-3. The lowest yield was recorded at As-80 in MW-0 and the highest yield was recorded at As-0 in MW-6 (Figure 5.6a). The highest yield reduction (82.36 %) was recorded at As-80 in comparison to control treatment. However, less reduction was observed under MW treatment, such as only 32.86 % reduction was found when MW-6 treatment was applied at the same As-80 treatment. The MW-6

treatment increased yield by 92.59 % at As-0, compared with the control. Microwave treatment increased rice grain yield up to As-60 (2.78 %) while, yield decline was observed at As-20 with no MW treatments (Figure 5.7). The grain yield increased significantly ($p = 0.014$) in biochar treatments compared with the BC-0 (Figure 5.6b). The combined effect of MW and biochar was non-significant (Appendix 5.7).

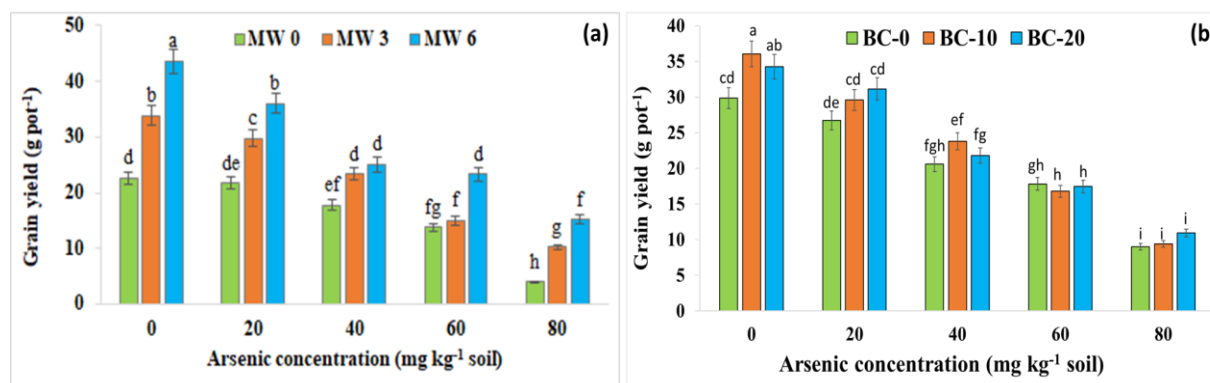


Figure 5.6. Effect of (a) microwave (MW) and (b) biochar (BC) treatments on rice grain yield at different soil arsenic (As) concentration. Replicated mean value are shown in bars with standard error. Different letter indicates the significant difference (LSD test at 5 % level of significance) among the treatments

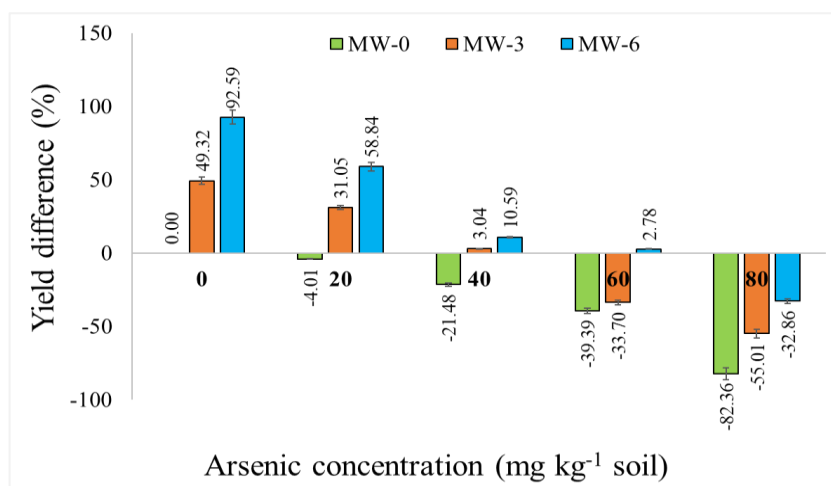


Figure 5.7. Grain yield difference at different soil arsenic (As) concentration in microwave (MW) treated soil. The mean values are shown in bar along with standard error and value of difference. Positive value indicates the increase in yield and negative value shows a reduction in grain yield

5.4.2 Grain total arsenic concentration

Grain As concentration increased significantly ($p < 0.001$) with increasing soil As concentration. Whereas, it was significantly ($p < 0.001$) lower in the MW treatments, compare with the MW-0 across all the soil As concentration. Grain As concentration was highest ($912.9 \mu\text{g kg}^{-1}$) at As-80 without MW treatment while, it was significantly lower ($442.4 \mu\text{g kg}^{-1}$) in MW-6 at same As treatment (As-80). The lowest grain As concentration was observed at As-20 with MW-6 treatment (Figure 5.8a). Thus, the reduction of As concentration was significantly higher in MW treated pots compared with the MW-0 treatment throughout the soil As concentration (Figure 5.9a). Furthermore, biochar application also had a significant ($p < 0.001$) effect on grain As concentration (Figure 5.8b). The BC-10 treatment recorded less grain As concentration at As-20 and As-40 treatments whereas, biochar could not reduce grain As concentration at higher soil As treatment (As-60 and As-80) (Figure 5.9b). On the other hand, grain As concentration was higher in the BC-20 treatment compared with BC-0 and BC-10 at all soil As concentration (Figure 5.8b). The combined effect of biochar and MW was non-significant (Appendix 5.8).

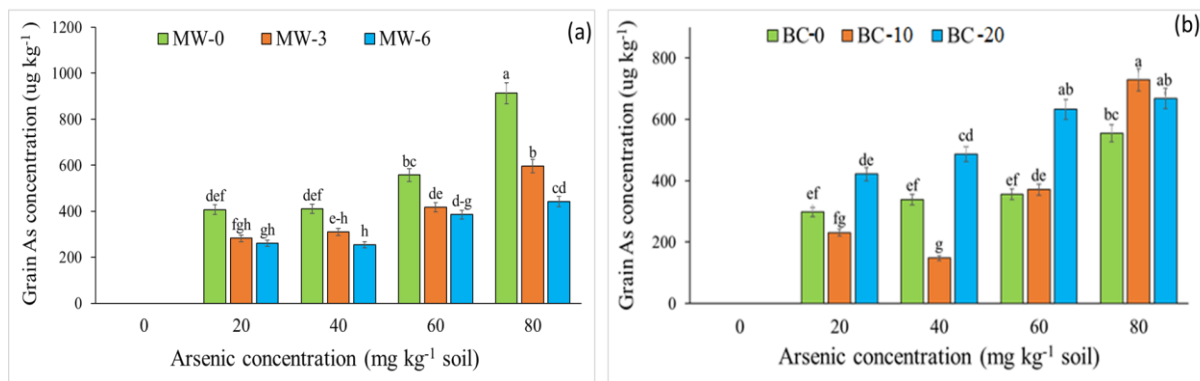


Figure 5.8. Effect of soil arsenic (As) on grain As concentration in response to (a) microwave (MW) and (b) biochar (BC) treatments. Replicated mean value are shown in bars with standard error. Different letter indicates the significant difference (LSD test at 5 % level of significance) among the treatments

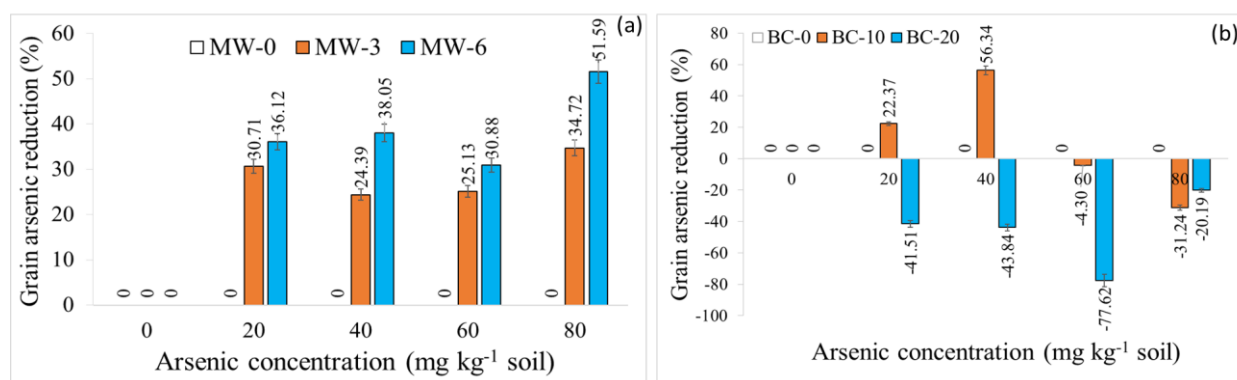


Figure 5.9. Grain arsenic (As) concentration reduction in response to (a) microwave (MW) and (b) biochar (BC) treatments at different soil As concentration. The mean values are shown in bar along with standard error and value of difference. Positive value indicates the reduction of grain As accumulation while, zero and negative value show no reduction and increase in concentration respectively

5.4.3 Correlation of plant growth and yield parameter with grain total arsenic concentration

Pearson's correlation coefficient (r value) showed that, all the plant growth parameters were positively correlated with the yield parameters and all the growth and yield parameters were negatively correlated with grain As concentration. Two-sided test of correlations different showed that all the correlation coefficients (r value) were statistically significant except correlation ($r = -0.1048^{ns}$) between leaf chlorophyll content and grain sterility (Table 5.6).

Table 5.6. Pearson's correlation matrix of different plant growth and yield parameter with grain arsenic (As) concentration

Variables		<i>r</i> -value							
Grain As concentration	1	—							
Grain yield	2	− 0.6176***	—						
Shoot biomass at T	3	−0.4572***	0.7184***	—					
Shoot biomass at PM	4	−0.5916***	0.8372***	0.6902***	—				
Plant height at 90 DAS	5	−0.5065***	0.5704***	0.4922***	0.582***	—			
Panicle number	6	−0.484***	0.8324***	0.6559***	0.869***	0.4872***	—		
Tiller number	7	−0.4748***	0.7564***	0.6568***	0.8065***	0.3941***	0.7866***	—	
Grain sterility	8	0.4463***	−0.6523***	−0.4182***	−0.3826***	−0.3235***	−0.4224***	−0.4412***	—
Leaf chlorophyll content	9	−0.2325**	0.3406***	0.1939**	0.4013***	0.5958***	0.4063***	0.2383**	−0.1048 ^{ns}
		1	2	3	4	5	6	7	8

*, ** and *** indicate significance at $p < 0.05$, < 0.01 and < 0.001 , respectively and ^{ns} indicate non-significant. T = tillering, PH = physiological maturity, DAS = days after sowing

5.5 Discussion

5.5.1 Effect of soil arsenic on plant growth, grain yield, and grain arsenic concentration

It is evident from this study that, rice plant growth and grain yield reduce significantly with the increase of soil As concentration from 20 to 80 mg kg⁻¹ soil. Higher concentration of As is toxic to most of the plants, which can interfere with the plant metabolic processes and inhibit plant growth and development through As induced phytotoxicity (Rahman et al., 2007a). Previous research revealed that As can significantly decrease the rice plant growth and grain yield along with reducing the leaf number, length, and area, panicle number, filled grain and total grain weight when grown in As contaminated soil (Asaduzzman et al., 2010; Vromman et al., 2013; Upadhyay et al., 2016). These above findings undoubtedly shown that the reduction of plant growth was ultimately the result of As phytotoxicity which agrees with the results of this experiment. Straighthead is a most common disease in rice, which is due fewer filled grains and the panicle remain upright when plants are expose to high concentration of soil As (Li et al., 2016b). Straighthead disease was observed (data not provided) in this experiment at the higher concentrations of soil As treatments. Therefore, higher spikelet sterility was recorded at higher soil As concentration which results in fewer filled grain and ultimately low grain yield in As treated pots. It is also evident from Table 5.6 that all the plant growth and yield contributing data were positively correlated with the grain yield except grain sterility. This agrees with the above discussion and findings of other researches.

From Figure 5.8 it was evident that rice grain As concentration increased significantly with increasing soil As concentration. As uptake by the plants is dependent on several factors such as plant species or variety, soil As concentration, soil pH, soil redox potential e.g. oxidized or reduced condition of soil, soil texture, other ions in soil solution, and the chemical form of As i.e. As speciation (Zavala and Duxbury, 2008; Bhattacharya et al., 2015; Khalid et al., 2017; Abbas et al., 2018). Among all these factors, soil As concentration is an important factor. Several studies assuredly concluded that rice grain As concentration increase when plant exposed to higher soil As. For example, rice grain As concentration of 0 – 2.60 mg kg⁻¹ dry weight at 1.9 – 40 mg kg⁻¹ soil As (Bhattacharya et al., 2013) and 0.69 – 1.6 mg kg⁻¹ dry weight at 0 – 50 mg kg⁻¹ soil As concentrations were reported (Azad et al., 2009). Thus, this discussion clearly shows that rice grains can accumulate more As when grown in highly As contaminated soil, which agrees with the current findings from this experiment.

Arsenic is a non-essential element for plants, which can affect plant growth and productivity through As induced phytotoxicity due to physiological, biochemical, morphological and molecular alteration if it is present in excessive amount in soil (Khalid et al., 2017; Srivastava et al., 2017). Rice plant can uptake the more toxic form of As [As(III)] in increased amount since it is grown under flooded (reduced) condition where As(III) become more available for plant uptake. Rice is one of the most efficient accumulators of Si and rice plants uptake As(III) through Si transporters, which facilitate greater As(III) uptake by rice plant and ultimately higher As accumulation in rice grain (Bakhat et al., 2017). In general, after uptaking the As from the soil to the plant, it can affect the plants by means of two process. One is disfunction of some key enzymes either by replacing the obligatory ions from their active site or through interaction with sulfhydryl groups. Another is indirectly through the eruption of reactive oxygen species (ROS), which results in a cataract effect of irreversible injuries in plants (Finnegan and Chen, 2012). Arsenate toxicity in the plant mainly happens due to an antagonistic relationship with phosphate (PO_4^{3-}), which is a consequence of a reaction with P or a P-ester as a substrate thereby disrupting vital biochemical processes include phospholipid metabolism, RNA/DNA metabolism, protein phosphorylation/dephosphorylation, oxidative phosphorylation, and glycolysis (Gresser, 1981; Finnegan and Chen, 2012). For example, As(V) can replace the PO_4^{3-} in the process of ATP synthesis by forming ADP-arsenate the ultimate inhibition of oxidative phosphorylation and increases As(V) toxicity (Rahman et al., 2014b). In addition, As(III) has higher affinity to react with sulfhydryl groups of enzymes and proteins than P, which leads to the deterioration of membranes, inhibition of cellular functions and subsequently cell death (Meharg and Hartley, 2002). In addition, higher soil As concentration can leads to a lower photosynthesis rate by decreasing the chlorophyll content, which consequences the reduced plant growth and grain yield (Rahman et al., 2007a). Increases in lipid peroxidation and hydrogen peroxide (Nath et al., 2014), decreases in both essential and non-essential amino acids (Dwivedi et al., 2012), reductions in protein and chlorophyll content (Ahmad et al., 2012b), and reductions in photosynthetic rate (Rahman et al., 2007a) of rice plants grown in As contaminated soil also reported. The results of the present experiment reveal that leaf chlorophyll content decreased with increasing soil As concentration (Table 5.4) which is in agreement with above statement. From Pearson's correlation analysis it is also evident that, the correlation between chlorophyll content and growth parameters was significantly positive (Table 5.6), which also support the above discussion.

5.5.2 Effect of microwave soil treatment on plant growth, grain yield, and grain arsenic concentration

Although the rice plant growth and grain yield reduced significantly with increasing soil As concentration, MW soil treatment showed a significantly beneficial effect on rice plants' growth and grain yield across all the soil As concentration. In particular, at the MW-6, plant growth and grain yield were significantly higher compared with the MW-3 and MW-0 treatment. A field study reported 1.20 – 1.50 t ha⁻¹ extra rice grain yield in MW treated plots where, 2 kW MW generator was used at 2.45 GHz frequency for 60s to treat the field soil to achieve the soil temperature of about 70 –75 °C in top soil layer (0 – 5 cm) (Khan and Brodie, 2018). The MW soil treatment in another rice field showed a significant increase in plant fresh biomass (50.90 %) and dry biomass (42.40 %), higher tiller numbers (387 m⁻²) compared with the control plot (268 m⁻²), and higher grain yield (7.80 t ha⁻¹) than the control plots (5.60 t ha⁻¹) (Khan et al., 2018). These findings agree with the results of this current experiment. In terms of grain As concentration, MW soil treatment significantly reduced the As concentration in rice grains across all the soil As concentrations (Figure 5.8).

The possible reasons for increased crop growth and grain yield of rice in MW treated soil can be explained by a couple of changes in soil after MW heating and its impact on other related circumstances. One of the possible reasons could be the increased soil nutrient (e.g. N, P, and S) availability in MW treated soil (Table 5.1) which could enhance the plant growth and grain yields. Similar findings were reported in the previous research where, MW treatment increased the availability of some soil nutrient e.g. N, S, and P (Gibson et al., 1988; Brodie et al., 2017; Khan and Brodie, 2018). To investigate the MW heating effect on soil N availability Khan et al. (Khan et al., 2016) designed an experiment where they imply that MW soil treatment mineralized soil indigenous N which resulted in a significant increase in crop biomass by 175 % and grain yield by 92 % of wheat compared with the control. A recent study revealed increased inorganic P (+1.2-fold compared with the control), and nitrate-N content in soil (Maynaud et al., 2019).

In terms of grain As concentration of current study, MW soil treatment significantly reduced the As concentration in rice grains across all the soil As concentrations (Figure 5.8). Several factors could contribute to reduce the As concentration in the rice grain in MW treatments. One factor could be the dilution effect in which plants could uptake the same amount of As from the soil, but this could be diluted after translocating into the grain because

of the higher grain yield in the MW treatment (Appendix 5.7 – 5.9). Beside the enhancement of crop growth and grain yield, the increased soil P and Si concentration after MW soil heating in this current study (Table 5.1) could explain the reduced grain As concentration. The PO_4^{3-} and Si is known as analogous to As(V) and As(III) respectively (Anawar et al., 2018). Therefore, increased PO_4^{3-} and Si availability in the soil results in enhanced competition for adsorption sites on soil particle surfaces and for plant uptake because of the similar uptake mechanism of PO_4^{3-} and As(V) through PO_4^{3-} transporter and Si and As(III) through aquaporin channels present in the plant root (Zhao et al., 2010a; Mosa et al., 2012). Thus, the increased soil P and Si concentration after MW soil heating could compete with As(V) and As(III) for plant uptake and reduce the accumulation in the grain. However, some different results are also reported after MW soil heating, like a declined in P concentration (Speir et al., 1986), and no significant effect on total N, P, K and S concentrations (Cooper and Brodie, 2009). Thus, further detail investigation is needed, in controlled conditions, to understand the MW heating effect on soil physicochemical properties at different temperature levels, because the soil temperature of around 75 – 85°C neither strongly modifies the soil properties (O'Brien et al., 2018) nor totally disinfects the soil (Voort et al., 2016).

Another important change in soil after MW heating is alteration of the physical and chemical properties of SOM, molecular composition (C, H, O and N), chemical structure, and enhanced humification of SOM (Hur et al., 2013; Kim and Kim, 2013). The formation of humic and fulvic acids, the more easily degradable forms of recalcitrant humic substances due to the thermal degradation induced by MW soil irradiation are the possible mechanism of SOM humification. This would have enhanced the amount of carbon and free amino acids for turnover in the carbon and NH_4^+ pool which may favor soil health (Kim and Kim, 2013; O'Brien et al., 2018). However, the particular mechanism behind this assumption is still in question. It has been also reported that, MW soil heating can increase soil organic carbon (Zagal, 1989), macromolecular organic substances that possess a higher number of functional groups (Kim and Kim, 2013), and synthesis of organometallic and coordination compounds (Taylor et al., 2005). These organic substances can retain, decrease mobility, reduce bioavailability and adsorb soil heavy metals (Yin et al., 2002). Hur et al. reported that, MW soil heating can enhance the binding efficiency of hydrophobic organic contaminants with the more humified SOM (Hur et al., 2013). Therefore, there were possibilities of As adsorption by adsorption sites of the soil and less accumulation into the grain. Thus, the above changes in

SOM could enhance the adsorption of As and reduce plant uptake. Further detailed investigations are needed to understand As behavior in soil after MW treatment.

Furthermore, MW also has adverse impacts on microbial communities in the soil. For example, MW soil heating can create a transient disturbance in soil bacterial communities and may affect their composition and function (O'Brien et al., 2018). However, due to the higher diversity and rapid growth in the bacterial communities, the rapid recovery of community structure and function could result in long-term negative effects of MW heating on soil bacteria (Fierer, 2017) although the long-term effects of MW heating would depend on heating duration and intensity (Nunes et al., 2018). MW irradiation induced disintegration of microbial cells, which can release the intracellular and extracellular macromolecules, may increase the soluble OM and organic-N (org-N) mineralization in the soil (Zhou et al., 2010). Previous research reported three pathways of org-N transformation: (1) microorganisms based org-N mineralization to ammonium, (2) release of org-N due to cell lysis, and (3) ammonium excreted from the bacterial grazing on soil fauna (Schimel and Bennett, 2004). Research also showed that, the org-N mineralization following MW irradiation of soil is of microbial origin (Speir et al., 1986). However, MW heating impacts on soil microbes needs to be further investigated for a better understanding of its role in crop growth and yield.

5.5.3 Effect of biochar on plant growth, grain yield, and grain arsenic concentration

Addition of sawdust biochar at 10 t ha^{-1} soil significantly increased plant growth and grain yield compared with the control treatment, while it decreased again at 20 t ha^{-1} of biochar application irrespective of As and MW treatment. Several studies showed that, biochar application in soil has the potential to increase rice crop growth and grain yield. For example, Khan et al. conducted a pot experiment with sewage sludge derived biochar and reported a significant increase in rice shoot biomass, grain yield, and total number of tillers (Khan et al., 2013). They used 5 and 10 % (w/w) biochar and found a 71.3 and 71.3 % increase in shoot biomass compared with the control soil, respectively. In the view of grain As concentration, biochar application showed both negative and positive effect based on the application rate. At BC-10, lower grain As was observed in As-20 and As-40 soil As concentration compared with the BC-0. Whereas, no difference was observed in grain As concentration at higher soil As concentrations (As-60 and As-80) in BC-10 (Figure 5.9b). On the other hand, grain As concentration increased in the BC-20 treatment compared with the BC-0 across all the soil As concentration. Numerous researches confirmed the use of biochar as an effective material for

As remediation, due to its tremendous ability to sort As with subsequent alleviation of As phytotoxicity (Beesley et al., 2011; Zheng et al., 2012; Gomez-Eyles et al., 2013; Waqas et al., 2014). For example, significant reduction in the concentrations of As(III), As(V), and DMA by 72, 62, and 74 % respectively in rice was reported using sewage sludge biochar (2 %, 5 % and 10 % on a dry weight basis) (Waqas et al., 2014).

Biochar has been widely reported as a soil amendment to alleviate the bioavailability of heavy metals due to its huge surface area, alkaline pH, strong sorption capacity and high cation exchange capacity (Zhang et al., 2013; Bian et al., 2014). The general benefits of biochar application are, increase soil organic carbon, increased water holding capacities, retention of nutrients and prevent their loss, direct nutrient supply, increased microbial population and microbial activity in soils, and improve soil structure, which ultimately improves the soil fertility and increases crop growth and yield (Lehmann et al., 2002; Atkinson et al., 2010; Sohi et al., 2010; Stavi and Lal, 2013). For instant, biochar application is known to increase soil C, N and P content (Chan and Xu, 2012; Zhang et al., 2012b), and enhanced availability of Ca, Mg and Zn (Major et al., 2010; Gartler et al., 2013). These essential plant nutrients in biochar are usually conducive to crop production. In addition, biochar has many surface functional group, which can increase the CEC of soil (Laghari et al., 2015) and reduce nutrient leaching from the soil profile and increase nutrient availability to plants' roots (Laird et al., 2010). Furthermore, biochar can harbor a suitable habitat for useful soil microbes (Laghari et al., 2016), and provides nutrients for their growth and stimulates their activity (Zhu et al., 2017), thus enhancing soil fertility and crop productivity (Singh et al., 2015a).

Both mobilization and immobilization of As by biochar application in soil has been reported. Arsenic can be immobilized by ion exchange (surface inner sphere complex or co-precipitation with biochar), surface complexation with different functional groups of biochar (carboxyl, alcoholic hydroxyl or phenolic hydroxyl groups), and chemical or physical sorption (Lu et al., 2012; Zhang et al., 2013; Vithanage et al., 2014). For example, biochar can decrease the As content in plant tissues by retaining As on its surface (Namgay et al., 2010; Beesley and Marmiroli, 2011). Even though surface adsorption and complexation are identified to play significant roles in the interaction among biochar and As (Ahmad et al., 2014; Beesley et al., 2014), the mechanisms of As immobilization by biochar are usually complex and differ from soil properties and types of biochar (Hale et al., 2012; Khan et al., 2015). The enhanced bioavailability of soil P for plant uptake after addition of biochar has been reported (Sohi et al., 2010; Wang et al., 2012). Being a PO_4^{3-} analog, increased P can reduce As(V) uptake and

accumulation in rice grain. In addition, application of biochar found to be increases the soil S content and its availability (13 – 16 fold), which could have interacted with As and reduced its uptake by the rice plants (Zhang et al., 2010a). Furthermore, increased Si concentration in soil solution after biochar application has also been reported, which may participate in As immobilization via formation of silicate precipitates and may reduce As uptake by competing with As(III) for same uptake transporter (Houben et al., 2014).

On the other hand, biochar application can results in As mobilization in rice soils under anaerobic conditions (Choppala et al., 2016). Zheng et al. (Zheng et al., 2012) also reported an increase (327 %) in As accumulation in rice grain due to biochar application prepared from rice residues. Likely some other studies also reported the increase availability of As after biochar application (Beesley et al., 2013; Zheng et al., 2015) resulting in the high As toxicity to rice plant (Chen et al., 2016). Arsenic can mobilize due to reactions with ionizable functional groups of biochar and interaction with DOC release due to biochar application. As(V) can reduce to As(III) by interacting with DOC could be serving as electron donors (Choppala et al., 2016; Yang et al., 2016), which enhances As mobility (Park et al., 2011b; Zhang et al., 2013). It was reported that biochar was sometimes unable to adsorb As(III) (Zhu et al., 2016) and is more challenging to immobilize than As(V) because of high mobility (Zhu et al., 2018). This results in a bio-accessibility increase of As in soil and phytotoxicity in crops (Vithanage et al., 2014). Moreover, it is widely reported that the addition of biochar to soils has resulted in pH increases (Jones et al., 2012; Kim et al., 2018). During the pyrolysis process, at high temperature, cations are transformed into oxides, hydroxides or carbonates in the biochar and (Yuan et al., 2011) dissolution of these alkaline substances increase the soil pH (Houben et al., 2013). In the soil solution, As is mainly present as hydro anion and increased soil pH after biochar application can reduce As sorption capacity by decreasing the positively charged sites on soil minerals, which increases As mobilization and release from the soil (Wilson et al., 2010). Khan et al (Khan et al., 2013) reported the mobilization of As after application of sewage sludge biochar into soil due to rise in soil pH. Hartley et al. (Hartley et al., 2009) also reported the mobility of As with biochar results from the rise in soil pH. The pH of the current experimental soil also increased after the saw dust biochar application (Appendix 5.2). At BC-10 and BC-20 treatment the average soil pH was 8.15 and 9.00 respectively while 7.73 was observed in the BC-0 treatment. Therefore, based on the above discussion, higher soil pH at BC-20 could enhance the availability of As in the soil solution and ultimately higher rice grain As accumulation was observed. Thus, reduction or increase of As mobility, bioavailability, and

plant uptake depends on the application rate of biochar. Therefore, it requires precise studies in terms of As binding, transformation, and releases into the soil after adding of saw dust biochar.

5.6 Conclusions

Rice plant growth and grain yield decreased significantly due to As phytotoxicity and grain As concentration increased significantly with increased soil As concentration. However, MW soil treatment had the potential to alleviate this As phytotoxicity and reduce the grain As concentration. Especially, MW-6 treatment found to be effective than the MW-3 compared with MW-0. Sawdust biochar can be used at the rate of 10 t ha⁻¹ in low As contaminated soils (20 – 40 mg kg⁻¹) to alleviate As phytotoxicity in rice and to reduce grain As concentration. Higher application rate (BC-20) of sawdust biochar can increase As phytotoxicity and grain As concentration in rice. In combination of MW and biochar, MW-6 with BC-10 exhibit better plant growth and grain yield and low As accumulation in grain. However, for a better understanding of the enhanced growth in MW treated soil, in combination with biochar, further validation experiments are needed especially in the field condition.

Residual effects of MW and biochar are important for the sustainability of the technology and the frequency of the application. Thus, along with the effect of MW and biochar in the actual treatment year, it is also important to evaluate the residual effect on As phytotoxicity alleviation. Therefore, the next chapter was designed to explore the residual effect of MW and sawdust biochar on the phytotoxicity alleviation in the wheat and rice.

Chapter 6 – Residual effect of microwave and biochar soil treatment on arsenic phytotoxicity alleviation in wheat and rice

6.1 Abstract

Microwave (MW) soil heating, at a threshold temperature, has been proposed as a unique technique, which can significantly enhance plant growth in addition to the process of arsenic (As) phytotoxicity alleviation in wheat and rice. In combination with MW, biochar application at 10 t ha⁻¹ soil rate was also found to be effective in alleviating As phytotoxicity in rice. Original treatment year pot experiments have demonstrated these favorable outcomes in wheat and rice; however, understanding the residual effect of MW and biochar is crucial to determine the application frequency of the treatment. Therefore, wheat and rice were grown in the next year (one year follow on from the initial treatment) using the same pots, without the addition of further MW and biochar treatment to investigate the residual effects. The results after 360 days of MW soil heating revealed the potential to alleviate As phytotoxicity in both wheat and rice, as shoot biomass and grain yield, increased significantly and facilitated less grain As concentration compared with the control treatment. For example, in wheat, 14.66 % and 19.01 % higher shoot biomass and grain yield, respectively, and 35.75 % less grain As concentration were recorded at MW-6 compared with MW-0 treatment. Whereas, in rice, 31.25 % and 39.11 % higher shoot biomass and grain yield, respectively, and 38.43 % less grain As concentration were recorded at MW-6 compared with MW-0 treatment. It was also evident from the result that 360 days after biochar application in rice, shoot biomass and grain yield were 10.39 % and 9.38 % higher, respectively, in the BC-10 treatment whereas, it was 14.82 % and 3.81 % higher in the BC-20 treatment, compared with the BC-0 treatment. Furthermore, 13.51 % less grain As concentration was recorded in the BC-10 treatment while, it increased by 8.29 % at BC-20 compared with BC-0. Thus, either MW soil treatments alone or in combination with BC-10 treatment have the potential to alleviate As phytotoxicity and decrease As concentration from wheat and rice grains harvested in the following year without further MW treatment and biochar application. However, further study is needed to investigate the long-term effect of MW and biochar in response to As phytotoxicity alleviation.

Keywords: Microwave soil heating, Biochar, Arsenic phytotoxicity alleviation, Residual effect, Wheat, Rice.

6.2 Introduction

Toxic elements such as arsenic (As) often pollute agricultural soils and pose a risk to human health through the soil–plant–food chain (Banerjee et al., 2013). Besides geogenic sources, anthropogenic activities also increase As in soil (Mitra et al., 2017). For instance, shallow groundwater, often rich in As, is widely used for crop irrigation in some highly As contaminated areas (e.g. Bangladesh). In the long term, this increases the As contents in soil and food grains (Williams et al., 2007). Although there are several techniques that have been used to remove As from drinking water, treating huge quantity of irrigation water in the same way is prohibitive. In comparison with other remediation methods, the *in-situ* immobilization of soil As could be an effective technique (Beesley and Marmiroli, 2011), particularly for huge areas of polluted agricultural land (Zheng et al., 2015). In this context, our previous studies (Chapter 4 and 5) revealed that microwave (MW) soil treatment has the potential to alleviate As phytotoxicity and subsequently lower grain As accumulation in wheat and rice.

In addition, saw dust biochar, produced from a MW assist pyrolysis process, was used in the previous rice experiment, in combination with MW soil heating treatment (Chapter 5). Increased plant growth and grain yield and decreased grain As concentration was observed in the MW treatments compared with the control. Similar results were recorded at the lower rate of biochar application (10 t ha^{-1}) compared with the control and higher biochar rate (20 t ha^{-1}). Understanding the residual effect of MW soil treatment on crop productivity is crucial for the sustainability, implementation, and application frequency of this new technique. However, the residual impact of a one-time MW soil heating treatment of As contaminated soil on crop growth, grain yield, and grain As accumulation has not yet been studied. In addition, the residual effect of MW pyrolyzed biochar on the immobilization of soil As have not been well studied. Therefore, this study aimed to investigate the residual impacts of MW soil heating and MW pyrolyzed biochar on the phytotoxicity and accumulation of As in wheat and rice grain.

6.3 Materials and methods

6.3.1 Application of arsenic, microwave, and biochar treatment

Since the main aim of this experiment was to explore the residual effect of MW and biochar treatment on the alleviation of As phytotoxicity, crops were grown in the same pots as the previous year's experiment without further MW and biochar application. The first wheat experiment was conducted in 2017 with MW treatment and the residual experiment was

conducted in 2018 without further MW treatment. In the same manner, the first rice experiment was conducted in 2018 with MW and biochar treatment and the residual experiment was conducted in 2019 without further MW and biochar treatment. Also, no further As was added in the residual experiment since the soils were contaminated with As from the original treatment year experiment.

6.3.2 Experiment setup

After completing of the original treatment wheat and rice experiment, the soils were kept undisturbed until the start of the residual experiment and soil moisture was maintained at field capacity during this period. The residual experiments were conducted in the same glasshouse where the previous experiments were conducted with the same design (completely randomised design), treatment replication (four replications), and crop variety. The EGA Gregory wheat variety (*Triticum aestivum* L.) and the YRM_70 rice variety (*Oryza sativa* L.) were grown. Before the seed sowing, the basal doses of fertilizers were applied to each pot as per standard practices for Australian wheat and rice cultivation. Other intercultural operations were followed as practiced in the year of the original experiment. The details of the intercultural operations are described in the previous chapters (Chapter 4 for wheat and Chapter 5 for rice). Abbreviated forms were used for As treatments (As-0, As-20, As-40, As-60 and As-80), biochar treatment (BC-0, BC-10 and BC-20) and MW treatment (MW-0, MW-3 and MW-6) to describe the treatment combination more conveniently.

6.3.3 Recording of agronomic data

At the physiological maturity stage, the wheat and rice crops were harvested. Fresh shoot biomass weight of all plant samples was recorded prior to drying at 60 °C in a dehydrating oven (Thermoline Scientific, TD-500F) for 48 hours to determine the dry shoot biomass weight. Wheat and rice grains were collected by hand threshing and grain yield (g pot⁻¹) was calculated as described previously in a glasshouse pot experiment of rice crop (Rahman et al., 2007a).

6.3.4 Grain total arsenic analysis

Total As concentration determination of wheat (whole grain) and rice grain (dehusked) samples was performed by following the procedure described previously (Chapter 4).

6.3.5 Statistical analysis

Statistical analysis of data was performed in the R statistical environment (R version 3.6.2) (Team, 2013). Normality and homogeneity tests of the experimental data were performed to examine the distribution of the data. The analysis of variance (ANOVA) test was performed to determine the significance of tested treatments on variables. The Least Significant Difference (LSD) test was performed to compare the treatment means at 5 % level of significance.

6.4 Results

In this experiment the residual effect of MW and biochar on the plant growth, grain yield and grain As concentration was studied. Results revealed that there was a positive residual effect of MW and biochar beyond 360 days after application. Detailed results are discussed below.

6.4.1 Residual effect of microwave on wheat plant growth and grain yield

It was evident from the results that, in the residual year, the wheat plant growth (Table 6.1) and grain yield (Figure 6.1) increased significantly in MW treatments, compared with the MW-0 treatment, irrespective of soil As concentration. For instance, MW soil treatment significantly increased shoot biomass ($p = 0.001$), and grain yield ($p < 0.001$) compared with the MW-0 treatment. The highest shoot biomass (48.54 g pot^{-1}) and grain yield (33.52 g pot^{-1}) were recorded in MW-6 treatment compared with MW-0 (44.97 g pot^{-1} and 29.58 g pot^{-1} respectively) at As-0, although the lowest shoot biomass (30.49 g pot^{-1}) and grain yield (23.81 g pot^{-1}) were obtained at As-80 with MW-0 treatment. A similar trend in the shoot biomass result was observed in the year of the original treatment. Wheat shoot biomass and grain yield decreased with increasing soil As concentration in the residual year. A similar trend in the results were observed in the original treatment year. In general, the grain yield was relatively higher in the residual year compared with the original treatment year irrespective of treatment combinations (Table 6.3).

Table 6.1. Residual effect of microwave (MW) soil treatment on shoot biomass of wheat at different soil arsenic (As) concentration

MW (min)	Original treatment year (2017)					Residual year (2018)				
	Soil As concentration (mg kg ⁻¹)									
	0	20	40	60	80	0	20	40	60	80
	Shoot biomass (g pot ⁻¹)									
0	40.11 ^{ef}	39.64 ^{ef}	40.27 ^{ef}	38.97 ^{ef}	32.05 ^f	44.97 ^{ab}	41.49 ^{bc}	43.59 ^{abc}	37.92 ^c	30.49 ^d
3	42.61 ^{de}	40.56 ^{def}	45.27 ^{cde}	40.85 ^{def}	38.15 ^{ef}	44.54 ^{abc}	45.62 ^{ab}	44.17 ^{abc}	41.85 ^{abc}	40.27 ^{bc}
6	49.37 ^{bcd}	57.57 ^{ab}	58.94 ^a	54.00 ^{abc}	44.96 ^{de}	48.54 ^a	48.51 ^a	45.14 ^{ab}	45.29 ^{ab}	40.08 ^{bc}
LSD _{0.05}			8.97					6.72		

Mean value with superscript different letter indicates the significant difference among the treatments. Least Significant Test (LSD) was performed at 5 % level of significance for comparisons.

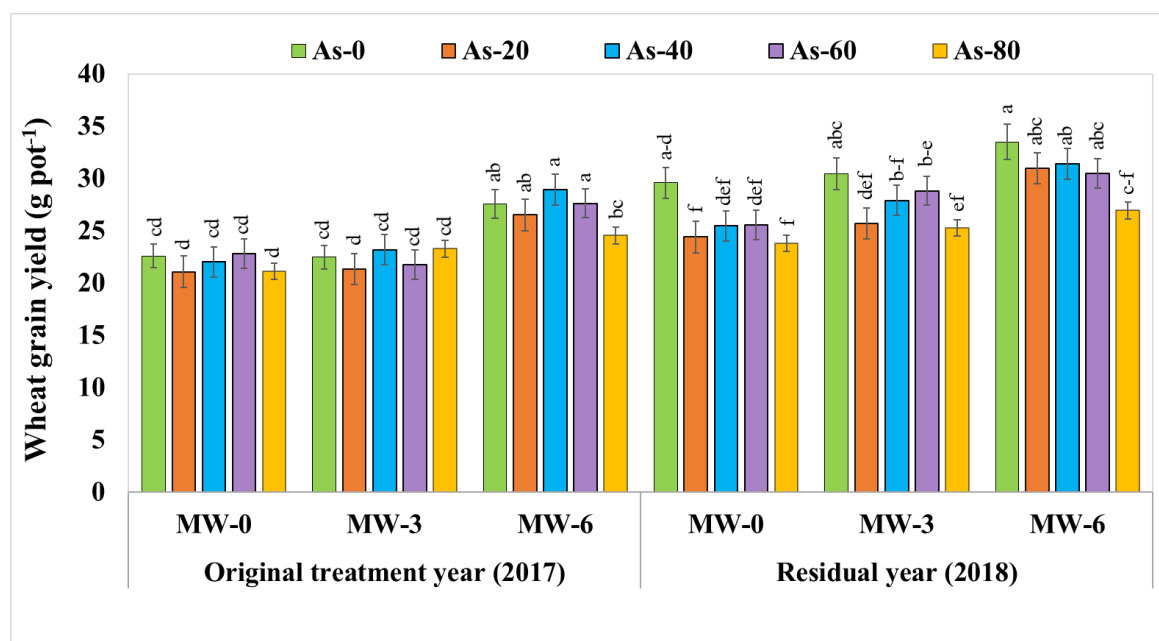


Figure 6.1. Residual effect of microwave (MW) soil treatment on wheat grain yield at different soil arsenic (As) concentration. The mean grain yield values are shown in bars along with standard error. Different letters indicate the significant difference (LSD at 5 % significant level) among the treatments

6.4.2 Residual effect of microwave treatment on wheat grain arsenic concentration

In the residual year, grain As concentration increased significantly ($p < 0.001$) with increasing soil As concentration. Whereas, significantly ($p < 0.001$) lower grain As concentration was recorded in the MW soil treatments, compared with the MW-0 treatment across all the soil As concentration (Figure 6.2). For example, at As-80 treatment, lower grain

As concentration ($321.59 \mu\text{g kg}^{-1}$) was recorded in MW-6 treatment compared with MW-3 ($394.34 \mu\text{g kg}^{-1}$) and MW-0 ($470.07 \mu\text{g kg}^{-1}$) treatment. A similar trend in the grain As concentration result was observed in the original treatment year. However, the grain As concentration was lower in the residual year than the original treatment year across all the treatment combinations (Table 6.3).

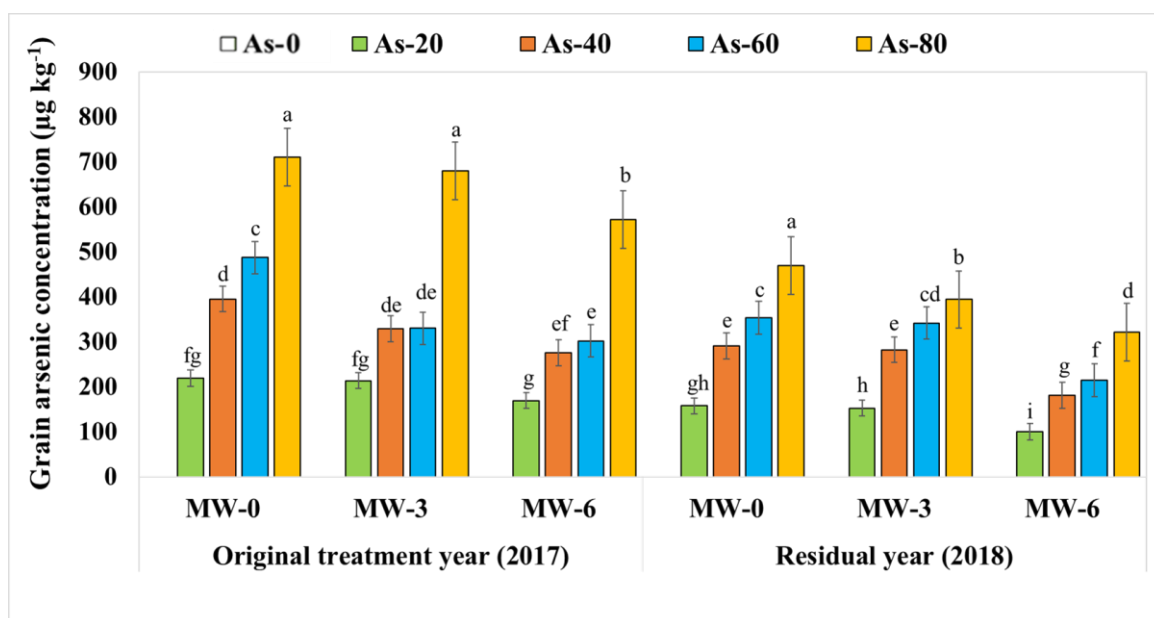


Figure 6.2. Residual effect of microwave (MW) soil treatment on wheat grain arsenic (As) concentration at different soil As concentration. The mean grain yield values are shown in bars along with standard error. Different letters indicate the significant difference (LSD at 5 % significant level) among the treatments

6.4.3 Residual effect of microwave on rice plant growth and grain yield

In the residual year, MW soil treatment significantly increased ($p < 0.001$) rice shoot biomass and grain yield, irrespective of soil As concentration, compared with the MW-0 treatment, although shoot biomass and grain yield decreased significantly ($p < 0.001$) with increasing soil As concentration (Table 6.2). The highest shoot biomass (37.25 g pot^{-1}) was recorded at MW-6 treatment, compared with MW-3 (32.51 g pot^{-1}) and MW-0 (27.59 g pot^{-1}) treatments at As-0. However, the lowest shoot biomass (18.84 g pot^{-1}) was obtained at As-80 with MW-0 treatment. A similar results of shoot biomass and grain yield were observed in the original treatment year. Overall, the grain yield was higher in the residual year for all treatment combinations, compared with the original treatment year (Table 6.3).

6.4.4 Residual effect of biochar on rice plant growth and grain yield

In the residual year, shoot biomass increased significantly ($p < 0.001$) in the biochar treatments (BC-10 and BC-20) compared with the BC-0 treatment (Table 6.2). The highest shoot biomass (24.79 g pot^{-1}) was recorded in the BC-20 treatment while, the lowest shoot biomass (21.82 g pot^{-1}) was obtained in BC-0 treatment at As-80. Furthermore, grain yield increased significantly ($p = 0.003$) in the biochar treatments, compared with BC-0. The highest grain yield (42.07 g pot^{-1}) was obtained in BC-10 compared with BC-20 (35.21 g pot^{-1}) and BC-0 (38.87 g pot^{-1}) whereas, the lowest grain yield (23.72 g pot^{-1}) was observed in the BC-0 at As-80. Overall, biochar treatment increased shoot biomass in the residual year whereas, decreased shoot biomass in biochar treatments was observed in the original treatment year compared with BC-0 (Table 6.3). However, the grain yield was higher in the residual year for all treatment combinations, compared with the original treatment year (Table 6.3).

Table 6.2. Residual effect of microwave (MW) and biochar (BC) treatment on shoot biomass and grain yield of rice at different soil arsenic (As) concentration

MW / BC	Original treatment year (2018)					Residual year (2019)				
	Soil As concentration (mg kg ⁻¹ soil)									
	0	20	40	60	80	0	20	40	60	80
Effect of MW on shoot biomass (g pot ⁻¹)										
0	29.63 ^{ef}	29.08 ^{efg}	25.05 ^{gh}	21.14 ^{hi}	17.66 ⁱ	27.59 ^{def}	26.71 ^{efg}	25.22 ^{fgh}	22.41 ^h	18.84 ⁱ
3	38.60 ^b	37.36 ^{bc}	31.35 ^{de}	26.26 ^{fg}	21.51 ^{hi}	32.51 ^{bc}	29.47 ^{cde}	28.51 ^{de}	24.12 ^{gh}	24.13 ^{gh}
6	46.54 ^a	41.63 ^b	34.11 ^{cd}	32.26 ^{de}	29.15 ^{efg}	37.25 ^a	35.49 ^{ab}	30.69 ^{cd}	28.70 ^{de}	26.43 ^{efg}
LSD _{0.05}			4.41					3.17		
Effect of biochar on shoot biomass (g pot ⁻¹)										
0	37.32 ^{ab}	38.57 ^a	31.59 ^{cd}	28.35 ^{def}	24.22 ^{fgh}	32.09 ^a	27.3 ^{cd}	24.72 ^{def}	22.63 ^{ef}	21.82 ^f
10	39.66 ^a	35.49 ^{abc}	29.36 ^{de}	24.72 ^{fgh}	22.43 ^{gh}	32.34 ^a	30.81 ^{ab}	28.81 ^{bc}	27.17 ^{cd}	22.79 ^{ef}
20	37.79 ^{ab}	34.01 ^{bc}	29.56 ^{de}	26.60 ^{efg}	21.66 ^h	32.92 ^a	33.57 ^a	30.89 ^{ab}	25.44 ^{de}	24.79 ^{def}
LSD _{0.05}			2.23					3.16		
Effect of MW on grain yield (g pot ⁻¹)										
0	22.67 ^d	21.76 ^{de}	17.80 ^{ef}	13.74 ^{gh}	4.00 ⁱ	31.94 ^{ef}	31.25 ^{ef}	30.4 ^{fg}	27.38 ^{gh}	21.06 ⁱ
3	33.85 ^b	29.71 ^c	23.36 ^d	15.03 ^{fg}	10.20 ^h	38.04 ^{bc}	34.29 ^{cde}	33.36 ^{ef}	31.05 ^{efg}	24.43 ^{hi}
6	43.66 ^a	36.01 ^b	25.07 ^d	23.30 ^d	15.22 ^{fg}	46.17 ^a	40.59 ^b	39.04 ^b	37.75 ^{bcd}	34.04 ^{def}
LSD _{0.05}			3.98					3.81		
Effect of biochar on grain yield (g pot ⁻¹)										
0	29.87 ^{cd}	26.72 ^{de}	20.6 ^{fgh}	17.83 ^{gh}	9.03 ⁱ	38.87 ^{ab}	33.73 ^{cde}	33.21 ^{cde}	30.36 ^{ef}	23.72 ^g
10	36.05 ^a	29.6 ^{cd}	23.83 ^{ef}	16.79 ^h	9.42 ⁱ	42.07 ^a	36.13 ^{bcd}	34.93 ^{cd}	33.38 ^{cde}	28.41 ^f

20	34.26 ^{ab}	31.15 ^{bc}	21.81 ^{fg}	17.45 ^h	10.97 ⁱ	35.21 ^{bcd}	36.27 ^{bc}	34.65 ^{cd}	32.45 ^{de}	27.41 ^{fg}
LSD _{0.05}			3.99					3.80		

The unit of MW and biochar treatment was minutes and $t\ ha^{-1}$ respectively. Mean value with superscript different letter indicates the significant difference among the treatments. Least Significant Test (LSD) was performed at 5 % level of significance for comparisons.

6.4.5 Residual effect of microwave treatment on rice grain arsenic concentration

Rice grain As concentration reduced significantly ($p < 0.001$) in the MW treatments compared with MW-0 in the residual year although, grain As concentration increased significantly ($p < 0.001$) with increased soil As concentration (Figure 6.3). The lowest grain As concentration ($454.99\ \mu g\ kg^{-1}$) was observed in MW-6 treatment compared with the MW-3 ($648.45\ \mu g\ kg^{-1}$) and the MW-0 ($812.86\ \mu g\ kg^{-1}$) treatments the same soil As concentration (As-80). A similar trend in the grain As concentration result was observed in the original treatment year (Figure 6.3). Nevertheless, the grain As concentration was higher in the first year than in the second year in all treatment combinations (Table 6.3).

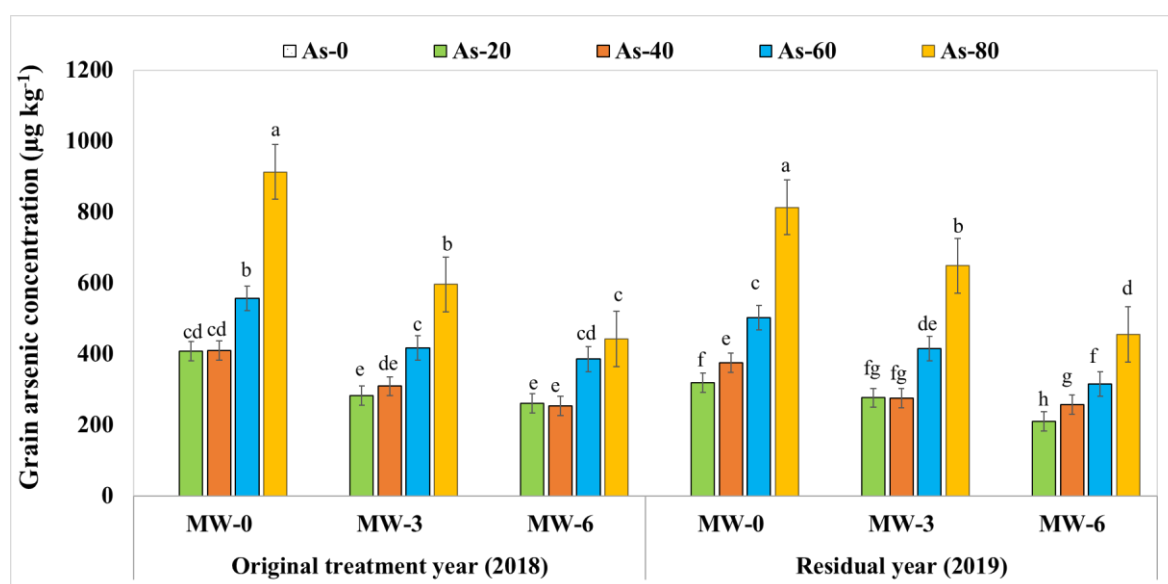


Figure 6.3. Residual effect of microwave (MW) treatment on rice grain arsenic (As) concentration at different soil As concentration. The mean values are shown in bars along with standard error. Different letters indicate the significant difference (LSD at 5 % significant level) among the treatments

6.4.6 Residual effect of biochar on rice grain As concentration

Rice grain As concentration decreased significantly ($p < 0.001$) at BC-10 whereas, at BC-20 grain As concentration significantly ($p < 0.001$) increased compared with the BC-0 treatment in the residual year (Figure 6.4). For instance, the lowest grain As concentration ($200.80\ \mu g\ kg^{-1}$) was observed in BC-10 compared with BC-0 ($301.98\ \mu g\ kg^{-1}$) and BC-20 ($302.60\ \mu g\ kg^{-1}$).

A similar trend was observed in the grain As concentration in the original treatment year (Figure 6.4). Although, the grain As concentration increased at BC-20 compared with BC-0 treatment in the residual year, this increment was lower (8.29 %) than the original treatment year (42.68 %) (Table 6.3). Overall, the grain As concentration was lower in the residual year compared with the original treatment year (Figure 6.4).

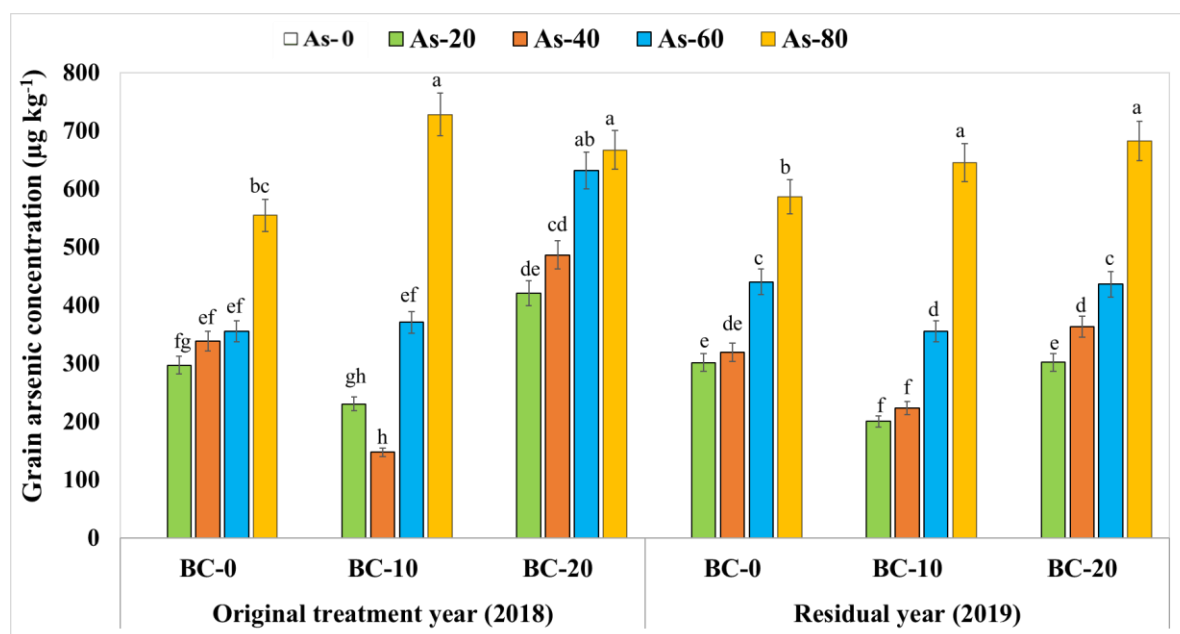


Figure 6.4. Residual effect of biochar treatment on the rice grain arsenic (As) concentration at different soil As concentrations. The mean values are shown in bars along with standard error. Different letters indicate the significant difference (LSD at 5 % significant level) among the treatments

Table 6.3. Comparison of wheat and rice shoot biomass, grain yield, and grain arsenic (As) concentration in response to residual impact of microwave (MW) and biochar (BC) treatment

Parameters	Difference compare to control (%)				Difference compare to original treatment year (%)		
	Original treatment year		Residual year		Residual year		
	MW-3	MW-6	MW-3	MW-6	MW-0	MW-3	MW-6
Wheat							
Shoot biomass	8.58	38.63	9.07	14.66	3.87	4.34	-14.08
Grain yield	2.19	23.29	7.26	19.01	17.46	23.28	13.38
Grain As	-14.27	-27.20	-7.98	-35.75	-29.75	-24.60	-38.01
Rice							
Shoot biomass	26.56	49.90	14.86	31.25	-1.43	-10.54	-13.69
Grain yield	40.28	79.17	13.45	39.11	77.67	43.69	37.94
Grain As	-29.83	-41.33	-19.59	-38.43	-12.13	0.70	-7.78

	BC-10	BC-20	BC-10	BC-20	BC-0	BC-10	BC-20
Rice							
Shoot biomass	-5.25	- 6.53	10.39	14.82	-19.68	- 6.43	- 1.34
Grain yield	11.20	11.15	9.38	3.81	53.68	51.17	43.54
Grain As	- 4.43	42.68	-13.51	8.29	6.62	-3.51	- 19.07

Positive and negative value indicates increase and decrease respectively.

6.5 Discussion

Pre-cropping MW soil heating treatment has been proposed for the alleviation of As phytotoxicity in wheat (Chapter 4) and rice (Chapter 5). In addition, biochar application, along with MW treatment, was found to be an effective technique to alleviate As phytotoxicity in rice (Chapter 5). However, understanding the residual impact of MW and biochar treatment on plant growth and grain yield and subsequent As accumulation in the grain is crucial for the sustainability and determining the application frequency of these techniques. Therefore, the main objective of this study was to investigate the residual effect of MW and biochar treatment on wheat and rice productivity and grain As concentration.

It was evident from the results that, MW soil heating significantly increased the plant growth and grain yield of wheat and rice in the residual year of study, regardless of soil As concentration. This positive residual effect of MW soil heating is supported by the findings of Khan et al. (Khan et al., 2019b), where they reported that MW soil heating at 75 – 85 °C not only significantly increased wheat shoot biomass and grain yield in the first main treatment season but also in the first (440 days after MW treatment) and second residual season (740 days after MW treatment) of crop cultivation. Additionally, they stated that the indigenous N supply was 171.8 % higher in the MW treated soil, compared with the control soil in the first main treatment season, which was even higher (about two times) in first residual cropping season. They reported this increased N in MW treated soils as one of the reasons why a one-off MW treatment had a positive residual effect in the following two cropping seasons, which was also supported by the higher N accumulation in the dry biomass of the MW treated pots. Some previous research also revealed the increase of indigenous N supply through soil heating (70 – 100 °C) treatments (Zagal, 1989; Glass et al., 2008; O'Brien et al., 2018). Furthermore, increased availability of soil nutrients after MW soil heating was reported in several studies. For instance, increased total N levels (106 mg N g⁻¹ soils) (Speir et al., 1986), NH₄⁺-N

concentrations (Zagal, 1989; Yang et al., 1990), NO_3^- -N, P and S availability (Cawse and Crawford, 1967; Wainwright et al., 1980; Cooper and Brodie, 2009), and NO_3^- -N and inorganic P (Maynaud et al., 2019) in MW treated soil was observed. Thus, considering these findings, the increased nutrient availability after MW soil heating could be a possible reason for the continuing positive effect of MW treatment on plant growth and grain yield in the residual year of crop cultivation as well. However, some exceptions like a declined in P concentration (Speir et al., 1986), and no significant effect of MW on total N, P, K and S concentrations (Cooper and Brodie, 2009) were also reported. Which suggest that further investigation needed to understand the sustained effect of MW heating on soil physicochemical properties at different temperature levels.

The positive residual effect of MW can also be explained by the rapid humification of soil organic matter (SOM), which may favor the persistent soil fertility. It has been demonstrated that, MW soil heating can enhance the humification of SOM by enhancing the amount of carbon and free amino acids for turnover in the carbon and NH_4^+ pool (Kim and Kim, 2013; O'Brien et al., 2018). Thus, the ample SOM may increase the mineralization process of organic N (org-N) after MW soil heating. Amino acids, amino sugars, peptides, ammonia, pyrenes, and proteins are the main source of soil indigenous N (Schulten and Schnitzer, 1997). Plants are unable to utilize these org-N unless converted into mineral forms. The conversion of org-N to mineral form (mineralization) depends on the decomposition of SOM. Thus, MW heating can enhance the N mineralization process through rapid decomposition of SOM which may contribute to better crop growth and development for a couple of cropping seasons. Furthermore, MW also has adverse impacts on microbial communities in the soil. For example, MW soil heating can affect the bacterial community composition and their function (O'Brien et al., 2018). Nevertheless, due to the higher diversity and rapid growth, the heat tolerant bacterial communities could recover rapidly where the sensitive community could recover after substantial time, which may contribute to the full action in the residual season of crop cultivation. Also, rapid humification of SOM may increase the substrate availability for soil microorganisms which may favor the activity of nutrient cycling microbes. Thus, rearrangement in the microbial community, after MW soil heating, could enhance the plant growth and grain yield in this residual experiment. However, MW heating impacts on soil microbes needs to be further investigated for a better understanding of its role in crop growth and yield since there will be possibilities of negative effects, depending on the MW intensity and treatment duration.

In terms of grain As concentration, MW soil treatment significantly reduced the As concentration in wheat (0.08-fold in MW-3 and 0.35-fold in MW-6) and rice (0.20-fold in MW-3 and 0.38-fold in MW-6) grains compared with the MW-0 treatment in the residual year of crop cultivation. The lower grain As concentration could be explained by two likely reasons: (1) a dilution effect in which plants could uptake the same amount of As from the soil but this could be diluted after translocating into the grain because of the higher grain yield in the MW treatment; and (2) synthesis of macromolecular organic substances that possess a higher number of functional groups (Kim and Kim, 2013), and synthesis of organometallic and coordination compounds (Taylor et al., 2005) as a result of the enhanced humification of SOM (Hur et al., 2013; Kim and Kim, 2013) where, more As could be adsorbed by the adsorption sites in the soil, resulting in less translocation into the grain. Another study also reported an increase of organic carbon in the soil after MW soil irradiation (Maynaud et al., 2019), which could also enhance the adsorption of As and reduce accumulation in plants. Considering the above discussions, it can be summarized that the rapid increase in soil temperature after MW irradiation may have some immediate positive effects on SOM, hence, this effect could last for the residual cropping year, despite the degrading effect on SOM. However, further detailed investigations are needed to understand As behavior in soil, in relation to changes in SOM after MW treatment.

The addition of biochar significantly increased rice shoot biomass and grain yield and reduced grain As concentration in the residual year. Biochar application at BC-10 had positive impact, while BC-20 had negative impact, however, this negative impact of BC-20 was more in the original treatment year than in the residual year of the study. Application of biochar can increase soil organic carbon, supply nutrients, increased microbial populations and microbial activity in soils, and improve soil structure, which ultimately improves the soil fertility and increases crop growth and grain yield (Atkinson et al., 2010; Sohi et al., 2010; Verheijen et al., 2010; McHenry, 2011). Eventually, these beneficial effects of biochar could continue for couple of cropping years and result increase in shoot biomass and grain yield in the residual year of this current study. This statement was in agreement with the findings of Nzediegwu et al. (Nzediegwu et al., 2020), where increased soil pH was observed in the residual year of biochar application, which suggested that biochar was still active in immobilizing heavy metals. The recalcitrant nature of biochar and slow process of mineralization may also explain the positive effect of biochar in the residual year of this current study. Normally, biochar has a larger specific surface area due to high porosity, strong sorption capacity due to its abundant

functional groups, and high cation exchange capacity (Zhang et al., 2013; Bian et al., 2014). Because of these characteristics of biochar, it can adsorb more As and reduce grain accumulation in the residual year of the study. This sustained effect of biochar in the residual year could be explained in support of the findings reported by Nzediegwu et al. (Nzediegwu et al., 2020), where identical functional groups were identified in both original treatment and residual cropping seasons. Thus, organo-metallic complexation may also be observed in the residual year, thereby immobilizing As in the residual year, as well.

However, no significant variations in rice plant growth and grain yield, after adding some biochar, was also reported in other studies, where increased soil pH, and dissolved organic carbon (DOC) was attributed to the negative impact of biochar (Zheng et al., 2015; Peng et al., 2018). During the pyrolysis process at high temperature base cations are transformed into oxides, hydroxides or carbonates in the biochar and (Yuan et al., 2011) dissolution of these alkaline substances increase the soil pH (Houben et al., 2013). In the soil solution, As is mainly present as hydro anion and increased soil pH after biochar application can reduce As sorption capacity by decreasing the positively charged sites on soil minerals, which increases As mobilization and release from the soil (Wilson et al., 2010). In the original treatment year of the study, the soil pH increased significantly at the higher rate (BC-20) of biochar application, which is consistence of with the above findings and could give rise to the negative impact of saw dust biochar in the residual year, as well. Some other studies reported the increased availability of As after biochar application resulting in high As phytotoxicity in rice plants and higher grain As accumulation (Beesley et al., 2013; Zheng et al., 2015; Chen et al., 2016). For instant, Zheng et al. (Zheng et al., 2012) reported an increase (327 %) in As accumulation in rice due to biochar application prepared from rice residues. In addition, the release of P and Si from biochar could have competed with As for binding sites at the iron oxide surfaces in the soils, which may have released more As into the soil solution (Jain and Loeppert, 2000; Waltham and Eick, 2002; Zheng et al., 2012). Thus, it was also reasonable in this study that As was mobilized at higher biochar application, which ultimately increased grain As accumulation in the residual year, as well.

Furthermore, with increased soil As concentration, reduced shoot biomass and grain yield and increased grain As concentration were observed in both original treatment year and residual year, in wheat and rice. A similar result was reported by Islam et al. (Islam et al., 2004b), where reduced shoot biomass and grain yield and increased grain As concentration of rice was observed in the residual cropping season. This result was consistent with the other findings

(Hossain et al., 2007; Huq et al., 2008). Overall, the grain yield of wheat and rice were higher and grain As concentration was lower in the residual year regardless of all treatment combinations, compared with the original treatment year. This could be explained by the fate of As after application into the soil in the original treatment year. In the residual year, soil As concentration may reduce after uptake by the plant in the original treatment year which could be the main fate of As in the soil. The remaining As in soil from the original treatment year attributed to the grain As accumulation in the residual year, although As was not added into the soil in the residual year. Soil is a highly heterogenous environment both spatially and temporally which may influence the bioavailability and interaction of soil As with soil nutrient elements and microorganisms. Thus, detailed investigations are needed to understand the interaction mechanism of As along with the temporal dynamics of MW and biochar treatment in the soil. Especially, in field soils, where the experimental conditions of this current study may not necessarily represent true field conditions, which gets regular exposure to environmental events such as rainfall, fluctuating temperature, and the influences of surrounding soils etc.

6.6 Conclusions

The negative impact of As on wheat and rice productivity was observed, not only in the original treatment year but also in the residual year of crop cultivation. However, MW soil treatments has the potential to consistently sustain the wheat and rice crop productivity and decreased grain As concentration for the residual year, 360 days after heating, regardless of the soil As concentration. A biochar application rate of 10 t ha⁻¹, in combination with MW soil heating at 80 – 90 °C in the original treatment year, could be considered as an effective option to alleviate As phytotoxicity in rice for the residual year, without further application. Further study is needed to investigate the effect of MW and biochar in the additional residual years, in response to As phytotoxicity alleviation and subsequent grain As accumulation.

When MW irradiation is applied it produces heat by the rotation of the water molecules in the soil. Thus, the As phytotoxicity alleviation is mainly mediated by the thermal effect. This thermal treatment could be applied through conventional means in addition to MW irradiation. Hence, it is important to understand the heating effect of MW and conventional heating on As phytotoxicity alleviation. Therefore, the experiment was designed to evaluate the effect of MW and conventional soil heating on As phytotoxicity alleviation which presented in the next chapter.

Chapter 7 – Comparison between microwave and conventional electric oven soil heating in response to arsenic phytotoxicity alleviation in rice

7.1 Abstract

Rice grain arsenic (As) is considered to be a potential pathway of dietary based As exposure for humans. This has encouraged strategies for rice grain As mitigation, despite there being choices of technique with key drawbacks. Microwave (MW) soil heating is an innovative way for As phytotoxicity alleviation. However, it is unclear whether MW soil treatment is just a heating effect or if there is more involved since MW has a faster heating rate, a higher penetration, and an inside-out heating mechanism compared with conventional heating. Therefore, this glasshouse pot experiment was designed to investigate the effect of MW and conventional electric oven (EO) soil heating on As phytotoxicity alleviation and grain As accumulation in rice. The soil was spiked with three As concentrations (As-0, As-40 and As-80 mg kg⁻¹ soil) prior to applying MW and EO heat treatments, to achieve the soil temperature around 80 – 90 °C along with control treatments (without heating treatment). The results showed that both the MW and EO soil heating alleviated As phytotoxicity as plant growth and grain yield increased significantly and facilitated less grain As accumulation, compared with the control, across all the soil As concentrations. For instance, grain As accumulation was highest (926.11 µg kg⁻¹) at As-80 without any heating treatment while, it was significantly lower in the MW (523.54 µg kg⁻¹) and EO (586.34 µg kg⁻¹) treatments at the same soil As concentration. Although, no statistically significant difference was noticed between MW and EO treatment, an increasing trend in rice plant growth and grain yield and decreasing trend in grain As concentration was observed in MW treatment compared with EO treatment. Also, EO required 4.59 times more energy (140.97 MJ) than the MW chamber (30.72 MJ) and EO required more time (2.5 times) and energy cost (4.59 times) to apply the heat treatment than the MW. Thus, MW soil heating could be better option than the EO heating to alleviate As phytotoxicity in rice. However, for full-scale field application and industrialization of MW soil heating system, further scaling-up, design, and modelling of MW system is required.

Keywords: Microwave, Electric oven, soil heating, Soil arsenic, Rice grain arsenic, Cost of soil heating, Energy efficiency

7.2 Introduction

Soil arsenic (As) contamination, through either geogenically or anthropogenically origins, is a source of grain As and exposure to As biomagnification in humans, alongside the drinking water pathway (Banerjee et al., 2013). Management of anthropogenic sources is crucial; however, control of geogenic sources is far as less controllable. Crop irrigation with As contaminated water is considered to be a major anthropogenic source of soil As contamination (Williams et al., 2006). Although different techniques have been efficiently practiced for removing As from drinking water, irrigation water treatment is not feasible in terms of cost and extent of application (Lim et al., 2014). Especially, in those areas (e.g. Bangladesh) where farmers do not even have access to As contamination-free drinking water, they are bound to use As contaminated water for crop production. Therefore, a technique is needed, which could reduce As uptake by the plants from the soil. Plants with a genetically lower accumulator of As could be an option for scientists and plant breeders to working on, even though the scenario could be different if crops are grown in highly contaminated soils.

In this occasion, rice (*Oryza sativa* L.) is reported to accumulate more As in the grain, which is often ten times higher than other cereals (Guillod-Magnin et al., 2018), because the anaerobic cultivation of rice where, As species, soil redox potential, and pH control the As mobility in soil favor plant uptake (Yamaguchi et al., 2011; Kumarathilaka et al., 2018). As(V) is predominant in aerobic soils; however, in anaerobic soils, As(V) reduces to more toxic (around 60 times) and mobile form [As(III)]. The accumulation characteristics of this more toxic form, which is found in rice, is a significant health risk where rice is the major dietary source of carbohydrates (Islam et al., 2017). Hence, no more than two or three servings of rice each week was suggested in a recent study, to remain under the safe limit of As ingestion; however, eating less is not the solution especially where rice is an irreplaceable part of the culture, diet, and lifestyle in many parts of the world (Sohn, 2014). Therefore, techniques related to soil treatment would be an alternative to decrease the rice As uptake, especially for lower As accumulation in grain even when grown in As contaminated soil. In this context, our previous studies (Chapter 5) revealed that microwave (MW) soil heating treatment has the potential to alleviate As phytotoxicity in plants and subsequently lower As accumulation in the rice grain.

Microwave is non-ionizing electromagnetic radiation with frequencies lying between 300 MHz to 300 GHz and wavelengths ranging from 1 m to 1 mm (Ramaswamy and Tang,

2008) which, can produce heat by absorption of the energy in the treated materials. Dielectric heating of a material is the main mechanism of MW heating, which depends on dipolar and interfacial polarization effects (Metaxas and Meredith, 1983). When MW radiation is applied, it causes rotation of the dipoles of polar (e.g. water) molecules because of the oscillating electromagnetic field (dipolar polarization), and produces heat by intermolecular friction and collision (Meredith, 1998; Falciglia et al., 2016). While kinetic energy accompanied with this dipolar rotation is responsible for the generation of thermal energy and dissipated in the form of heat, interfacial polarization effects are known as the Maxwell-Wagner effect (Komarov, 2012).

In contrast, conduction and convection or radiation are the main mechanisms of conventional heating (CH) where, the exposed surface area of a material heated first and transfer of heat into the interior of the material by thermal conduction (Menéndez et al., 2010). As a consequence, CH usually takes a longer time to achieve a certain temperature in comparison to MW heating since CH needs to overcome the heat transfer barrier (Li et al., 2016a). Thus, material heating with MW energy has been demonstrated to be a unique and promising alternative to CH because of its advantages such as rapid heating with quick start-up, material selective heating, precise control, volumetric heating, ease of operation by instant on/off control, and being environment-friendly (Menéndez et al., 2010; Zhou et al., 2010). Due to these major advantages, MW heating is gaining popularity since it has been applied to diverse fields such as food processing, medical application, drying of wood, textiles and leather processing, paper and cardboard processing, plasma and solvent-free chemistry, pest control, enhancing seed germination, and remediation of contaminated soils (Chandrasekaran et al., 2013; Falciglia et al., 2016; Patrascu et al., 2018; Zhou et al., 2020).

It was evident from the literature that, MW soil heating treatment in the agricultural farming system could be a potential technique to control weed infestation by destroying the soil weed seed bank as a non-chemical means, especially for herbicide resistance weed control (Brodie et al., 2017; Jamal et al., 2017). Besides weed control, MW soil heating has been known to increase crop growth and yield as reported in some previous studies, where increased availability of soil nutrients (e.g. N, S, and P) was reported as the main reason of better plant growth in MW treated soils (O'Brien et al., 2018; Khan et al., 2019b; Maynaud et al., 2019). Furthermore, humification of soil organic matter (SOM), mineralized soil indigenous nitrogen and subsequent release of ammonium (NH_4^+), enhanced carbon, free amino acids, increased organic matter, and increased organic nitrogen (org-N) mineralization results from dead

microorganisms could contribute to better plant growth (Zhou et al., 2010; Kim and Kim, 2013; O'Brien et al., 2018). Our recent study showed that MW soil heating significantly increased rice plant growth and grain yield in addition of the process of As mitigation (Chapter 5). Thus, MW soil heating could be a novel technique to alleviate As phytotoxicity and reduce As buildup in rice grain. But the question remains: “Was it only a heating effect or was there some unique MW effect involved?”. Therefore, this experiment was designed to find out the preliminary answer of this question by evaluating the effect of MW and EO soil heating on plant growth, grain yield, and the grain As accumulation of rice.

7.3 Materials and methods

7.3.1 The experimental site, soil collection, and preparation

The glasshouse pot experiment was conducted at Dookie campus, The University of Melbourne, Australia. Experimental soil was collected from a clay soil paddock (H12: 36°23'51"S; 145°43'17"E) at a depth of 0 – 15 cm. The soil was a grey to grey-brown clay and was classified as a Congupna clay (Downes, 1949) or a Grey Vertosol (Isbell, 2016). After collection, the soil was air-dried, pulverized, mixed thoroughly by hand, and sieved through a 4 mm mesh to minimize the undesired effects of dry root, grasses, stones, sticks, and clods. After sieving, 8.5 kg soil (with around 13 – 15 % moisture) was thoroughly mixed and shifted into unperforated pots (diameter 27 cm and height 30 cm), to prevent the loss of water-soluble As from the pots (Rahman et al., 2007b).

7.3.2 Physicochemical properties of experimental soil

To determine the soil physicochemical properties, soil samples were sent to the Nutrient Advantage Laboratory, a NATA (National Association of Testing Authorities, Australia) accredited laboratory (Lab number: 11958, ISO/IEC 17025). The physicochemical properties of soils are presented in Table 7.1.

7.3.3 Arsenic application

The soils were spiked at three different levels of As concentration (0, 40, and 80 mg kg⁻¹ dry soil) as sodium arsenate heptahydrate (Na₂HAsO₄·7H₂O) (Bhattacharya et al., 2015). Arsenic was applied by following the method described previously (Chapter 4). Since the initial As content of the pre-experimental soils, prior to treatment, was < 0.01 µg kg⁻¹ dry weight (Table 7.1), there was no chance of further As being available from the soil. To establish an

equilibrium condition between soil and applied As, soil moisture was maintained at field capacity for two weeks prior to applying the MW treatment.

Table 7.1 Physicochemical properties of experimental soil before (H-0) and after microwave (H-MW) and electric oven (H-EO) soil heating treatments

Soil properties	Analytical method	Units	Soil heating treatments		
			H-0	H-EO	H-MW
Organic carbon (OC)	Walkley & Black	%	1.57	1.51	1.38
Organic matter (OM)	Walkley & Black	%	2.70	2.60	2.37
Electrical conductivity (EC)	Saturated extract	dS/m	0.80	1.10	1.60
pH	1:5 CaCl ₂	N/A	6.50	6.70	6.60
Cation exchange capacity (CEC)	BaCl ₂ exchange	cmol(+)/kg	9.95	9.17	10.40
Nitrate nitrogen (NO ₃ ⁻ -N)	Kjeldahl	mg kg ⁻¹	39.00	28.00	36.00
Ammonium nitrogen (NH ₄ ⁺ -N)	Kjeldahl	mg kg ⁻¹	7.50	65.00	160.00
Available Potassium (K)	Atomic emission	mg kg ⁻¹	290.00	250.00	310.00
Sulphur (S)	0.25M KCl at 40°C	mg kg ⁻¹	7.00	19.00	38.00
Phosphorus (P)	Colwell	mg kg ⁻¹	45.00	150.00	260.00
Calcium (Ca)	Ammonium acetate	cmol(+)/kg	3.70	3.50	3.80
Magnesium (Mg)	Ammonium acetate	cmol(+)/kg	4.60	4.30	5.00
Potassium (K)	Ammonium acetate	cmol(+)/kg	0.75	0.64	0.80
Sodium (Na)	Ammonium acetate	cmol(+)/kg	0.62	0.54	0.67
Aluminium (Al)	Ammonium acetate	cmol(+)/kg	0.26	0.19	0.15
Copper (Cu)	DTPA	mg kg ⁻¹	1.60	1.80	1.70
Zinc (Zn)	DTPA	mg kg ⁻¹	1.00	1.30	1.30
Manganese (Mn)	DTPA	mg kg ⁻¹	66.00	100.00	82.00
Iron (Fe)	DTPA	mg kg ⁻¹	92.00	110.00	97.00
Boron (B)	DTPA	mg kg ⁻¹	0.90	0.83	0.93
Silicon (Si)	CaCl ₂ Soluble	mg kg ⁻¹	75.00	84.00	100.00
Arsenic (As)	HG-AFS	µg kg ⁻¹	< 0.01	< 0.01	< 0.01

7.3.4 Soil heating application

A four kW MW chamber, operating at a frequency of 2.45 GHz, was used for MW treatment, while an electric drying oven, of 9.3 kW (TR 1050, Nabertherm, Germany), was used for EO treatment to heat the soil to a temperature around 80 – 90°C. The individual unperforated plastic pot (diameter 27 cm and height 30 cm) with 8.5 kg of soil was placed inside the MW chamber and exposed for six minutes of soil heat. Unlike the MW treatment,

an aluminium tray with the same amount of soil (8.5 kg per tray), instead of the plastic pot, was used for EO heating to get the maximum surface area of soil for heat circulation, since the heating mechanism in EO is different from the MW. In EO heating, all the trays were placed inside the electric oven together (on the different racks) at the same time and exposed for four hours to achieve the anticipated soil temperature. The target soil temperature was considered from previous work (Chapter 5) where soil heating to around 80 – 90 °C increased the rice plant growth and yield and decreased grain As concentration. At the time of MW treatment, soil moisture was maintained at around 17 – 18 % (w/w) since soil moisture is an important factor that affects the MW radiation effect on soil (Kabir et al., 2020). The duration of the MW treatments to achieve the anticipated temperature was determined by a separate set of the same soil with the same moisture content. To monitor the soil temperature, a liquid-in-glass thermometer was inserted into the soil immediately after MW energy exposure (Cooper and Brodie, 2009). A similar technique was followed to determine the EO treatment duration; however, the thermometer was inserted into the tray soil during the whole time of heating to follow the temperature increment. Also, thermal images were taken with an infrared thermal camera (FLIR T1050SC) to inspect the temperature distribution across the soil (Figure 7.1).

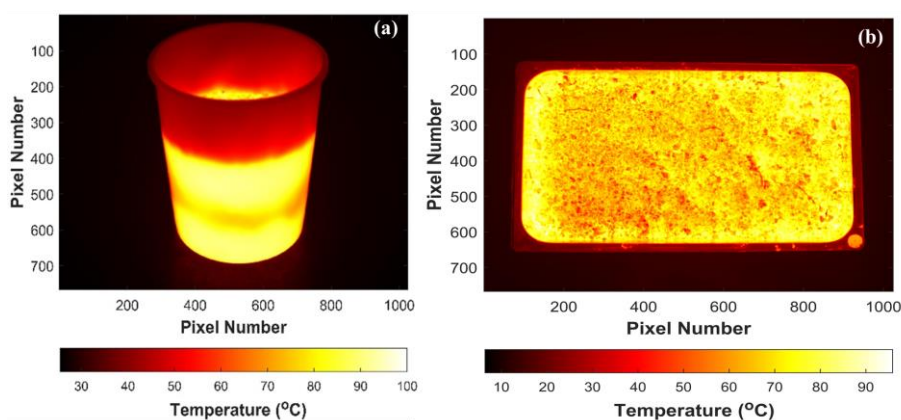


Figure 7.1. Thermal images of microwave and conventional electric oven soil heating at a temperature around 80 – 90 °C, (a) side view of a pot of microwave soil heating, (b) top view of a tray of electric oven soil heating. Thermal images were captured with an infrared camera (FLIR T1050SC model)

7.3.5 Experiment setup

After the application of As and heating treatments, a total of 36 pots, including control treatments, with four replications of each treatment and their combinations, were placed in a glasshouse following a completely randomized design (CRD). To describe the treatments more conveniently, abbreviated forms were used for As treatments (As-0, As-40, and As-80) and

heating treatments (H-0, H-MW, and H-EO for control, microwave, and electric oven heating respectively). All the fertilizers were applied according to the standard practices for Australian rice cultivation (Dunn et al., 2018). Seeds of YRM_70 rice variety (*Oryza sativa* L.) were sown on the 15th of November 2018. At the three-leaf stage, extra seedlings were removed, leaving 12 seedlings per pot. Soil moisture was maintained at field capacity (weight basis) up to the three-leaf stage of plant growth. Flooding irrigation was started at the three-leaf stage and maintained at a certain level throughout the growing period and stopped 10 days before harvesting the plants (Dunn et al., 2018). Normal tap water was used for irrigation purposes. This water source contained As below the detection limit of AFS ($< 0.01 \mu\text{g l}^{-1}$); therefore, there were no possibilities of As addition from the tap water to the potting soil. After 150 days of the growing period at physiological maturity stage the crop was harvested on the 14th of April 2019.

7.3.6 Recording of agronomic data

The plant height was measured as a distance from the soil to the top of a plant using a measuring scale at the tillering stage. Chlorophyll Meter-SPAD-502Plus (Soil-Plant Analysis Development) was used to measure the leaf chlorophyll content as SPAD value (Yuan et al., 2016) at the tillering stage. To get the plant height and leaf chlorophyll content, five plants within a pot were selected randomly and data was recorded as mean value of these five plants. At the tillering stage, plant samples (3 hills per pot) were collected for plant dry biomass determination and the total tiller number was recorded prior to sample collection. At the physiological maturity stage, the total number of panicles per pot were recorded prior to crop harvest. Along with shoot samples, root samples were also collected at the crop harvest. Grains were collected from the panicle by hand threshing and yield (g pot^{-1}) was calculated as described previously in a glasshouse pot experiment (Rahman et al., 2007a). Total filled grain and sterile spikelet was counted to calculate the spikelet sterility percentage. Fresh weight of all plant samples was recorded prior to drying at 60 °C in a dehydrating oven (Thermoline Scientific, TD-500F) for 48 hours to determine the dry biomass weight.

7.3.7 Grain total arsenic analysis

The atomic fluorescence spectrometry (AFS) was used for total As analysis of rice grain samples. The detail procedure of total As concentration determination is described in our previous experiment (Chapter 4).

7.3.8 Energy calculation of heat treatments

Energy requirement to apply the MW heat treatment was calculated based on the selected power and heating time by Equation (7.1) as follows (Ma et al., 2009):

$$E = Pt \quad (7.1)$$

Where, E is output energy (Joule), P is output power (watt), and t is the heating time (second)

For MW soil heating treatment, the electrical energy (E_{el}) needed to dissipate constant heat power (P_d) within the soil for a specific time (t) was calculated by Equation (7.2) as follows (Cherbański, 2011):

$$E_{el} = \frac{P_d}{\eta_{el \rightarrow mw} \eta_{mw \rightarrow heat}} t \quad (7.2)$$

Where, E_{el} is output electric energy (Joule), P_d is the dissipated power (watt), t is the heating time (second), $\eta_{el \rightarrow mw}$ is the efficiency factor of electrical energy conversion into MW, and $\eta_{mw \rightarrow heat}$ is the efficiency factor of MW energy conversion into thermal energy.

The efficiency factor of MW energy conversion into thermal energy, $\eta_{mw \rightarrow heat}$, was calculated from the following Equation (7.3) (Cherbański, 2011):

$$\eta_{mw \rightarrow heat} = \frac{P_d}{P_{mw}} \times 100 \% \quad (7.3)$$

Where, P_d is the dissipated power (watt), P_{mw} is the MW power produced by the magnetron (watt).

For EO heating, the electrical energy requirement was also calculated by the Equation 7.2. Total efficiency factor ($\eta_{el \rightarrow heat}$) of electrical energy conversion into heat was calculated by following Equation (7.4) (Cherbański, 2011):

$$E_{el} = \frac{P_d}{\eta_{el \rightarrow heat}} t \quad (7.4)$$

Where, E_{el} is output electric energy (Joule), P_d is the dissipated power (watt), t is the heating time (second), and $\eta_{el \rightarrow heat}$ is the efficiency of electrical energy conversion into thermal energy.

To calculate the cost of application of MW and EO heat treatment the electricity cost was assumed @ \$0.10 per kWh, which may differ from location to location.

7.3.9 Statistical analysis

Statistical analysis of data were performed in the R statistical environment (R version 3.6.2) (Team, 2013). Normality and homogeneity tests of the experimental data were performed to examine the distribution of the data. An Analysis of Variance (ANOVA) test was performed to determine the significance of tested treatments on variables. A Least Significant Difference (LSD) test was performed to compare the treatment's means at 5 % level of significance. Thermal images were captured with an infrared camera (FLIR T1050SC model) and post-processed in MATLAB software (version: R2015b; MathWorks, Inc., USA) (MATLAB, 2015).

7.4 Results

The results showed that the heat treated (MW and EO) soils showed better plant growth and higher grain yield with lower grain As concentration compared with the control, across all the soil As concentrations. Although, similar results were noticed in MW and EO treatment, an increasing trend in rice plant growth and grain yield and decreasing trend in grain As concentration was observed in the MW treatment compared with the EO treatment. The detailed results are given below.

7.4.1 Plant growth and biomass production

7.4.1.1 Plant height

It is evident from Table 7.2 that, the mean plant height decreased significantly ($p = 0.002$) with increasing soil As concentration while, soil heating treatments (MW and EO) increased the plant height significantly ($p < 0.001$) irrespective of soil As concentration. For example, the plant height for the As control treatment (As-0) was 79.70 cm, while 70.75 cm was recorded at the highest soil As treatment (As-80). In contrast, the plant heights at the same level of soil As were 88.35 cm and 78.60 cm in the MW treatment and 82.60 cm and 80.55 cm in the EO treatment, respectively.

7.4.1.2 Leaf chlorophyll content (SPAD value)

There was a significant effect ($p = 0.038$) of soil heating where, leaf chlorophyll content increased in the MW and EO heating treatments compared with the control (H-0) treatment (Table 7.2). However, there was no statistically significant difference observed between MW and EO heating for leaf chlorophyll content.

7.4.1.3 Tiller number

Table 7.2 suggests that the effect of soil As concentration was statistically non-significant ($p = 0.064$). However, the tiller number increased significantly ($p < 0.001$) in MW and EO soil heating treatments in comparison with the control treatment.

Table 7.2. Mean plant height (cm), leaf chlorophyll content (SPAD) and tiller number (per pot) in response to microwave (MW) and electric oven (EO) soil heating treatment at different soil arsenic concentration

Soil heating treatment	<u>Plant height (cm)</u>			<u>Leaf chlorophyll content</u>			<u>Tiller number^T</u>		
	Soil As concentration (mg kg ⁻¹ soil)								
	0	40	80	0	40	80	0	40	80
Control	79.70 ^b	78.55 ^b	70.75 ^c	40.80 ^a	36.80 ^{ab}	32.78 ^b	31 ^{ab}	27 ^{bc}	22 ^c
MW	88.35 ^a	83.55 ^{ab}	78.60 ^b	41.38 ^a	38.65 ^a	40.25 ^a	34 ^{ab}	35 ^a	31 ^{ab}
EO	82.60 ^{ab}	85.75 ^{ab}	80.55 ^b	39.18 ^a	38.93 ^a	39.33 ^a	35 ^a	34 ^{ab}	34 ^{ab}
LSD _{0.05}	6.82			4.43			6.5		

Mean data with superscript same letter are not significantly different. Least Significant Difference (LSD) test was performed at 5 % level of significance to determine the difference between the treatments. T: data recorded at tillering stage of rice plant growth.

7.4.1.4 Shoot Biomass

At tillering stage (Table 7.3), significantly ($p = 0.006$) lower shoot biomass was recorded when plants are grown in As treated soils compared with the control soil, while there was no significant effect ($p = 0.077$) of soil heating treatments on shoot biomass. At crop harvest (Table 7.3), shoot biomass reduced significantly ($p < 0.001$) in As treated soils compared with the control treatment, although significantly ($p = 0.032$) higher biomass was recorded in the soil heating treatments compared with the control, irrespective of soil As concentration.

7.4.1.5 Root biomass

From Table 7.3 it was evident that root biomass reduced significantly ($p < 0.001$) with increasing soil As concentration whereas in MW and EO heat treated pots, significantly ($p < 0.001$) higher root biomass was recorded from the control.

Table 7.3. Effect of soil arsenic (As) concentration on mean shoot biomass (g pot⁻¹) and root biomass (g pot⁻¹) in response to microwave (MW) and electric oven (EO) soil heating treatment

Soil heating treatment	<u>Shoot biomass^T</u>			<u>Shoot biomass^{CH}</u>			<u>Root biomass^{CH}</u>		
	Soil As concentration (mg kg ⁻¹ soil)								
	0	40	80	0	40	80	0	40	80
Control	5.84 ^{ab}	5.12 ^{bc}	4.27 ^c	32.11 ^b	36.02 ^{ab}	18.71 ^c	16.33 ^b	10.98 ^e	9.05 ^f
MW	6.75 ^a	5.69 ^{abc}	5.12 ^{bc}	42.94 ^a	32.76 ^b	30.17 ^b	20.14 ^a	15.45 ^{bc}	14.61 ^{cd}
EO	6.24 ^{ab}	5.44 ^{abc}	5.69 ^{abc}	35.47 ^{ab}	34.94 ^{ab}	29.67 ^b	19.13 ^a	14.19 ^{cd}	13.13 ^d
LSD _{0.05}	1.29			8.26			1.63		

Mean data with superscript same letter are not significantly different. T: data recorded at tillering stage, CH: data recorded at crop harvest. Least Significant Difference (LSD) test was performed at 5 % level of significance to determine the difference between the treatments.

7.4.1.6 Panicle number

From Table 7.4 it was evident that, with increasing soil As concentration, the number of panicles per pot decreased significantly ($p = 0.001$) while, a significantly ($p = 0.006$) higher panicle number was observed in the MW and EO treated pots compared with the control, even at the highest soil As concentration (As-80).

7.4.1.7 Spikelet sterility

Spikelet sterility percentage increased significantly ($p < 0.001$) with increasing soil As concentration, while significantly ($p < 0.001$) lower grain sterility was recorded in the MW and EO soil heating treatments compared with the control (Table 7.4). The highest grain sterility of 63.51 % was observed at As-80 treatment; whereas, at the same soil As concentration, the sterility was 25.01 % and 27.93 % in the MW and EO treatments, respectively.

7.4.1.8 100 seed weight

It was evident from Table 7.4 that, 100 seed weight increased significantly ($p < 0.001$) when soil was treated with MW and EO. In contrast, 100 seed weight decreased significantly ($p < 0.001$) with increasing soil As concentration. For example, at As-80, 100 seed weight was 1.82 g and 1.74 g in the MW and EO treatments while, in control treatment, the 100 seed weight was 0.97 g.

Table 7.4. Influence of soil arsenic (As) concentration on panicle number (per pot), spikelet sterility (%), and 100 seed weight (g) in response to microwave (MW) and electric oven (EO) soil heating treatments

Soil heating treatment	<u>Panicle number</u>			<u>Spikelet sterility %</u>			<u>100 seed weight</u>		
	Soil As concentration (mg kg ⁻¹ soil)								
	0	40	80	0	40	80	0	40	80
Control	23 ^{ab}	22 ^b	15 ^c	9.38 ^{cd}	23.11 ^b	63.51 ^a	2.39 ^{bc}	1.92 ^e	0.97 ^g
MW	28 ^a	22 ^{ab}	22 ^b	5.61 ^d	12.07 ^{cd}	25.01 ^b	2.41 ^{ab}	2.21 ^{cd}	1.82 ^{ef}
EO	26 ^{ab}	25 ^{ab}	22 ^b	4.87 ^d	13.69 ^c	27.93 ^b	2.60 ^a	2.14 ^d	1.74 ^f
LSD _{0.05}		4.76			8.04			0.20	

Mean data with superscript same letter are not significantly different. Least Significant Difference (LSD) test was performed at 5 % level of significance to determine the difference between the treatments.

7.4.2 Grain yield

Rice grain yield reduced significantly ($p < 0.001$) in the response of soil As concentration increases from As-0 to As-80, while significantly ($p = 0.007$) higher grain yield was recorded in the MW and EO soil heating treatments, irrespective of soil As concentration (Figure 7.2a) in comparison with the control. The highest grain yield was recorded in MW soil treatment (H-MW) at As-0; whereas, the lowest grain yield was observed in the As-80 in no heating treatment (H-0). Higher grain yield was recorded in MW treatment than the EO treatment though the difference was not statistically significant.

7.4.3 Grain total arsenic concentration

Grain As concentration increased significantly ($p < 0.001$) with increasing soil As concentration, while it decreased significantly ($p < 0.001$) in the MW (H-MW) and EO (H-EO) soil heating treatments, compare with the control (H-0), irrespective of soil As concentration (Figure 7.2b). For instance, the highest grain As concentration (926.1 µg kg⁻¹ dry grain) was observed at As-80 in the control soil heat treatment (H-0), while the grain As concentration in the MW and EO treatments were 523.5 µg kg⁻¹ and 586.3 µg kg⁻¹, respectively, at the same soil As concentration. In contrast, the lowest grain As concentration (200.1 µg kg⁻¹ dry grain) was recorded in MW treatment at As-40.

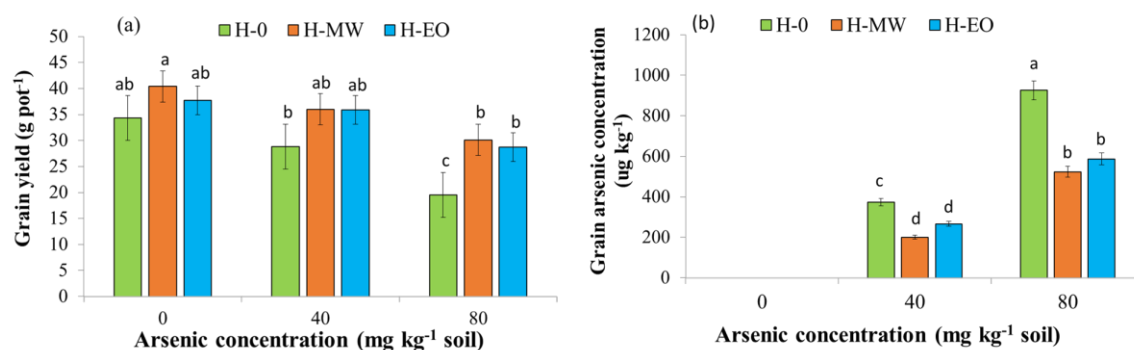


Figure 7.2. Rice grain yield (a) and grain arsenic concentration (b) in response to microwave (H-MW) and electric oven (H-EO) soil heating treatments at different soil arsenic concentrations. Bar represents the mean value with standard error and different letter indicate the significant difference (LSD at $p = 0.05$) among the treatments

7.4.4 Correlation of growth and yield parameters with grain arsenic concentration

Pearson's correlation coefficient (r value) showed that all the growth parameters were positively correlated with the yield parameters and all the growth and yield parameters were negatively correlated with grain As concentration except spikelet sterility percentage which was positively correlated with grain As concentration and negatively correlated with all other growth and yield parameters (Figure 7.3). Two-sided tests of correlations showed all the correlation coefficients (r value) were statistically significant (at 0.01 significant level) except the correlation among leaf chlorophyll content (SPAD), tiller number, and shoot biomass at tillering stage.

7.4.5 Energy consumption comparison of heating treatments

Results from the

Table 7.5 demonstrate that the electric oven (EO) required 4.59 times more energy (140.97 MJ) than the MW chamber (30.72 MJ) to heat the same amount of soil (102 kg) to 80 – 90°C temperature. Furthermore, the EO required more time (2.5 times) and energy cost (4.59 times) to apply heat treatment than MW. Thus, MW soil heating was more energy, time, and cost efficient compared to EO soil heating.

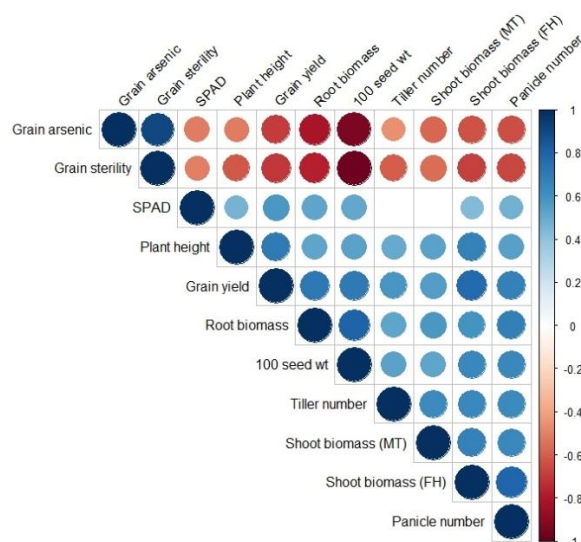


Figure 7.3. Pearson's correlation matrix of different growth and yield parameter with grain arsenic concentration. Red color showed negative correlation and blue color indicate the positive correlation. Blank box indicates the correlation was not statistically significant at 0.01 level of significance. MT: maximum tillering, FH: final crop harvest

Table 7.5. Comparison between microwave and electric oven soil heating in response to energy consumption

Parameters	Unit	Microwave chamber	Electric oven
Power	kW	4.00	9.30
Soil load	Kg	102.00	102.00
Heating time	Min	96.00	240.00
Soil temperature	°C	80.00 – 90.00	80.00 – 90.00
Efficiency factor $\eta_{el \rightarrow mw}$	—	0.75	—
Efficiency factor $\eta_{el \rightarrow heat}$	—	—	0.95
Energy required	Mega Joule (MJ)	30.72	140.97
Cost of operation (\$0.10 per kWh)	\$	0.64	3.72

7.5 Discussion

Results from this present study revealed that rice plant growth and grain yield reduced significantly and grain As concentration increased significantly in As treated soils. Several studies reported that an elevated level of soil As concentration can reduce rice plant height, tiller number, shoot and root biomass, leaf chlorophyll content, photosynthetic rate, and even cause plant death. Studies also imply that soil As concentration has a negative effect on rice

grain yield and yield contributing traits (Rahman et al., 2004; Rahman et al., 2007a; Vromman et al., 2013). These findings agree with the results of current experiment where, significantly reduced plant height, leaf chlorophyll content, tiller number, shoot and root biomass, panicle number, and grain yield were observed. As uptake by the rice plant primarily depends on the soil As concentration since several studies assuredly concluded that rice grain As concentration increases when plants were exposed to higher soil As (Duxbury et al., 2003; Rahman et al., 2004). Higher As uptake was found to induced phytotoxicity due to physiological, biochemical, morphological and molecular alteration in rice plant (Srivastava et al., 2016). Arsenic can also impede numerous metabolic processes that can constrain the plant growth through As induced phytotoxicity (Rahman et al., 2007a). Disfunction of some key enzymes can occur through interaction of As with sulfhydryl groups (Williams et al., 2005). For example, arsenite [As(III)], one of the most toxic inorganic form of As, has great affinity to react with sulfhydryl groups (–SH) of enzymes and proteins, which leads to the deterioration of cell membranes, inhibition of cellular functions and subsequently cell death (Meharg and Hartley, 2002). Other toxic effects, including the, increase generation of reactive oxygen species (ROS) and subsequent induction of oxidative stress (Shri et al., 2009), competition with phosphate (PO_4^{3-}) and replacing PO_4^{3-} in ATP synthesis by forming ADP-arsenate, thereby disrupting the energy flow in the cell (Meharg and Hartley, 2002), disruption of vital biochemical processes such as phosphorylation/dephosphorylation, phospholipid metabolism, RNA/DNA synthesis, glycolysis, oxidative phosphorylation, and protein synthesis (Gresser, 1981; Finnegan and Chen, 2012), and decrease photosynthesis rate by declining the chlorophyll content due to change in chloroplast shape and flow of assimilates (Rahman et al., 2007a) are mostly reported adverse effects of As on plants through As phytotoxicity, which ultimately reduces plant growth and grain yield.

In contrast, although rice plant growth and grain yield are significantly reduced with increasing soil As concentration, MW and EO soil heating treatments significantly increased the rice plants' growth and grain yield compared with the control, across all the soil As concentration. Pre-sowing MW soil heating has been known to increase crop growth and grain yield, as reported in some previous studies (Jamal et al., 2017; Brodie et al., 2019; Khan et al., 2019a). The better plant growth and grain yield could be attributed to the increased availability of soil nutrients (e.g. N, S, and P) after MW and EO soil heating treatment (Table 7.1). This result is in agreement with the previous findings where, more availability of nutrients (e.g. N, S, and P) for the plants (O'Brien et al., 2018; Khan et al., 2019b; Maynaud et al., 2019) after

MW soil heating treatment was reported. Rapid humification of SOM (Kim and Kim, 2013; O'Brien et al., 2018) also reported as another reason of better plant growth in MW treated soil. In view of this, increase mineralization of soil indigenous org-N was reported after MW soil heating as the source of increased N concentration in soil (O'Brien et al., 2018). Additionally, the disintegration of soil microorganisms, due to heating, can release nutrients, increase soluble organic matter, intracellular and extracellular macromolecules, and org-N mineralization in the soil (Zhou et al., 2010). Soil heating (below 200 °C) has also been reported to increase the availability of soil nutrients (e.g. $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$, P, Ca, Mg, Na, and K) for plant uptake (Alcañiz et al., 2016; Alcañiz et al., 2018). The decomposition of SOM was identified as the main reason for the increases of these soil nutrients. However, decreased soil nutrients were also reported at temperature range 100 – 400 °C as a consequence of significant reduction of SOM (10 – 80 %) due to combustion at high temperature (Yi et al., 2016). In this study the soil heating temperature was 80 – 90 °C, which might be responsible for enhancing the decomposition of SOM instead of its reduction through combustion and increasing soil nutrients.

In terms of grain As concentration, MW and EO soil treatment significantly reduced the As concentration in rice grains compared with the control treatment, across all the soil As concentrations (Figure 7.2b). The lower grain As concentration could be attributed to the growth dilution effect, where grain As can be diluted because of the higher grain yield in heat treated soils. Previous studies also reported growth dilution effect as one of the reasons of lower As accumulation in grain due to the higher plant growth, biomass, and grain yield (Pigna et al., 2009; Wu et al., 2015). In addition, increased availability of soil nutrients such as P, S, and Si (Table 7.1) after MW and EO heating could alleviate As phytotoxicity and reduce As accumulation in grain. For example, P (as PO_4^{3-}) and Si have antagonistic relationship with arsenate [As(V)] and arsenite [As(III)] respectively because As(V) and As(III) is taken up and transported by the PO_4^{3-} and Si transporters respectively (Ma et al., 2008; Zhao et al., 2010b). Hence, increase of P and Si in MW and EO treated soils can reduce the As(V) and As(III) uptake and ultimately less As accumulation in the grain. In addition, S is a vital constituent of phytochelatins (PCs), a nonprotein thiols (NPTs), which have high affinity to form complex with As(III). This PC-As(III) complex can transported into the vacuole through the ATP-binding cassette (ABC) transporters present in tonoplast as an As detoxification mechanism in plant (Song et al., 2014). Thus, increased S in MW and EO heated soils could enhance the PC complexation and subsequent vacuole sequestration which consequences As phytotoxicity

alleviation. Furthermore, the physical and chemical changes in SOM in that way which could enhance the adsorption of As and reduce accumulation in plants after soil heating. Thus, from the above discussion, it can be concluded that better plant growth, higher grain yield and lower grain As concentration could be attributed to some common beneficial heating effects of both MW and EO, since the soil heating temperature was the same (80 – 90 °C) in both cases.

It was evident from the results that, there was no significant difference between MW and EO heating, in response to plant growth, grain yield, and grain As concentration. However, higher grain yield and lower grain As concentration was recorded in MW treatment than the EO treatment though the difference was not statistically significant (Figure 7.2). Which indicates that there could be some other effects of MW soil heating compared with conventional heating. This differential effect in MW compared with EO heating could be explained by the more nutrient availability, heating mechanism, changes in SOM, and effect on soil microorganisms since this would be the ultimate difference between MW and EO heating effect. In view of this, the availability of some key soil nutrients (e.g. N, P, S, and Si) were higher in MW treated soils compared with control and EO treated soils (Table 7.1) which could be attributed to the more beneficial effect of MW over EO treatment.

In terms of heating mechanism, the application of MW radiation into the soil induces rotation of the water molecules and produces heat by intermolecular friction and collision (Meredith, 1998; Falciglia et al., 2016), ionic conduction, and interfacial polarization mechanisms (Taylor et al., 2005). Thus, MW heating is no longer limited by exterior temperature, temperature gradients, or thermal conductivity. This indicates a fast heating rate of soil and heat is produced throughout the volume of the material during a MW treatment. On the other hand, conventional heating processes take a longer time to achieve the anticipated temperature because of overcoming the heat transfer barriers (Li et al., 2016a). Thus, the changes in soil properties, especially SOM due to different heating mechanisms and the quick heating during MW treatment, might not be the same as in EO heating. For example, the physical and chemical properties of SOM can transform markedly after exposure to MW irradiation, which includes but is not limited to changes in molecular composition (C, H, O and N) and chemical structure, enhanced humification (Hur et al., 2013; Kim and Kim, 2013), increased soil organic carbon (Zagal, 1989), synthesis of macromolecular organic substances that possess a higher number of functional groups (Kim and Kim, 2013), and formation of organometallic and coordination compounds (Taylor et al., 2005). These modified organic substances retained, decreased the mobility of, and reduced the bioavailability of soil heavy

metals (Impellitteri et al., 2002). Thus, the above changes in SOM could enhance the adsorption of As and reduce plant uptakes. On the contrary, no such changes were previously reported in conventional heating at the temperature used in this experiment (80 – 90 °C). However, in conventional heating at 100 – 200 °C, SOM degradation (lignin and hemicellulose) could occur, while more than 300 °C soil temperature is needed for decarboxylation of humic and fulvic acids (Pérez et al., 2004). Thus, conventional heating temperature should be around three times higher than the treatment temperature in this current experiment (80 – 90 °C) to enhance SOM humification. Hence, differential changes of SOM in MW treated soil, compared with EO treated soil, could be responsible for reduced grain As concentration by enhancing the adsorption of soil As into humified SOM due to MW soil treatment. However, further detailed investigations are needed to understand As behavior and interaction with SOM in the soil after MW treatment.

Soil microorganisms plays a vital role for soil fertility. Soil heating can alter the diversity and richness of the soil microorganisms either in positive or negative way. It has been reported that the soil heating (75 – 85 °C) can reduce the microbial community diversity and richness as an immediate effect while, community reshaping or restructuring occurred after a period of soil heating where heat-tolerant species appeared with greater abundance (Khan et al., 2019d). Water soluble carbon (WSC), a dynamic fraction of SOM, may regulate heterotrophic microbial activity, since most of WSC is readily available for soil microorganism's to take up (Wagai and Sollins, 2002). Soil heating was found to increase the solubilization of WSC either from SOM or dead microorganisms (Tanaka et al., 2003; Khan et al., 2010), which could increase heat tolerant microorganisms growth and number, leading to their rapid recovery. However, a decrease in or alteration of the microbial community may not always be negative since the soil fertility depends on the functionality of microorganisms. Changing of the soil microbial community, in response to heating, may depend on the heating temperature, duration, intensity, penetration depth, and source of the heating. Primary interactions by the penetration of MW, their propagation, and spatial distribution into the living system may reflect the interaction of entire organisms, cellular, subcellular, molecular, and atomic levels in a different way to conventional heating. Although, there are some established effects and mechanisms of MW heating on the biological system, some speculative effects are also reported. Several researches revealed the effect of MW as a thermal source (Dreyfuss and Chipley, 1980; Mishra et al., 2013), but studies refuting the non-thermal effect of MW must also need to be considered (Shazman et al., 2007).

In comparison with conventional heating, the differential effect of MW heating on soil microorganisms has been reported. This differential effect of MW could be attributed to the dielectric heating mechanism of MW where, besides water molecules, the rotational motions of biological molecules, such as amino acids, peptides, and proteins of the microorganisms, have been reported to participate in heat production when soil is treated with MW irradiation. Whereas, such mechanism of heating was not reported in conventional heating (Takashima et al., 1984). The differential effect of MW heating than the conventional heating on soil microorganisms may include changes in structural and molecular components. In line of this, different cell morphology of *Escherichia coli* (Shamis et al., 2011), change in structural and molecular components of *Bacillus subtilis* spores (Celandroni et al., 2004), and increased enzyme activity and growth of enterobacteria (Spencer et al., 1985) were reported in MW heat treatment compared with conventional heat treatment. Furthermore, increased synthesis of peptides, DNA, and RNA of *Bacillus mucilaginous* was noticed in MW radiation treatment compared with conventional heat treatment (Kothari et al., 2012).

Furthermore, it was evident from the result that, EO required more energy (4.59 times), time (2.5 times), and energy cost (4.59 times) to apply heat treatment than the MW (

Table 7.5). Therefore, MW could offer an attractive alternative to EO owing to its energy transfer rather than heat transfer (Menéndez et al., 2010), rapid, selective, and volumetric heating, precise control, as well as quick start up (Metaxas and Meredith, 1983), along with energy, time and cost efficient. The efficiency factor $\eta_{el \rightarrow mw}$ describes the efficiency of electrical energy conversion into MW energy. In general, the value of this factor varies from 0.5 – 0.7 (Nüchter et al., 2004) though a well-designed MW system can achieve efficiencies more than 0.75 when operating at 2.45 GHz and even in excess of 0.90 when operating at 922 MHz in some industrial system (Patil et al., 2012). In this study, 0.75 was used as the value of the factor $\eta_{el \rightarrow mw}$. A recent study reported 40 % efficiency with a 0.214 energy transfer coefficient of MW soil heating using a horn antenna, where the poor coupling coefficient of the horn antenna and the poor efficiency of the MW oven system was described as the reason of the lower efficiency of MW heating (Jamal et al., 2017). Eventually, the efficiency of a MW system depends on the design of the applicator and operating frequency. Theoretically, the efficiency factor of electrical energy conversion into the heat of an EO is 1 because all the energy embodied in the electric current is converted into heat in the resistance wire of a heater (Holme and Caruthers, 2013). However, EO is usually equipped with a fan blowing the heat, which can consume some electric energy (usually very little). Thus, the total efficiency factor

for the heater is lower than 1. In this study, 0.95 was assumed as the total efficiency factor ($\eta_{\text{el} \rightarrow \text{heat}}$) of electrical energy conversion into heat. A similar efficiency factor (0.95) was also used previously, where conventional heating efficiency was compared with MW heating (Cherbański, 2011). Microwave heating was demonstrated to be more energy efficient than conventional heating, which agrees with the current study. However, proper MW engineering and careful process design could increase the MW heating efficiency.

7.6 Conclusions

Microwave and conventional EO soil heating treatment have the potential to alleviate As phytotoxicity in rice. However, MW treatment performed better than EO treatment although the difference was not statistically significant. Hence, further investigation is required to explore the mechanisms and reasons for the more beneficial effect in MW soil heating than the EO soil heating. Besides, MW soil heating can be more time and energy cost efficient compared with EO soil heating. However, for full-scale field application and industrialization of MW soil heating system, further characterization, scaling-up, design, and modelling of MW system is required.

Toxicity of As not only depends on the concentration but also depends on the forms of As present in the environment. Thus, to understand and successful application of the remediation strategies, it requires the speciation analysis of the As in the system. Therefore, experiment was designed to determine different As species concentration in wheat and rice grain to explore the effectiveness of MW and sawdust biochar in As phytotoxicity alleviation as the function of the As speciation which is presented in the next chapter.

Chapter 8 – Arsenic speciation in wheat and rice grain grown in microwave and biochar treated soil

8.1 Abstract

Inorganic As species are more toxic than organic species and among inorganic species, arsenite [As(III)] is more toxic than arsenate [As(V)] to plants and humans. Thus, it is necessary to determine the As specie, especially in the food grain, to evaluate the related risk. In this study, the As speciation of wheat and rice grain samples, from previous glasshouse experiments, was performed. In both experiments, the same levels of soil As concentration (As-0, As-20, As-40, As-60, and As-80 mg kg⁻¹) were used. Microwave (MW) soil treatments (0, 3 and 6 minutes) were applied in the wheat experiment and a combination of MW and biochar (BC-0, BC-10, BC-20 t ha⁻¹) treatments were applied in the rice experiment. Results showed that, in wheat grain, As(III) and As(V) was detected and their concentrations decreased in MW treatments, especially in MW-6, compared with the control treatment. In the rice grain samples, As was mainly present (70.59 %) as DMA (dimethyl arsinic acid), an organic As form, and the remaining (29.38 %) was As(III). Significantly lower grain As(III) concentration was observed in the MW treatments compared with the control treatment. Furthermore, rice grain As(III) increased in the biochar treatments (BC-10 and BC-20) compared with the control (BC-0) while, the DMA concentration decreased in the BC-10 treatment, and it increased again in the BC-20 treatment. In both the wheat and rice grain, the species percentage of the more toxic As form [As(III)] was significantly lower in the MW-6 treatment compare with MW-3 and MW-0. Although, the As(III) percentage was higher in both BC-10 and BC-20 compare with the BC-0 treatment, the DMA percentage was lower in the BC-10 treatment compare with the BC-20 and BC-0 treatments. Thus, MW-6 treatment alone for wheat, or combined with BC-10 for rice, could be used for As phytotoxicity alleviation. However, a more detail investigations are needed, considering the MW effect on the different soil's rhizosphere system, interaction of As species with other soil nutrients, and application rate of sawdust biochar.

Keywords: Arsenic speciation, Phytotoxicity, Microwave, Biochar, Wheat, Rice

8.2 Introduction

Arsenic (As) contaminated drinking water is a major pathway of human As exposure (Suzuki and Mandal, 2002). Crops grown using As polluted irrigation water would facilitate the gradual build-up of As in agricultural soil, which could be a potential route of dietary As exposure through plant uptake (Abedin et al., 2002a; Williams et al., 2006). Among cereals, rice has been identified as a good scavenger of elevated As since it has about a 10-fold higher accumulation capacity than other crops because of its cultivation in a reduced condition, which can harbor more As than aerobic cultivation (Bakhat et al., 2017). In addition, wheat can also accumulate As when cultivated in As contaminated soils, which is also adding a means of As exposure as wheat is the second most important staple food after rice (Suman et al., 2020). Hence, besides the drinking water, rice and wheat could be major dietary sources of As for humans. Therefore, searching for cost effective and potentially mitigating options to immobilize As in soils is crucial.

Thus, we have proposed that the combination of MW heating along with biochar amendment could be an effective remediation method to immobilize soil As, which ultimately abates As accumulation in crops including wheat (Chapter 4) and rice (Chapter 5). The result showed that, MW soil heating, especially at 80 – 90 °C heating, had potential to alleviate As phytotoxicity and significantly reduce the grain total As concentration in both wheat and rice. Biochar application demonstrated both negative and positive effect at 10 and 20 t ha⁻¹ soil rate respectively on As phytotoxicity alleviation in rice. However, despite the biochar properties and humified of SOM, as the results of MW soil heating, soil As species could also be an influencing factor of plant growth and grain As concentration since the phytotoxicity and mobility of As not only depends on its total concentration but also on its chemical forms (Abbas et al., 2018).

In the soil and water environment, As is mainly present in two forms, inorganic and organic. Arsenate [As(V)] and arsenite [As(III)] are the inorganic forms while monomethyl arsonic acid (MMA), dimethyl arsinic acid (DMA), trimethylarsine oxide (TMA), arsenobetaine (AB), arsenocholine (AC), and arsenosugars are the most common forms of organic As. The inorganic forms are more toxic to plant, animal, and human than the organic forms and among the inorganic forms As(III) is about 60 times more toxic, soluble, and mobile than the As(V). Several factors such as soil pH, redox condition (Eh), iron hydroxide chemistry influenced the nature of As (Jiménez et al., 2012; Khalid et al., 2017). As(V) is a predominant form present

in aerobic conditions (Khalid et al., 2017) which can form arsenic acid (H_3AsO_4) at very low pH (2 – 3) while it disassociates to H_2AsO_4^- and HAsO_4^{2-} in the range $3 > \text{pH} > 11$. As(III) (e.g. H_3AsO_3) is mostly present in anaerobic condition (Jiménez et al., 2012; Khalid et al., 2017). Whereas, methylation of inorganic As species in soil are mediated by microorganisms to form organic species (MMA, DMA, and TMA) (Huang et al., 2011b). In terms of plant uptake and transportation, As(V) is mainly taken up and transport by the plant through phosphate transporters (PHTs) such as OsPHT1;1 (Kamiya et al., 2013) which is identified in rice roots for As(V) uptake from soil, while different PHT proteins were also reported for root-to-shoot As(V) transport (Wu et al., 2011). In contrast, As(III) and methylated As species are transported via aquaglyceroporins such as nodulin 26-like intrinsic protein (NIP) (Zhao et al., 2010a; Mosa et al., 2012). More specifically, OsNIP2;1 (Lsi1) was identified as a major member for As(III) uptake, which is well known for silicic acid (Si) transport (Ma et al., 2008). Hence, changes of these soil conditions and plant uptake significantly control the As speciation and their bioavailability.

Since, As availability in soil and subsequent uptake by the plant and phytotoxicity not only depends on its concentration but also its chemical speciation and bioavailability, the measurement of total As concentration in plant, thus, is unable to deliver adequate information for the assessment of toxicity and alleviation strategies. Therefore, the main objectives of this study were to determine the different As species in wheat and rice grain to investigate the effect of MW soil heating and biochar on the As speciation in wheat and rice and their relationship with As phytotoxicity alleviation.

8.3 Materials and methods

In this study, the As speciation of wheat and rice grain samples, from previous glasshouse experiments (Chapter 4 and Chapter 5), was performed. Separate glasshouse pot experiments were conducted for wheat and rice at Dookie campus, The University of Melbourne, Australia. In both experiments, the soils were spiked with the same levels of soil As concentration (0, 20, 40, 60, and 80 mg kg^{-1}). Microwave soil treatments (MW irradiation for 0, 3 and 6 minutes) were applied in the wheat experiment and a combination of MW and biochar (0, 10, 20 t ha^{-1} soil) treatments were applied in the rice experiment. The details of soil collection, soil preparation, soil physicochemical properties, application of As into soils, MW application, and experiment setup were described in the Chapter 4 (Wheat) and Chapter 5 (Rice). In addition, biochar preparation, characterization, and application details were described in the rice

experiment (Chapter 5). To describe the treatment combination more conveniently, abbreviated forms were used for As treatments (As-0, As-20, As-40, As-60 and As-80), biochar treatments (BC-0, BC-10 and BC-20) and MW treatments (MW-0, MW-3 and MW-6). Atomic fluorescence spectrometry (AFS) was used for total As analysis of wheat and rice grain samples. The detailed description of the AFS method was given in Chapter 4. The total As concentration data of wheat and rice grain samples, used in this study to calculate As species percentage and recovery percentage, was from previous experiments (Chapter 4 and Chapter 5).

8.3.1 Grain arsenic speciation

The As speciation analysis was performed with the procedure utilized by Signes-Pastor et al. (Pastor et al., 2016). The detail procedure was discussed in previous publication (Islam et al., 2017). High performance liquid chromatography (HPLC) coupled with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used for As speciation. Before the ICP-MS detection, As species were extracted from wheat and rice grain samples using MW assist digestion method.

8.3.2 Extraction of arsenic species

A MW digestion system (MARS 6; CEM Corp, Matthews, NC, USA) with a rotor of 40 Teflon digestion vessels (MARSEXpress TFM) was used for sample extraction. About 0.25 g of finely ground powdered sample was taken into a dry, clean Teflon (PTFE) high pressure digestion vessel and 10 ml of 1 % conc. nitric acid (HNO_3) was added and allowed to stay overnight (12 h). A batch of 36 samples including blank, duplicate, and SRM, were placed in the rotor and the extraction procedure was run for 30 min at 95 °C using a three-stage slow heating program at the progressive power ramp from 290 – 1550 W: ramping to 55 °C in 5 min. holding for 10 min., ramping to 75 °C in 5 min. holding for 10 min., and ramping to 95 °C in 5 min. and holding for 30 min. After cooling down, the digested solution was transfer to a 10 ml sterile polypropylene tube and diluted to the mark with MilliQ water. Then, the solution was filtered through a 0.45 μm Millipore membrane (RC, 0.45 μm , 47 mm; Thermo Fisher Scientific) and 1ml was transferred to a 2 ml amber colored HPLC grade glass vials and stored at 4 °C for further chromatographic analysis.

8.3.3 HPCL-ICP-MS species detection

For chromatographic separation of As species, an Agilent 1260 Infinity liquid chromatography module (Agilent Technologies), equipped with a guard column (Hamilton PRP-X100) and Hamilton PRP-X100 (10 μm , 4.1 X 250 mm) separation column, was used. The samples (20 μl) were injected using an auto-sampler. The mobile phase contained 30 mM phosphate buffer solution prepared from ammonium dihydrogen ortho-phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) and pH was adjusted at 5.6 by adding NH_4OH . The outlet of the separation column was connected to a Babington nebulizer to supply the solution to an Agilent 7900 ICP-MS (Agilent Technologies) for detection of respective As species. The operating conditions of HPLC and ICP-MS are given in Table 8.1. Standard solutions of respective As species were prepared from arsenite (NaAsO_2 , Sigma, St. Louis, MO), arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, Sigma), monomethylarsonate (MMA; $\text{CH}_3\text{AsNa}_2\text{O}_3$, Sigma), and dimethylarsinate [DMA; $(\text{CH}_3)_2\text{AsO}(\text{OH})$, Sigma] and kept at 4°C in darkness until used. For analysis and standard curve preparation dilution of these standard solution were prepared daily. ICP-MS MassHunter software was used to collect the data and chromatographic peak area was calculated in offline mode. From a typical chromatogram of $100 \mu\text{g l}^{-1}$ the retention times were 2.52, 3.07, 4.95 and 8.15 min for As(III), DMA, MMA, and As(V) respectively (Appendix 8.1). The detection limit of the As species in standard solution when using ICP-MS ranged between $0.10 - 0.30 \mu\text{g l}^{-1}$ (Chen et al., 2008).

Table 8.1. HPLC and ICP-MS operating conditions

ICP-MS operating condition	
RF power (W)	1550.00
Plasma gas flow rates (l min^{-1})	15.00
Auxiliary gas flow rates (l min^{-1})	0.80
Carrier gas flow rates (l min^{-1})	1.21
Nebulizer type	Glass concentric
Make up gas flow rates (l min^{-1})	0.10
Sampling depth (mm)	7.90
Sample and skimmer cones	Ni
Spray chamber temperature ($^\circ\text{C}$)	2.00
Peristaltic pump (rev min^{-1})	0.40
Collision cell HEHe (ml min^{-1})	10.00

Data acquisition mode	Time-resolved, m/z 75 for $^{75}\text{As}^+$, and m/z 35 for $^{35}\text{Cl}^+$
Dwell time	0.80 s (m/z 75), 0.20 s (m/z 35)
Energy discriminator (volts)	7.00
HPLC parameters	
Separation column	Hamilton PRP-X100 (10 μm , 4.1 X 250 mm)
Guard column	Hamilton PRP-X100
Mobile phase	30 mM $\text{NH}_4\text{H}_2\text{PO}_4$, pH: 5.60
Mobile phase flow rate (ml min^{-1})	1.00
Sample injection volume (μl)	20.00
Column temperature	Ambient
Acquisition time (min.)	12.00

All the glassware and MW digestion vessel was cleaned with laboratory grade cleaning solution (decon 90) followed by 10 % nitric acid (HNO_3) solution and finally rinsed with MilliQ water. High purity analytical grade chemicals and gases (99.99 % pure) were used for the analysis. All the solutions were prepared with MilliQ water (Milli Q Plus system; Millipore 18.2 $\text{M}\Omega \text{ cm}^{-1}$ resistivity, Bedford, MA). The 1568b SRM (Standard Reference Material) from NIST (National Institute of Standard and Technology, USA) was used for the validation of the digestion method and quality control of speciation analysis. Duplicate and blank samples were also used to compare the actual values in each sample. If recovery of SRM As was $\pm 10\%$ of its certified value and the calculated relative standard deviation (RSD) of sample duplicate was no greater than 5 % it was considered to be a good quality digestion. The observed values were in good agreement with the certified values (Table 8.2).

8.3.4 Statistical analysis

Statistical analysis of data was performed in the R statistical environment (R version 3.6.2) (Team, 2013). Normality and homogeneity tests of the experimental data was performed to examine the distribution of the data. The analysis of variance (ANOVA) test was performed to determine the significance of tested treatments on variables. The Least Significant Difference (LSD) test was performed to compare the treatment means at 5 % level of significance.

8.4 Results

8.4.1 Arsenic determination and quality control

The 1568b rice flour SRM was used for the quality control of As analysis of both wheat and rice grain samples since no wheat SRM was available. The use of rice flour SRM 1568b in the wheat grain As analysis was previously reported (Suman et al., 2020). During the total As determination, the observed mean total As in SRM rice flour was $280.43 \pm 8.57 \mu\text{g kg}^{-1}$ ($n = 9$) with the recovery of 98.40 %, which was within the range of the certified value $285 \pm 14 \mu\text{g kg}^{-1}$ (Table 8.2). Furthermore, during the speciation analysis, the observed mean of DMA and MMA were $194.51 \pm 9.34 \mu\text{g kg}^{-1}$ and $10.33 \pm 4.96 \mu\text{g kg}^{-1}$ yielding a recovery of 108.06 % and 89.04 % respectively. While, the observed value of inorganic As species was $98.32 \pm 9.88 \mu\text{g kg}^{-1}$, which was within the range of the certified value with 106.87 % recovery (Table 8.2).

Table 8.2. Recovery percentage of total and arsenic species of SRM 1568b (Standard Reference Material)

Analyte	Certified value ($\mu\text{g kg}^{-1}$)	Observed value		Mean recovery (%)
		Mean $\pm$ SD	Range	
Total arsenic	285.00 ± 14.00	280.43 ± 8.57	272.11 – 296.25	98.40
DMA	180.00 ± 12.00	194.51 ± 9.34	187.01 – 206.79	108.06
MMA	11.60 ± 3.50	10.33 ± 4.96	7.33 – 16.05	89.04
Inorganic arsenic*	92.00 ± 10.00	98.32 ± 9.88	90.49 – 111.05	106.87

*Total of As(III) and As(V); SD: Standard deviation, DMA: Dimethyl arsine acid, MMA: Monomethyl arsonic acid.

8.4.2 Wheat grain arsenic speciation

In the wheat grain samples, only inorganic As species [As(III) and As(V)] were detected with no evidence of methylated As species (Table 8.3). Arsenite was the dominant As species with 55.96 % (45.62 – 78.14 %) while 27.28 % (21.86 – 54.38 %) was As(V) across all the samples (Table 8.3). The mean recovery of As species was 61.68 % (62.59 – 98.39 %) compared with the total As concentration (Table 8.3). With the increasing of soil As concentration, both the inorganic As species concentrations increased significantly ($p < 0.001$). In contrast, both the As(III) and As(V) concentrations decreased in the MW treatments (MW-3 and MW-6) compared with the control (MW-0) soil treatment. Although it was significant ($p = 0.020$) for As(III), it was not significant ($p = 0.066$) for As(V) (Figure 8.1). For example, at As-80, grain

As(III) concentration was 362.93 $\mu\text{g kg}^{-1}$ while, it was 142.92 $\mu\text{g kg}^{-1}$ at As-20 whereas, the concentrations were 167.40 $\mu\text{g kg}^{-1}$ and 120.63 $\mu\text{g kg}^{-1}$ in MW-6 treatment, respectively. The mean species percentage of As(V) was higher in MW-6 treatment compared with the control though it was not significant ($p = 0.791$) while, mean As(III) percentage was more or less similar in the control and the MW-6 treatment (Table 8.3).

Table 8.3. Total arsenic (As) and As speciation in wheat grain sample in response to microwave (MW) soil treatment at different soil As concentrations

Soil As (mg kg^{-1} soil)	MW (min)	Wheat grain As concentration ($\mu\text{g kg}^{-1}$) (mean $\pm$ SD)			As species (%)		Recovery %
		Total As	As(III)	As(V)	As(III)	As(V)	
0	0	222.48 $\pm$ 24.65	142.92 $\pm$ 51.56	55.41 $\pm$ 36.56	71.67	28.33	88.74
	20	215.92 $\pm$ 16.86	153.74 $\pm$ 36.31	41.21 $\pm$ 10.67	78.14	21.86	90.01
	6	169.66 $\pm$ 19.99	120.63 $\pm$ 45.56	34.43 $\pm$ 24.32	76.19	23.81	91.45
40	0	403.96 $\pm$ 18.60	185.33 $\pm$ 71.41	114.60 $\pm$ 20.99	60.44	39.56	74.56
	3	323.93 $\pm$ 74.47	174.25 $\pm$ 49.73	106.65 $\pm$ 19.61	61.44	38.56	87.65
	6	275.99 $\pm$ 22.91	144.46 $\pm$ 31.51	126.34 $\pm$ 30.43	53.31	46.69	98.39
60	0	432.08 $\pm$ 119.35	210.56 $\pm$ 15.59	177.10 $\pm$ 8.78	54.29	45.71	90.31
	3	329.68 $\pm$ 14.24	198.20 $\pm$ 75.20	59.52 $\pm$ 31.74	75.12	24.88	78.79
	6	314.65 $\pm$ 53.57	186.70 $\pm$ 66.82	64.26 $\pm$ 39.92	73.61	26.39	82.89
80	0	683.1 $\pm$ 89.63	362.93 $\pm$ 123.03	219.28 $\pm$ 82.79	62.38	37.62	83.67
	3	653.31 $\pm$ 75.72	184.60 $\pm$ 39.80	220.05 $\pm$ 44.42	45.62	54.38	62.59
	6	544.97 $\pm$ 101.33	167.40 $\pm$ 16.16	174.48 $\pm$ 94.11	51.62	48.38	62.66
LSD _(0.05)		86.27	88.20	66.92	19.48		20.83

LSD: Least significant difference; SD: Standard deviation; As(III): Arsenite; As(V): Arsenate.

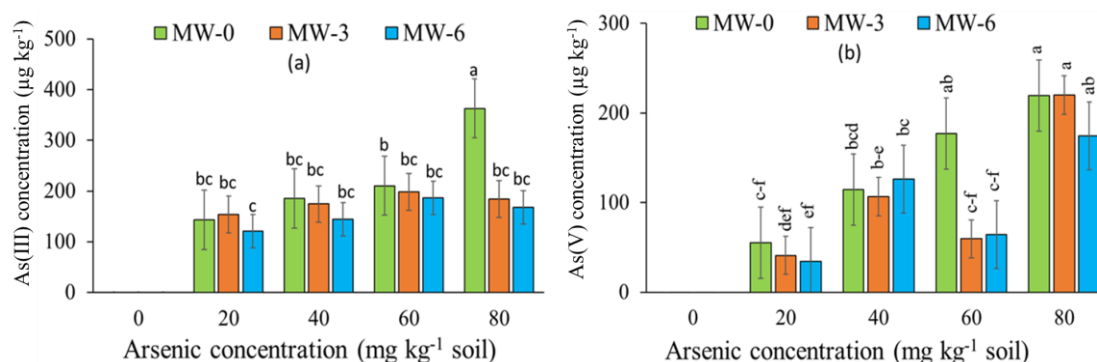


Figure 8.1. Wheat grain (a) Arsenite [As(III)] and (b) Arsenate [As(V)] concentration in response to microwave (MW) soil treatments in arsenic contaminated soils. The mean values are shown in bars along with standard error. Different letters indicate the significant difference (LSD at 5 % significant level) among the treatments

8.4.3 Rice grain arsenic speciation

In the rice grain samples, both the inorganic and organic As species were found, with As(III) accounting for 29.38 % (3.47 – 66.61 %) and the remaining being mainly DMA, accounting for 70.59 % (33.39 – 96.53 %) across all the samples (Table 8.4). The mean recovery of As species was 92.05 % (56.55 – 132.89 %) compared with the total As concentration (Table 8.4). With increasing soil As concentration both the grain As(III) and DMA concentration increased significantly ($p < 0.001$). However, As(III) concentration was significantly ($p < 0.001$) lower in the MW treatments compared with the control treatment, across all the soil As concentrations (Table 8.4) while, the effect of MW on DMA concentration was not significant ($p = 0.196$) (Figure 8.2c). For example, the grain As(III) concentration at As-80 in MW-0 was $416.48 \mu\text{g kg}^{-1}$ whereas it was 109.38 and $107.43 \mu\text{g kg}^{-1}$ in MW-3 and MW-6 treatment, respectively (Figure 8.2a). In the MW treatments, the mean species % of As(III) was significantly ($p = 0.015$) lower and DMA was significantly ($p = 0.014$) higher compared with the control treatment (Table 8.4).

On the other hand, the biochar treatments had a significant ($p < 0.001$) effect on grain As(III) and DMA concentration where, As(III) increased in the biochar treatments compared with the control (Figure 8.2b) and DMA concentration decreased in the BC-10 (10 t ha^{-1} soil) treatment but increased again in the BC-20 (20 t ha^{-1} soil) treatment (Figure 8.2d). For example, the As(III) concentration at As-80 in BC-0 was $167.01 \mu\text{g kg}^{-1}$ whereas it was $220.56 \mu\text{g kg}^{-1}$ in the BC-20 treatment (Figure 8.2b) while $368.47 \mu\text{g kg}^{-1}$ DMA was found at As-80 in BC-0, which decreased ($315.73 \mu\text{g kg}^{-1}$) in the BC-10 and increased ($513.07 \mu\text{g kg}^{-1}$) again in the BC-20 treatment (Figure 8.2d). In the biochar treatments, the mean species % of As(III) was relatively higher ($p = 0.058$) and DMA was lower ($p = 0.058$) compared with the control treatment (Table 8.4). The combined effect of MW and biochar on rice grain As(III) ($p = 0.440$) and DMA ($p = 0.786$) concentration was not significant.

Table 8.4. Rice grain total arsenic (As) and As speciation in response to microwave (MW) and biochar application at different soil As concentration

Soil As (mg kg ⁻¹)	Biochar (t ha ⁻¹)	MW (min)	Total As concentration (µg kg ⁻¹) (mean ± SD)	Arsenic species concentration (µg kg ⁻¹) (mean ± SD)		As species %		Recovery %
				As(III)	DMA	As(III)	DMA	
20	0	0	342.97 ± 78.21	134.82 ± 125.02	192.41± 57.02	36.17	63.83	95.24
		3	287.18 ± 62.67	45.43 ± 78.68	211.60± 100.46	19.59	80.41	91.55
		6	281.37 ± 32.64	56.20 ± 97.35	171.53± 104.79	24.37	75.63	80.55
	10	0	290.21 ± 67.48	113.03 ± 97.89	154.02± 79.58	40.67	59.33	95.88
		3	276.98 ± 43.21	33.12 ± 57.37	193.53± 104.01	19.05	80.95	83.03
		6	226.70 ± 36.55	55.54 ± 96.19	149.86± 63.01	22.77	77.23	91.55
	20	0	631.68 ± 215.34	183.24 ± 127.22	141.32± 26.02	51.99	48.01	56.55
		3	412.17 ± 80.02	114.01 ± 35.22	150.47± 85.30	46.10	53.90	68.03
		6	392.21 ± 46.77	187.67 ± 61.03	103.00± 34.94	64.66	35.34	72.88
40	0	0	419.36 ± 139.79	113.91 ± 113.93	237.33± 106.40	31.71	68.29	83.38
		3	316.15 ± 67.55	8.54 ± 11.78	239.04± 13.46	3.47	96.53	81.11
		6	279.08 ± 26.87	17.31 ± 19.00	205.24± 18.53	7.76	92.24	80.16
	10	0	258.74 ± 85.19	99.02 ± 87.71	179.80± 139.35	39.48	59.49	112.79
		3	192.78 ± 43.16	33.57 ± 58.15	171.00± 87.66	19.08	80.92	107.50
		6	207.23 ± 47.00	33.69 ± 50.21	154.72± 62.85	18.66	81.34	92.35
	20	0	707.56 ± 42.56	207.39 ± 121.19	476.39± 177.08	31.38	68.62	96.84
		3	600.99 ± 207.73	174.90 ± 91.65	479.63± 167.27	27.16	72.84	111.83
		6	501.22 ± 48.62	142.63 ± 11.27	356.01± 51.07	28.79	71.21	100.43
60	0	0	402.77 ± 122.96	106.58 ± 56.75	310.21± 86.23	26.09	73.91	107.71
		3	328.16 ± 93.71	16.47 ± 28.53	303.50± 117.58	7.04	92.96	97.91
		6	329.73 ± 62.10	34.55 ± 59.84	302.03± 159.94	14.85	85.15	104.49
	10	0	493.38 ± 18.49	176.92 ± 192.06	273.94± 157.77	37.02	62.98	91.58
		3	358.13 ± 35.75	112.15 ± 194.25	243.55± 193.22	30.88	69.12	99.50
		6	250.35 ± 15.01	102.91 ± 178.25	209.64± 141.98	28.35	71.65	124.14
	20	0	853.57 ± 65.96	182.98 ± 67.27	326.34± 110.90	36.51	63.49	60.45
		3	609.63 ± 86.63	130.99 ± 63.53	355.82± 19.20	26.01	73.99	81.13
		6	643.09 ± 182.54	154.53 ± 64.18	358.78± 154.88	31.37	68.63	81.92
80	0	0	867.65 ± 22.67	405.37 ± 81.75	391.73± 79.94	50.84	49.16	91.90
		3	419.89 ± 208.60	46.74 ± 57.02	403.52± 24.07	9.81	90.19	132.89
		6	368.63 ± 159.91	48.93 ± 79.23	310.16± 142.19	16.37	83.63	110.05
	10	0	889.36 ± 54.90	327.31 ± 118.79	270.97± 176.69	66.61	33.39	88.59
		3	629.25 ± 28.39	102.51 ± 177.56	321.19± 195.39	25.41	74.59	67.33
		6	477.40 ± 26.25	117.87 ± 204.15	355.04± 247.15	27.36	72.64	99.65
	20	0	982.63 ± 86.31	516.75 ± 25.90	605.15± 44.02	35.14	64.86	95.27
		3	804.56 ± 47.39	178.88 ± 33.80	552.23± 194.30	26.12	73.88	91.55
		6	631.28 ± 87.46	155.49 ± 12.86	381.82± 18.31	28.95	71.05	86.23
LSD _(0.05)			137.54	144.49	174.88	40.22	40.30	33.58

LSD: Least significant difference; SD: Standard deviation; As(III): Arsenite; As(V): Arsenate, DMA: Dimethyl arsinic acid.

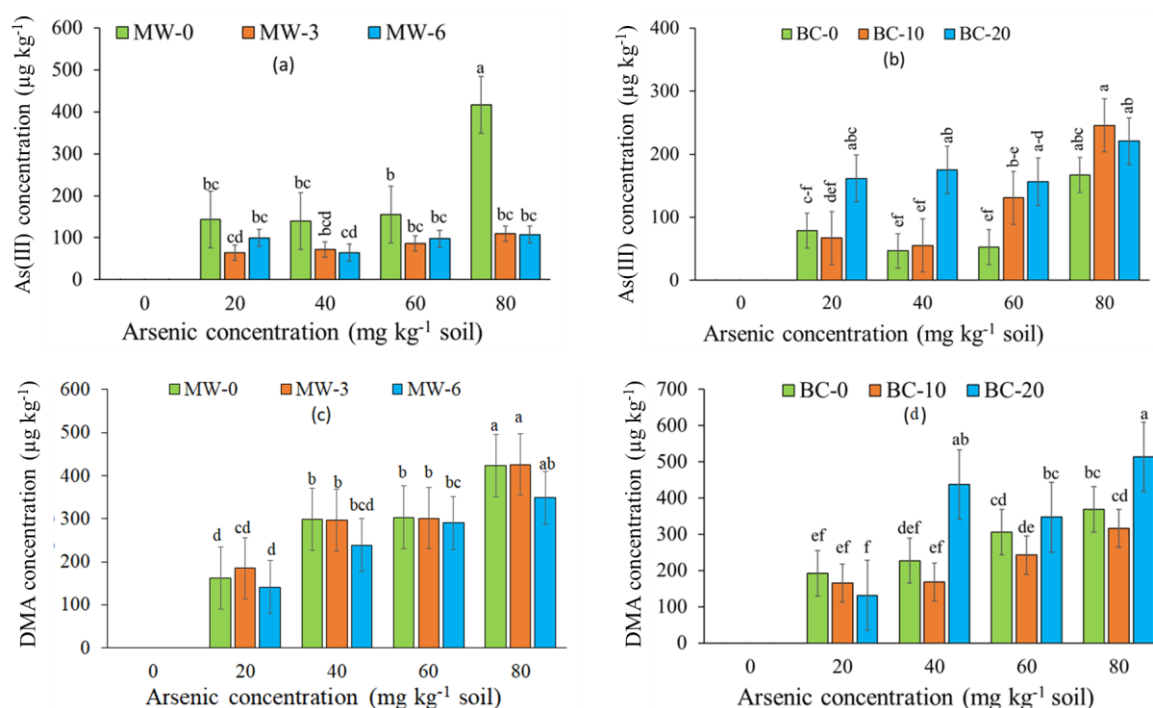


Figure 8.2. Arsenic (As) speciation of rice grain sample. Effect of (a) microwave (MW) and (b) biochar treatment on arsenite [As(III)] concentration and effect of (c) MW and (d) biochar on dimethyl arsenic acid (DMA) concentration. The mean values are shown in bars along with standard error. Different letters indicate the significant difference (LSD at 5 % significant level) among the treatments

8.4.4 Reduction of grain arsenic concentration in microwave treatments

In the wheat grain sample, the reduction of As(III) concentration ranged from 5.87 % to 53.88 % while it was 6.94 % to 66.39 % for As(V). The highest reduction (53.88 %) in As(III) concentration was observed in the MW-6 treatment at As-80 whereas in MW-3 treatment it was 66.39 % at As-60 for As(V). In the rice grain sample, the reduction of As(III) concentration ranged from 30.54 % to 74.21 % and it was 0.43 % to 19.87 % for DMA. The highest reduction (74.21 %) in As(III) concentration was observed in the MW-6 treatment at As-80 while it was 19.87 % in MW-6 treatment at As-40 for DMA (Table 8.5).

Table 8.5. Effect of microwave (MW) soil treatment on the reduction of wheat and rice grain arsenic (As) concentration

Wheat grain As reduction (%)						Rice grain As reduction (%)					
As species	MW (min)	Soil As concentration (mg kg ⁻¹)				As species	MW (min)	Soil As concentration (mg kg ⁻¹)			
		20	40	60	80			20	40	60	80
As(III)	3	+ 7.57	– 5.98	– 5.87	– 49.14	As(III)	3	– 55.33	– 48.37	– 44.34	– 73.74
	6	–15.60	– 22.05	– 11.33	– 53.88		6	– 30.54	– 53.93	– 37.40	– 74.21
As(V)	3	– 25.63	– 6.94	– 66.39	+ 0.35	DMA	3	+ 13.91	– 0.43	– 0.84	+ 0.72
	6	– 37.86	+ 10.24	– 63.72	– 20.43		6	– 12.99	– 19.87	– 4.40	– 17.42

Negative and positive value indicate the reduction and increase percentage respectively. As(III): Arsenite; As(V): Arsenate, DMA: Dimethyl arsinic acid.

8.4.5 Comparison between wheat and rice grain arsenic speciation

Only inorganic As species were detected in the wheat grain samples, where As(III) was the more dominant species than As(V), whereas both the inorganic and organic species were observed in the rice grain sample, among which DMA was the more dominant species than As(III). Although As(V) was detected in the wheat samples, it was not detected in the rice samples and no MMA was found in either the wheat or the rice samples. However, total As concentration in the rice grain there was 1.32 times that of the wheat grain and the As(III) concentration was 1.51 times higher in the wheat grain than the rice grain (Table 8.6).

Table 8.6. Comparison of arsenic (As) species concentration in wheat and rice grain at different soil As concentration

Soil As (mg kg ⁻¹)	Total As (µg kg ⁻¹)		As(III) (µg kg ⁻¹)		As(V) (µg kg ⁻¹)		DMA (µg kg ⁻¹)		Rice/ wheat	Wheat/ Rice
	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Total As	As(III)
20	202.69	349.05	139.10	102.56	43.68	–	–	163.08	1.72	1.36
40	334.63	387.01	168.01	92.33	115.86	–	–	277.68	1.16	1.82
60	358.00	474.31	198.47	113.12	100.29	–	–	298.20	1.33	1.75
80	627.13	674.52	238.29	211.09	204.60	–	–	399.09	1.08	1.13
Average	380.61	471.22	185.97	129.78	116.11	–	–	284.51	1.32	1.51
LSD _(0.05)	24.37	45.85	50.92	48.16	38.63	–	–	58.29	–	–

LSD: Least significant difference; As(III): Arsenite; As(V): Arsenate; DMA: Dimethyl arsinic acid.

8.5 Discussion

All the observed As species and total As concentration of the SRM were within the range of the certified value with 98.40, 108.06, and 106.87 % recovery for total As, DMA, and inorganic species respectively (Table 8.2). Several previous studies also used NIST, 1568b SRM to validate the extraction efficiency of total As and As species of rice and other environmental samples where similar results of this study were reported (Islam et al., 2017; Atiaga et al., 2020). For example, in a recent study $349 \pm 10 \mu\text{g kg}^{-1}$, $109 \pm 4 \mu\text{g kg}^{-1}$, $218 \pm 9 \mu\text{g kg}^{-1}$, and $18 \pm 0.1 \mu\text{g kg}^{-1}$ with 123 %, 107 %, 113 %, and 118 % of recovery in comparison with certified values of total As, inorganic As, DMA and MMA, respectively, were observed in SRM 1568b (Atiaga et al., 2020).

It was evident from Table 8.3 that, only inorganic As species were observed in the wheat grain samples with no detection of the organic forms of As. The majority of the extracted As species (55.96 %) was As(III) compared (27.28 %) with As(V) across all the samples. These findings agree with the previous results of wheat grain As speciation studies (Zhao et al., 2010b; Ruttens et al., 2018). For example, Zhao et al. detected only inorganic As species in wheat grain samples grown in As contaminated field ($1.3 - 28.5 \text{ mg kg}^{-1}$ soil) where As(III) was the most dominant (64 – 90 %) species (Zhao et al., 2010b). In present study, the detection of only inorganic species in wheat grain would be as a consequence of aerobic soil cultivation since there is some evidence that microbial methylation of As in soil greatly is enhanced by flooded condition (Mestrot et al., 2009), which would be further supported by the measly methylated As species accumulation in aerobic rice cultivation. The prospect of As methylation in plants remains contentious (Zhao et al., 2010a). Meanwhile it has also been reported that plants might have the ability to methylate As inside the plant body (Meharg and Zhao, 2012). Thus, very low amounts or no DMA was present in the wheat soil of this study, since the wheat plant can uptake higher DMA if it is present in the soil (Duncan et al., 2017). However, very low concentrations of DMA and MMA ($< 0.001 - 0.007 \text{ mg kg}^{-1}$) were also reported in wheat grain and wheat flour samples where the extraction method was methanol-water based (Tsai and Jiang, 2011) rather than the acid extraction used in the present study. It was also evident from Table 8.3 that wheat grain As concentration of both inorganic species increased with increasing soil As concentration. Similar results were observed in previous studies where it was clearly shown that higher wheat grain As concentration was the consequence of higher soil As (Zhao et al., 2010b; Karimyan et al., 2020).

Both inorganic and organic As species were detected in the rice grain samples. As(III) was the only detected inorganic species (29.38 %) and the remaining was mainly DMA (70.59 %) as the organic and dominant form of As across all the samples (Table 8.4). Similar results were also reported in some other research, where rice grain As was found to be mainly As(III) and DMA (Meharg et al., 2009; Syu et al., 2015). However, As(III), As(V), and DMA are the predominant species present in rice grain (Rahman et al., 2014a), while other organic species, such as MMA, AsB, and AsC, are rarely detected (Pizarro et al., 2003). Flooding condition in rice soil results in the mobilisation of As and more available in soil solution and ultimately higher uptake by rice plant (Koyama, 1975). The higher DMA in rice grain is mainly the consequence of higher DMA present in the soil due to microbial methylation of As (Jia et al., 2013), which is greatly enhanced by flooded condition (Mestrot et al., 2009).

Furthermore, DMA translocation efficiency from roots to shoots and finally export to the rice grain is higher than the other species of As due to the higher mobility of DMA within plants in both the phloem and the xylem, even though the plant uptake efficiency of DMA is lower than the others (Raab et al., 2007b; Carey et al., 2010). Arsenic species translocation into rice grain substantially dissimilar than the whole-plant system which could be elucidated based on thiol complexation, physiological barrier and preferential translocation, and localization and distribution of As species. Methylated As species have less affinity to thiol complexation than the inorganic As species when translocated through the plant (Raab et al., 2007a). Thus, poorer thiol complexation affinity of DMA in particular, results in less obstruction at translocating time and in due course leads to higher accumulation in the grain. Like other nutrients, As is also unloaded symplastically into the grain through the ovular vascular trace (OVT) to nucellar tissue apoplastically and then radially inwards to the filial tissues (aleurone, endosperm, and embryo). In this transfer process, there is a physiological barrier and As species cross this barrier with differential efficiency. In this occasion, DMA is more efficient than inorganic As species at crossing this barrier (Krishnan and Dayanandan, 2003; Lombi et al., 2009). After influx into the grain, DMA is more rapidly and efficiently distributed throughout the grain and into the nucellar and filial tissues while inorganic As is inclined to remain in the OVT (Carey et al., 2010; Carey et al., 2011). Thus, more DMA could upload into the grain more readily. It was also evident from Table 8.4 that, rice grain As concentration of both inorganic and organic species increased with increasing soil As concentration. It was also evident from other studies that the rice grain inorganic and organic As species concentration increased when rice is grown in higher As contaminated soil (Syu et al., 2015).

On the other hand, it was observed from Figure 8.1 and Figure 8.2 that both in the wheat grain and rice grain As species concentration decreased in the MW treatments compared with the control treatment. The reduced grain As concentration could be attributed to the increased soil nutrients (P, S, and Si) after MW soil heating (Table 4.1 and Table 5.1). It was evident from the previous studies that, MW soil heating has the potential to increase soil N as a consequence of soil indigenous N mineralization, P, and S availability in soil (Cooper and Brodie, 2009; Khan et al., 2019b). These more available nutrients could result the better plant growth and ultimately higher grain yield in MW treated soils. Eventually, higher grain yield would be one of the possible reasons for lower grain As concentration in MW treatments, because of the dilution effect, though the plants could uptake similar amounts of As.

Since, phosphate (PO_4^{3-}) is known as analogous to As(V) and Si to As(III) and DMA, the increased availability of P and Si could enhance competition of these elements with respective As species for plant uptake because of the similar uptake mechanism through phosphate and aquaglyceroporins transporter respectively present in the plant root (Zhao et al., 2010a; Mosa et al., 2012). Thus, the increased soil P and Si concentration after MW soil heating could compete with As(V) and As(III) for plant uptake and reduce the accumulation in the grain. However, a significant increase in DMA concentration in the rice grains after adding Si was also observed (Liu et al., 2014b). The Si efflux transporter Lsi2 is not permeable to DMA which was attributed to the reduction in As(III) uptake but not the DMA. Also, Si may increase the DMA concentration in the soil solution by reducing DMA adsorption by means of ligand exchange. Hence, an increased DMA concentration in soil solution and less competition for root uptake may result the higher DMA accumulation in the rice grain (Ma et al., 2008; Li et al., 2009b; Liu et al., 2014b). Thus, the above discussion could explain the higher decrease of As(III) concentration compare with the DMA concentration in the MW treatments of this present study. Also, As(III)-thiol complexation and subsequent sequestration to vacuoles is an As detoxification mechanism of plant (Song et al., 2010). Sulphur is an important constituents of thiol (Dixit et al., 2015) and increased S concentration could enhance the vacuole sequestration of As(III) and reduce its translocation into the grain. Hence, more availability of soil nutrients and less percentage of more toxic As form could explain the reason of minimizing As phytotoxicity and subsequent lower As the concentration of wheat and rice grain in MW treatment.

Moreover, it has been reported that MW soil heating noticeably altered the properties of SOM (Hur et al., 2013), enhanced its humification (Kim and Kim, 2013), increased soil

organic carbon (Zagal, 1989), synthesized the macromolecular organic substances with a higher number of functional groups (Kim and Kim, 2013) and synthesized the organometallic and coordination compounds (Taylor et al., 2005). These changes in SOM could reduce As bioavailability and mobility, like other heavy metals, by adsorbing them onto the adsorption sites of humified organic matter (Yin et al., 2002). For example, Hur et al. reported that, MW soil heating can enhance the binding efficiency of hydrophobic organic contaminants with the more humified SOM (Hur et al., 2013). Another study also implied that dissolved organic carbon (DOC) increased in soil after MW soil irradiation (Maynaud et al., 2019). Dissolved organic carbon has a higher affinity to combine with As, resulting in the formation of organo-As complexes (Kumarathilaka et al., 2018). Thus, the above changes in SOM, after MW heat treatment, could enhance the adsorption of As and reduce accumulation in plants.

Several studies have demonstrated that biochar application is an effective technique for As remediation due to their ability to sort As and subsequently alleviate As phytotoxicity (Khan et al., 2013; Waqas et al., 2014). After biochar application, As is mainly immobilized by ion exchange (surface inner sphere complex or co-precipitation with biochar), surface complexation with different functional groups (carboxyl, alcoholic hydroxyl or phenolic hydroxyl groups), and chemical or physical sorption and surface precipitation (Lu et al., 2012; Zhang et al., 2013; Vithanage et al., 2014). Significant reduction of As(III) (72 %), As(V) (62 %) and DMA (74 %) in rice was reported when using sewage sludge biochar (Waqas et al., 2014). Similarly, Khan et al (Khan et al., 2013) also reported the immobilization of As after application of sewage sludge biochar into soil and the average decreases in rice grain As concentration at 5 % and 10 % (w/w) biochar applications were 69.8 % and 74.1 %, respectively.

Unexpectedly, in this present study, the application of sawdust biochar had a negative effect on rice grain As concentration. The concentration of As(III) increased in both the BC-10 and BC-20 treatments compared with the BC-0 (Figure 8.2b) and although, DMA concentration decreased in the BC-10 treatment it increased again in the BC-20 treatment (Figure 8.2d). The increased concentration of grain As(III) in the biochar treatments of this present study could be attributed to various reasons. Application of biochar was found to increase the reduction rate of As(V) to As(III). Biochar has a larger exposed surface area with different surface functional groups. Some of these functional groups can transfer π -electrons to As(V) thereby reducing it to As(III) (Choppala et al., 2016). Several soil microorganisms have also been identified to reduce As(V) (Smith et al., 1998). Biochar application can increase soil

microbial activity because of the higher availability of carbon as an energy source for microbes (Choppala et al., 2012). For instance, biochar releases numerous organic molecules found to induce Fe(III) reducing bacteria, which can drive reductive resolution of FeOOH, leading to the release of As(III) into the soil solution (Mladenov et al., 2015; Chen et al., 2016). In addition, biochar usually possesses more negatively charged functional groups on the surface, which sometimes results in an inability to adsorb As(III) due to anion exclusion (Zhu et al., 2016). Thus, all these points may contribute to an increase in the soil As(III) concentration in biochar treated soils of this present study and thereby an increased As(III) concentration in the grain samples because of the higher solubility and mobility of As(III) than the other As species (Zhu et al., 2018).

Furthermore, Hartley et al. (Hartley et al., 2009) reported that the increased mobility of As after adding biochar, resulted from the rise in soil pH. During the pyrolysis process, at high temperature, base cations are transformed into oxides, hydroxides or carbonates in the biochar and (Yuan et al., 2011) dissolution of these alkaline substances increases the soil pH (Houben et al., 2013). Also, increased CO₂ evolution, due to increased microbial activity in biochar applied to soil, may attributed to more addition of labile C and increase the soil pH (Farrell et al., 2013). In the soil solution, As is mainly present as a hydro anion and increased soil pH, after biochar application, can reduce As sorption capacity by decreasing the positively charged sites on soil minerals, which increases As mobilization and release from the soil (Wilson et al., 2010). Our previous study (Chapter 5) also showed that, the application of saw dust biochar can increase the soil pH. At BC-10 and BC-20 treatment the average soil pH was 8.15 and 9.00 respectively, while it was 7.73 in the BC-0 treatment (Chapter 5). This increment of soil pH could increase the mobility and uptake of As by plant.

Moreover, the increased concentration of NO₃⁻ and PO₄³⁻ ions was reported when biochar was applied. The competitive effect of NO₃⁻ and PO₄³⁻ ions could be responsible for the desorption of As oxyanions from adsorption sites by an exchanging phenomenon (Bang and Meng, 2004; Tyrovola et al., 2006; Ahmad et al., 2017). A similar result was reported in a recent study where the application of sawdust biochar increased As mobility (Amin et al., 2020). Beiyuan et al. reported higher mobility of As when pine sawdust biochar was applied soil. They revealed that, increased soil pH and less oxygen-containing functional groups was responsible for higher As mobility (Beiyuan et al., 2017). Thus, it was also reasonable in this present study that As(III) and DMA mobilization was higher in both biochar treatments (BC-10 and BC-20), which ultimately increased their concentration in grain. However, reduced

concentration of DMA in the BC-10 biochar application treatment could be attributed to the lower increment in soil pH and lower competitive effect of NO_3^- and PO_4^{3-} ions due to the lower biochar application rate compare with BC-20.

However, total As concentration in the rice grain was 1.32 times higher than in the wheat grain and As(III) concentration was 1.51 times higher in the wheat grain than the rice grain (Table 8.6). Previous studies also reported that, rice has the capacity to accumulate more As (up to 10-fold) than wheat (Williams et al., 2007) although it was 1.32-fold in present study, which could be due to the genetically higher capacity for As accumulation by the wheat variety (Zhao et al., 2010b) or other dissimilarities in soil properties (Dai et al., 2016) compared to previous studies. The reason why rice has more capacity to accumulate As than the wheat are, rice is usually cultivated under flooded conditions, which is conducive to As(V), where As(V) reduced to As(III) and increases mobilization later, which is taken up by the membrane transporters for Si, OsLsi1 and OsLsi2 the highly expressed transportation mechanisms in rice roots (Ma et al., 2008). Although DMA is less toxic than inorganic As species (Hughes, 2002), its higher accumulation in rice grain would be a concern, especially from human health perspective given the toxicity of reduced methylated As species (Mass et al., 2001).

8.6 Conclusions

Although, rice has been reported as a good accumulator of As, in this study wheat was also found to accumulate a considerable amount of As in the grain even higher As(III) concentration was observed in wheat grain than rice grain. Contrasting with the rice, where DMA was dominant, As(III) was more abundant in wheat than As(V), despite the fact that wheat, unlike rice, is grown in aerobic soil conditions. Although, the less toxic DMA concentration in rice grain was higher compared with the more toxic As(III), it could be still toxic because of higher concentration. Both the As(III) and As(V) concentration in wheat and As(III) concentration in rice reduced in MW treatments whereas, such effect was not observed on the DMA concentration. Thus, MW soil treatment especially MW-6 could be applied to reduce As phytotoxicity in wheat and rice by decreasing the As(III) concentration, which is the more toxic form of As. However, a more detail investigation is needed on the effect of MW on the soil rhizosphere system and the interaction mechanisms of As species with other soil nutrients in different soil types. The application of sawdust biochar needs to be reconsidered may be with

different application rate for the As immobilization since a negative effect of this biochar was observed in this study.

It is evident from the previous findings that the main effect of the MW irradiation is the heating effect. Since soil heating has some adverse effect on the soil microorganisms, MW soil heating would have some detrimental effect as well. Hence, it is important to know the effect of MW soil heating on soil microorganisms while applying this MW technology for As phytotoxicity alleviation. Therefore, the experiment was designed to investigate the effect of MW soil heating on soil bacteria as one of the most important soil microorganisms, which is presented in the next chapter.

Chapter 9 – Bacterial community response to the microwave soil heating in arsenic contaminated soils

9.1 Abstract

Microwave (MW) soil heating has emerged as a novel technique for arsenic (As) phytotoxicity alleviation; however, the effect of MW soil heating on soil beneficial microorganisms, particularly on bacteria, is a topmost concern. Therefore, this experiment was designed to investigate the effect of MW heating on soil bacterial community diversity and composition in As contaminated soils (As-0, As-40, and As-80 mg kg⁻¹ soil). Twenty-five grams of soil in replicated (four) microcosms were incubated for four weeks prior to MW treatment (80 °C) and up to 56 days after MW treatment at 25 °C. Soil DNA was extracted at different time points (0, 14, 28, 42, and 56 days after MW treatment) for 16S rRNA amplicon sequencing and quantitative PCR (qPCR) analysis. Soil As speciation was performed to see the changes of As species after MW treatment. For statistical analyses of the data, R software was used. Bacterial community richness (α -diversity) decreased significantly in MW treated soils and did not recover to its original state, even after 56 days of the incubation period. The bacterial community composition (β -diversity) changed significantly in MW treated soils. The total number of 16S rRNA bacterial gene copies decreased significantly immediately after MW soil heating but recovered back to its previous number after 42 days of incubation. There was no significant effect of soil As concentration on α -diversity, β -diversity, and 16S rRNA bacterial gene copies was observed. The relative abundance of phylum Firmicutes increased significantly after MW treatment and remain dominant throughout the incubation period. The relative abundance of some beneficial bacteria (e.g. *Bacillus* and *Symbiobacterium*) were significantly higher in MW treated soils compare to MW untreated soils. Dimethyl arsinic acid (DMA), the methylated and less toxic form of As, was found only in MW treated soils. Nevertheless, the reduction in bacterial richness, diversity and composition indicated a detrimental impact of MW soil heating on some soil bacteria. However, heat resistance bacteria can reshape the community after MW treatment and may facilitate important function, especially biomethylation, one of the ways to reduce As phytotoxicity. Thus, MW soil heating can potentially be applied for As phytotoxicity alleviation without significantly destroying the ecologically important bacteria.

Keywords: Microwave soil heating, Arsenic, α -diversity, β -diversity, 16S rRNA

9.2 Introduction

Arsenic (As) is a toxic and carcinogenic metalloid ubiquitously distributed in soils and water environments. It has raised serious global concern because of its adverse effect on human health and the ecosystem (Abernathy et al., 2003; Naidu et al., 2006). Microwave (MW) soil heating at 80 – 90 °C revealed as a novel technique for As phytotoxicity alleviation in wheat and rice (Chapter 4 and 5). Besides this beneficial effect of MW, the temperature induced by MW irradiation could also result in undesirable side effects on soil biological properties (Cooper and Brodie, 2009; O'Brien et al., 2018). Soil is a highly complex and heterogeneous body, which provides habitat for bacteria, fungi, nematodes, and arthropods (Lu et al., 2014). These populations can be affected by MW soil heating (O'Brien et al., 2018). Various studies have investigated the effect of MW on soil microbial populations and found varying susceptibilities in response to MW heating. Specifically, MW soil heating could be detrimental to bacterial community by directly destroying the bacterial cell (Woo et al., 2000; Shamis et al., 2012). Furthermore, 2.45-GHz MW treatment was found to reduce the bacterial community in the top layer (0 – 10 cm) of soil whereas no effect was found on fungi, amoeba, and flagellate (Brodie et al., 2016). Previous study reported that, the population of *Escherichia coli* decreased by 10^5 in the top layer of soil treated with 500 J cm^{-2} of MW energy (Brodie et al., 2016). Another study revealed that, the bacterial population decreased by 78 % when soil was exposed to MW (750W, 2.45 GHz, 2 – 16 min) soil heating, however, the soil was not completely sterilize even after 16 minutes of MW irradiation which suggested bacterial population could recover after MW treatment (Cooper and Brodie, 2009). Since bacteria are vastly diverse entities in the soil, the community might undergo a rapid regrowth and recovery after any disturbance or stress condition, resulting no significant long term effect (Fierer et al., 2007).

Microwave soil heating can create stress condition for soil bacteria by increasing soil temperature. However, the effect of MW soil heating mainly depends on the soil moisture content, MW intensity, soil heating depth, treatment duration, and soil temperature (Nunes et al., 2018; Khan et al., 2019d; Kabir et al., 2020). Khan et al. reported that, bacterial community richness and composition can change significantly after MW soil heating at around 75 – 80 °C of the top soil layer (0 – 10 cm). They also revealed that, the community did not recover after 28 days of MW treatment which suggest more time required for community recovery (Khan et al., 2019d). Survival of the heat resistance bacteria (e.g. Firmicutes) and recovery and reshaping of the community after MW soil heating also observed (Nunes et al., 2018). Thus,

MW soil heating can create momentary alterations in bacterial communities which may recover over time (O'Brien et al., 2018).

In the environment, As is mainly present as inorganic [arsenate; As(V) and arsenite; As(III)] and organic [e.g. monomethyl arsonic acid (MMA) and dimethyl arsinic acid (DMA)] forms (Han et al., 2017). Inorganic As species are more toxic than the organic species and among the inorganic species As(III) is more toxic than As(V) due to higher mobility and solubility in soil and plant (Cai et al., 2016). The interchanges of these forms largely depend on the soil condition and soil microbes. Influences such as reduction, oxidation, and methylation, which are mainly arbitrated by soil microbes or plants, regulate the destiny of As species (Zhao et al., 2009; Yoshinaga et al., 2011). Biomethylation of inorganic As species to organic species is one of the ways of reducing As phytotoxicity (Mestrot et al., 2011). Although some soil microbes are responsible for the methylation of As species and formation of volatile MMA, DMA and TMA, far less is known about the microorganisms involved in this process in soil (Huang et al., 2011b). Some studies have shown that methylated As species (mostly DMA), which are present in the crop grain (e.g. Rice) (Zheng et al., 2011), most likely originated from the soil via the activities of methylating microorganisms, and are not from *in vivo* methylation in the plants (Lomax et al., 2012). Thus, soil microbial communities are key regulators of species dependent As toxicity in plants. For example, the microbial methylation process of As(III) is the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to As(III), which is catalysed by As(III) S-adenosylmethionine methyltransferase (ArsM) (Qin et al., 2006). Those soil microbes (e.g. bacteria, archaea, and fungi) which possess the ArsM homologues, have the ability of methylating the inorganic As species (Qin et al., 2006). However, very few studies have been conducted to known about the abundance and diversity of the *arsM* operon gene in the soil environment (Afroz et al., 2019). Since the soil bacterial community is affected by MW soil heating treatment, followed by the other soil microorganisms, the effect of MW soil heating on the bacterial communities that possess the *arsM* gene would be crucial to study and explain the alleviation of As phytotoxicity by MW soil treatment.

However, due to the limited research on the *arsM* gene and its involvement in As methylation in soil rhizosphere, it is still an experimental challenge to successfully investigate the *arsM* related bacterial abundance owing to the phylogenetic diversity and limited As methylation sequences available (Afroz et al., 2019). Hence, this study was mainly focused on the impact of MW soil heating on the bacterial communities, although the experimental soil

was treated with different As doses and soil As speciation was determined to see if there were any changes in As forms and related microbes in response to MW soil heating. Although still more detailed investigation needed to calibrate and standardize the MW soil heating before application, the response of bacterial community to MW heating and post-heating recovery of the communities are primarily needed to be studied. Hence, the hypotheses of this study were (i) MW soil treatment would alter the bacterial community based on the heat resistance and sensitive bacteria, (ii) community would recover to its original level within the 56 days incubation time, and (iii) the concentration of methylated As species will increase in MW treated soils. Therefore, this experiment was designed to investigate the effect of MW soil heating on (i) bacterial abundance, diversity and composition, (ii) subsequent recovery of bacterial community after heating, and (iii) As speciation in the soil.

9.3 Materials and Methods

9.3.1 Soil Sample Collection and preparation

Soil samples were collected with a soil core sampler (H:10 cm, D:3 cm) at the depth of 0 – 10 cm from a crop production paddock of the Dookie farm (Paddock H12, 36°23'51"S; 145°43'17"E), Dookie College Campus, The University of Melbourne, Australia. The detail soil properties described in previous chapter (Chapter 5).

9.3.2 Arsenic application

Since, the As concentration of pre-experimental soil was below the detection limit ($< 0.01 \mu\text{g kg}^{-1}$), there was no chance of adding As from soil. Hence, the initial bulk soil was spiked with three different As concentrations (0, 40, and 80 mg kg^{-1} soil) as sodium arsenate heptahydrate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) (Bhattacharya et al., 2015). Respective amounts of sodium arsenate were mixed with distilled water (required to make soil moisture at 45 % field capacity) to prepare the As solutions followed by mixing with the soil by spraying onto the soil and homogenized thoroughly by hand mixing.

9.3.3 Pre-microwave incubation of soil

Immediately after As application, 25 g of soil sample from each respective As treatment (As-0, As-40, and As-80) was taken in 270 ml cylindrical sterile microcosms (4 cm in diameter by 8 cm deep). The experimental design is depicted in Appendix 9.1. A total number of 120 microcosms (with four replication) were placed in a temperature-controlled incubator (PHCbi,

MIR-254-PE) at 25 °C for four weeks as the pre-incubation period. An extra ten microcosms were also placed in the incubator with the same amount of soil and moisture content to calibrate the MW treatment duration. All the microcosms were aerated by opening their lids every three days to maintain proper aerobic conditions, and moisture loss was replaced by adding distilled water to maintain a constant moisture (45 % field capacity) during the whole pre-microwave incubation period (Khan et al., 2019d).

9.3.4 Microwave soil treatment

Based on chapter 4 and 5 findings, after four weeks of incubation, the respective microcosms were treated with a MW oven (1100 W) for 25 s to rise the soil temperature at around 80 – 85 °C. Thus MW-80 was used as MW treated soil and MW-0 as the control soil. Since the soil moisture was maintained at 45 % field capacity, MW treatment duration was determined using the extra microcosms (Appendix 9.2). To monitor the soil temperature after MW irradiation, thermal images (Appendix 9.3) were taken immediately (within 2 – 5 s) after soil heating by using an infrared thermal camera (FLIR T1050SC). After MW treatment, the respective microcosms were taken for DNA extraction at 0 days of incubation and rest of the microcosms were again placed in the incubator for further incubation periods of 14, 28, 42 and 56 days. The incubation period of this current study was considered based on the findings of Khan et. al. (Khan et al., 2019d) where they incubated the soil for 0, 14, and 28 days and did not find the bacterial community recovery after 28 days of MW soil heating. Hence, two more weeks was considered for incubation in this present study. During the incubation period, proper soil aeration and moisture content (45 % field capacity) were maintained as described previously (Khan et al., 2019d).

9.3.5 Soil microbial DNA extraction

Soil microbial DNA was extracted from samples taken 0, 14, 28, 42, and 56 days after MW soil treatment. At day 0 incubation, DNA was extracted after the soil had cooled to room temperature (25 °C). DNA was extracted from 0.25 g of soil with the DNeasy® PowerSoil® Kit (QIAGEN) according to the manufacturer's instructions (QIAGEN), with an extra bead-beating step. DNA concentrations were quantified using a Nanodrop™ ND2000c spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) to ensure the quality of the DNA extraction. DNA samples were stored at – 80 °C for further qPCR and sequencing analysis. Respective soil samples were also stored at – 80 °C for further analysis of As speciation.

9.3.6 Determination of bacterial 16S rRNA gene copy numbers by qPCR

Quantitative PCR (qPCR) was used to determine the bacterial 16S rRNA gene copy number in the soil sample using the primers 1369F (5'-CGGTGAATACGTTTCYCGG) and 1492R (5'-GGWTACCTTGTTACGACTTT) as described previously (Zhai et al., 2017). A CFX384™ optical qPCR detection system (Bio- Rad, Laboratories Inc., Hercules, CA, USA) was used for all qPCR reactions and each sample was analysed in triplicate. For each reaction, the PCR mixture consisted of, 2 µl of 10-fold diluted DNA template, 1 µl of each primer, 10 µl iTaq Universal SYBR GREEN Supermix (Bio-Rad, USA), and 6 µl of RNase-free water. Thermal cycling conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15s, annealing at 54 °C for 30s, and final extension at 72 °C for 30s (Khan et al., 2019d). For each qPCR reactions standard curves were prepared from bacterial standard template DNA. The standard curves were linear over six orders of magnitude (10^4 – 10^9 gene copies μl^{-1}), with efficiency of 80 – 100 % and R^2 value of 0.97 – 0.99.

The bacterial standard template DNA was prepared by PCR amplification of 16S rRNA using the primer pair 1369F and 1492R. A reaction mixture of 25 µl contained 12.50 µl PCR master mix (Bio-Rad, USA), 1 µl of each 10 µM 1369F and 1492R primer pair (Sigma-Aldrich, USA), 2 µl of 10-fold diluted DNA template, and 8.5 µl of RNase-free water (QIAGEN, Germany) was prepare for PCR amplification using C1000 thermal cycler (Bio Rad) with the following condition: initial denaturation at 95 °C for 5 min, and 40 cycles of 95 °C for 15s, 60 °C for 20s, and 72 °C for 30s, followed by a final extension at 72 °C for 5 min (Zhai et al., 2017). The PCR amplicons were ligated into pGEM®-T easy vector system (Promega) by following the protocol mentioned in manufacturer's instruction manual. A 10 µl ligation reaction mixture was prepared as follows: 5 µl 2X rapid ligation buffer, 1 µl pGEM®-T easy vector (50 ng), 1 µl T4 DNA ligase, 1 µl PCR product, and 2 µl of RNase free water. The resultant plasmids were transformed into *E. coli* JM109 high efficiency competent cells (Promega) and cultured onto LB/ampicillin/IPTG/X-Gal plates. Plasmid DNA was extracted from selected white colonies using a plasmid DNA extraction kit (Promega) and the concentration ($143.6 \text{ ng } \mu\text{l}^{-1}$) was measure with Nanodrop™ ND2000c spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). These linearized plasmids were then sequenced and used as a calibration standard for the qPCR via serial dilutions of a known copy number (4.23×10^{10} copies μl^{-1}). The data of 16S rRNA gene copy numbers were converted to copies per gram of soil prior to statistical analysis and presented on a $\log_{10}$ axis.

9.3.7 Sequencing of 16S rRNA gene

The V3 – V4 hypervariable region of the 16S rRNA gene was amplified using universal primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) as described previously (Apprill et al., 2015). Image analysis was performed in real time using the MiSeq Control Software (MCS) v2.6.2.1 and Real Time Analysis (RTA) v1.18.54, running on the instrument's computer. RTA performs real-time base calling on the MiSeq instrument computer. Then the Illumina bcl2fastq 2.20.0.422 pipeline was used to generate the sequence data. Paired-end amplicon sequencing was performed in an Illumina MiSeq sequencer (San Diego, CA, USA). Paired-ends (300bp paired end run) reads were assembled by aligning the forward and reverse reads using PEAR (version 0.9.5) (Zhang et al., 2014). Primers were identified and trimmed. Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8.4) (Caporaso et al., 2010), USEARCH (version 8.0.1623), and UPARSE software (Edgar et al., 2011). Using USEARCH tools (Edgar, 2010; Edgar et al., 2011), sequences were quality filtered, full length duplicate sequences were removed and sorted by abundance. Singletons or unique reads in the data set were discarded. Sequences were clustered and chimeric sequences were filtered using the “rdp_gold” database as a reference. To obtain the number of reads in each OTU, reads were mapped back to OTUs with a minimum identity of 97 %. Taxonomy was assigned with the Greengenes database (DeSantis et al., 2006) using QIIME (Caporaso et al., 2010).

9.3.8 Soil arsenic speciation

A MW assisted extraction followed by high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) quantification technique was used for soil As speciation analysis by following the method as described earlier (Rahman et al., 2009). The detail procedure of soil As speciation analysis described in previous chapter (Chapter 8).

9.3.9 Statistical analysis

All statistical analyses were performed in the R statistical environment (R version 3.6.2) (Team, 2013) using the *vegan* (Oksanen et al., 2007) and *phyloseq* (McMurdie and Holmes, 2013) packages. Prior to analyses, sequencing data was rarefied to 13,791 reads per sample and one sample was lost in this process due to low read depth. The final dataset contained 7,323 operational taxonomic units (OTUs). α -diversity was calculated using the Shannon's diversity

index and Pielou's evenness. Analysis of variance (ANOVA) was used to test for differences between treatments in α -diversity and total 16S rRNA gene copy numbers, and post-hoc Tukey tests were performed to evaluate the pairwise differences among treatments. A principal coordinate analysis (PCoA) and a permutational multivariate analysis of variances (PERMANOVA, function *adonis*, package *vegan* (Khan et al., 2019d)) of Bray-Curtis distances were used to investigate the relative differences in community composition between MW soil heating, soil As concentration, and incubation time. The contribution of each of the treatment variables (MW heating, soil As, and incubation time) were estimated using the *varpart* function in the *vegan* package (Bienhold et al., 2012). To follow bacterial community recovery, principal response curves (PRCs) were used (Jurburg et al., 2018). An ANOVA test was performed for each phylum and genus in response to treatments and top 10 OTUs based on relative abundance was visualized in bar graphs.

9.4 Results

9.4.1 Total abundance of soil bacteria

Bacterial abundance was evaluated by quantifying the total number of 16S rRNA bacterial gene copies in the control and treated soils. From Figure 9.1 it is evident that MW heating ($p < 0.001$) and incubation time ($p < 0.001$) had significant effects on the total number of bacterial gene copies, while the effect of soil As concentration was nonsignificant ($p = 0.687$). The average gene copy number in MW-0 was $\log_{10} 9.24 \pm 0.06$ which did not change significantly throughout the incubation period. In contrast, gene copy number decreased significantly ($\log_{10} 7.99 \pm 0.04$) immediately after MW heating (MW-80) at 0 days of incubation and started to increase with increasing incubation time, reaching the highest number ($\log_{10} 9.25 \pm 0.05$) 42 days after MW heating.

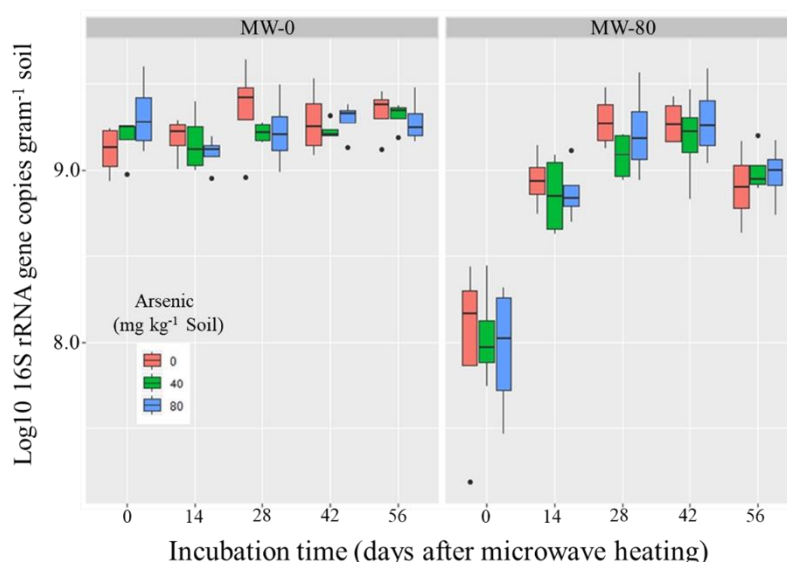


Figure 9.1. Influence of microwave (MW) soil heating (MW-0 as control and MW-80 as treated) on the total abundance of the 16S rRNA bacterial gene copy number over incubation time (days after MW heating) in arsenic (As) contaminated soil

9.4.2 Bacterial community richness (α -diversity)

Bacterial community diversity (α -diversity) was calculated as richness from the 16S rRNA gene sequence data (Figure 9.2). Shannon's diversity index evenness was also calculated to explain the community diversity. Microwave soil heating significantly ($p < 0.001$) reduced the bacterial diversity compared with the control (MW-0) soils. It is evident from the Shannon's diversity index that the control (MW-0) soils were richer than the MW treated (MW-80) soils ($p < 0.001$) where the mean values were 6.03 ± 0.26 and 3.47 ± 1.16 , respectively (Figure 9.3a). The control soils (MW-0) were also more even in diversity than the MW treated soils ($p < 0.001$), with a mean of Shannon's evenness of 0.82 ± 0.01 compared with 0.54 ± 0.17 for the treated soils (Figure 9.3b). The ANOVA test also showed a significant effect of incubation on richness ($p < 0.001$) and evenness ($p < 0.001$). Also, the microwave $\times$ incubation had a significant influence over richness ($p < 0.001$) and evenness ($p < 0.001$), while the effect of soil As concentration was nonsignificant on both richness ($p = 0.642$) and evenness ($p = 0.754$), suggesting that the soil bacterial community changes in response to the MW soil heating and incubation period irrespective of soil As concentration. However, in MW treated soil, bacterial community richness does not reach that of the control soil (MW-0) even after the 56 days of incubation period, although the recovery started at 42 days of incubation in As-0 and As-40 treatment and at 28 days in the As-80 treatment. Therefore, it is suggested that more time is needed for recovery of the bacterial community richness after MW soil heating.

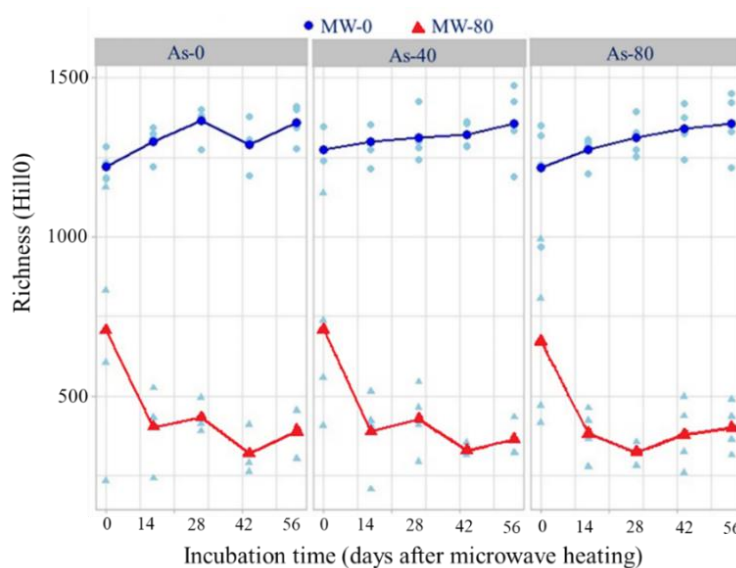


Figure 9.2. Changes of bacterial diversity over time, measured as the effective number of observed OTUs (operational taxonomic units), grouped by soil arsenic (As) treatment. Measurements are in sky-blue, and the mean for each incubation timepoint and microwave (MW) treatment combination is displayed in blue (MW-0) and red (MW-80) point connected with line. Richness was calculated from 16S rRNA gene sequence data

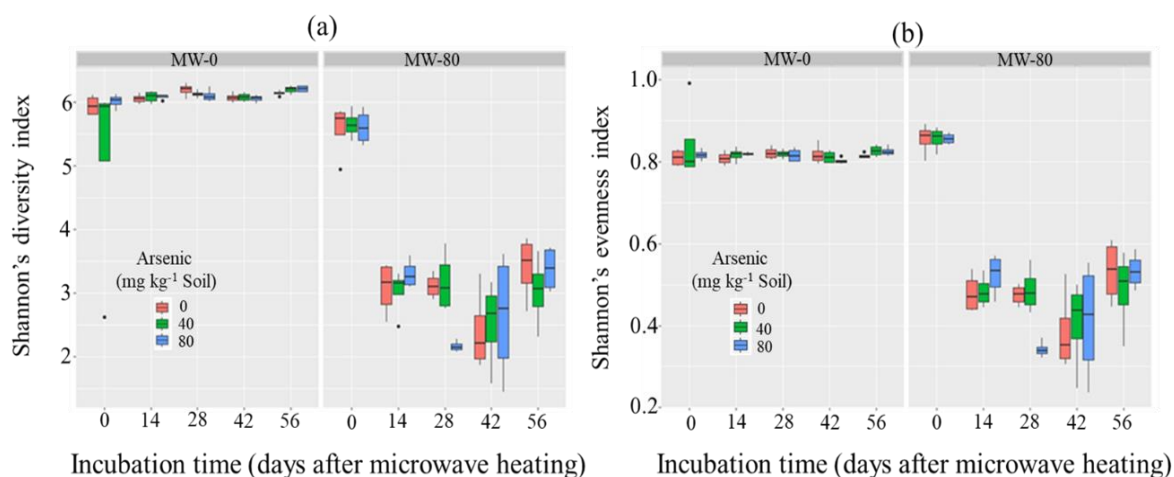


Figure 9.3. Effect of microwave (MW) soil heating (MW-0 as control and MW-80 as treated) on soil bacterial diversity measured as (a) Shannon's diversity index and (b) Shannon's evenness over time (days after MW soil heating treatment) in arsenic contaminated soils (As-0, As-40, and As-80)

9.4.3 Bacterial community composition (β -diversity)

The PCoA of Bray-Curtis distances of the 16S rRNA gene sequences were considered to estimate the overall variation in the bacterial community structure (Figure 9.4). Significant differences were observed between the community composition in response to MW soil heating and incubation time. The PCoA plot explained 60.10 % of the observed variation,

where the first axis explained 49.80 % of the variations and the second axis explained 10.30 % of the variation. Soil without MW heating (Control) tightly clustered together throughout the incubation time, suggesting no changes in community. In contrast, the community dispersed and clustered separately from the control in MW treated soil. Immediately after MW heating (0 days of incubation), the community clustered closely to the control samples but started separating from the control with the passing of incubation time. A PERMANOVA of the Bray-Curtis distances also showed significant differences between the community structures in response to MW heating ($p < 0.001$), incubation time ($p < 0.001$), and the interaction of MW heating x incubation time ($p < 0.001$). There was no significant effect (PERMANOVA, $p = 0.271$) of soil As concentration on the bacterial community composition (Figure 9.4).

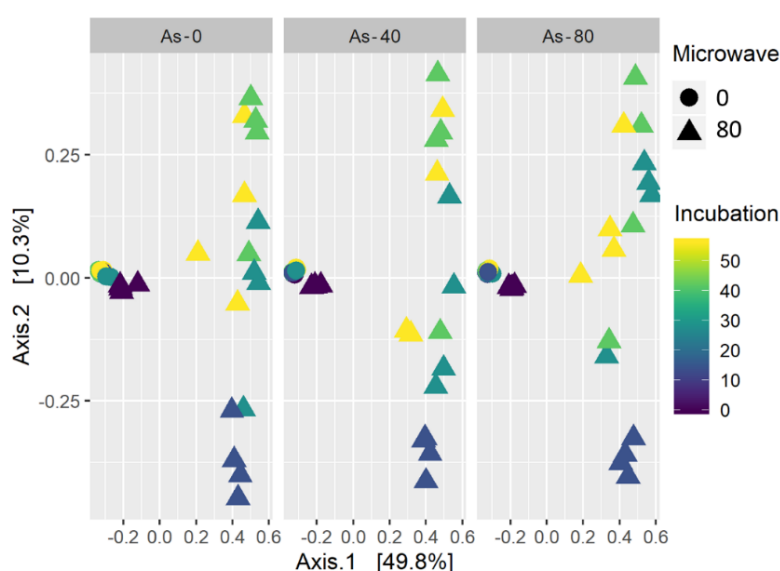


Figure 9.4. Bacterial community composition in response to microwave (MW) soil heating (MW-0 as control and MW-80 as treated) over the incubation time (days after MW soil heating treatment). The Principal Coordinate Analyses (PCoA) of Bray-Curtis dissimilarity index grouped by soil arsenic (As) concentrations (As-0, As-40, and As-80)

9.4.4 Bacterial community variance

Variation in bacterial community structure was assessed by calculating the variance partitioning (VP) with MW, As, and incubation time as explanatory matrices (Figure 9.5). The full VP model significantly (PERMANOVA; $p < 0.001$) explained about 44 % of the community variation, where MW soil heating explained most of the variance (38 %) and approximately 6 % of the variance was explained by the recovery dynamics during the incubation time (PERMANOVA; $p < 0.001$). The residual variation was about 57 % which was

not explained by the explanatory matrices. In MW treated soils, the community variance increased more quickly with increasing soil As concentration from 0 (after 42 days) to 40 (after 28 days) and 80 (after 14 days) (Figure 9.6).

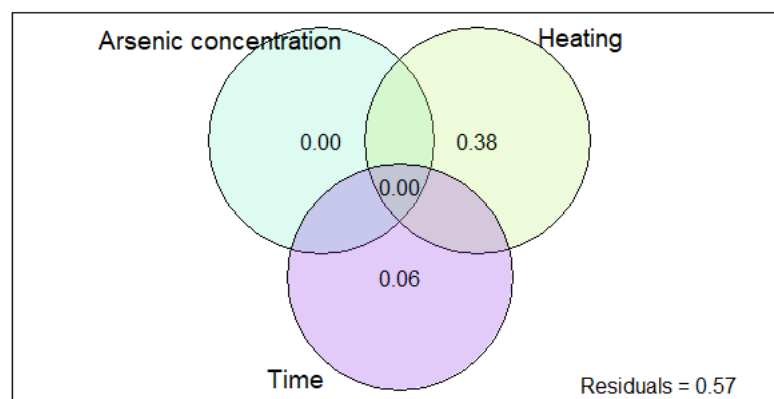


Figure 9.5. Fictional Venn diagram displaying the results of a variation partitioning analysis. Three explanatory matrices were used here, containing variables pertaining to microwave heating, incubation time, and soil arsenic concentration. The bounding rectangle represents the total variation in the response data while each circle represents the portion of variation accounted for by the explanatory matrices

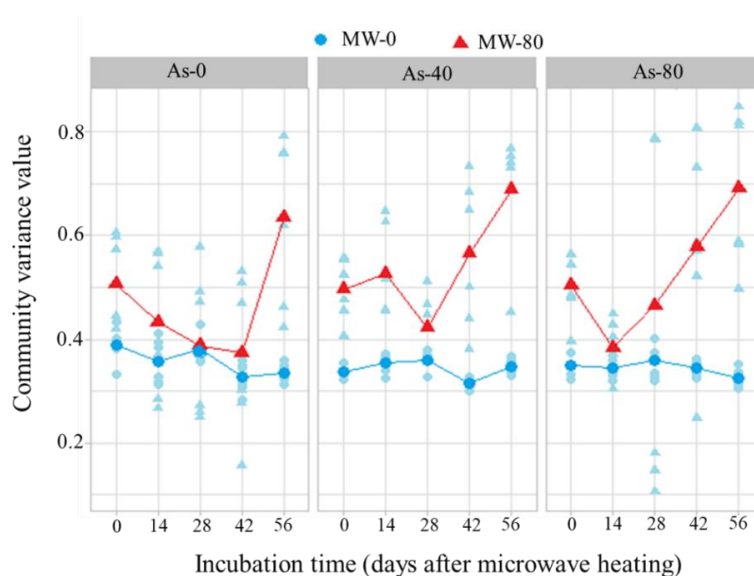


Figure 9.6. Differences in the community variances in response to soil arsenic (As) concentration during incubation time in microwave treated (MW-80) and untreated (MW-0) soil. Values are grouped by the soil arsenic concentrations (As-0, As-40, and As-80)

9.4.5 Relative abundance of bacteria (Phylum and genus level responses)

To explore the bacterial community dynamics in response to MW soil heating, incubation, and soil As, the relative abundance was estimated at phylum and genus level.

Firmicutes was the most abundant (32.10 %) phylum in all samples followed by Proteobacteria (23 %), Bacteroidetes (12.80 %), Actinobacteria (8.40 %), Acidobacteria (6.10 %), Verrucomicrobia (5 %), Gemmatimonadetes (4.40 %), and Chloroflexi (4.30 %) (Figure 9.7). The abundance of Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, and Gemmatimonadetes decreased significantly ($p < 0.001$) while, the abundance of Firmicutes increased significantly ($p < 0.001$) in MW treated (MW-80) soil compared with the control (MW-0) (Figure 9.7). Proteobacteria, and Verrucomicrobia also decreased significantly ($p < 0.001$) immediately after MW heating where Proteobacteria started recovering after 42 days of incubation time and recovered after 56 days, whereas Verrucomicrobia only started to recover after 56 days (Figure 9.7).

In terms of the most abundant genera across all the samples, the relative abundance of *Symbiobacterium* was highest (13 %) followed by an uncategorized genus of Bacteroidetes (8.6 %), *Bacillus* (5.4 %), *Brevibacillus* (4.1 %), uncategorized genus of Proteobacteria (2.8 %), Acidobacteria (2.9 %), and Verrucomicrobia (2.7 %), *Cohnella* (2 %), *Paenibacillus* (2 %) and uncategorized genus of Chloroflexi (1.5 %) (Figure 9.8). An uncategorized genus was most abundant in control (MW-0) soils, while the abundance of *Symbiobacterium*, *Bacillus*, *Brevibacillus*, *Cohnella*, and *Paenibacillus* increased significantly ($p < 0.001$) in MW treated (MW-80) soils compared with the control. The relative abundance of these reshaped genera survives and started increasing after MW heating, which again decreased after 42 days of incubation (Figure 9.8).

Soil As concentration dependent bacterial relative abundance, after MW heat treatment, was evaluated (Figure 9.9) to see if there was any effect of soil As concentration on bacterial relative abundance irrespective of incubation time, since the relative abundance of bacteria did not change significantly ($p = 0.354$) in response to soil As concentration during the pre-incubation time prior to MW heat application (Appendix 9.4 and S 9.5). It was evidence from Figure 9.9 that, bacteria clustered in three groups (a – c) in response to soil As concentrations (As-0, As-40, and As-80 mg kg⁻¹ soil). Cluster *a* was mostly represented by phyla Proteobacteria (β -, δ -Proteobacteria), along with Gemmatimonadetes, Chloroflexi, and Fibrobacteres in the highest soil As level (As-80). Cluster *b* assigned to the Proteobacteria (δ -Proteobacteria, Family: OM27) and Bacteroidetes in the control As level (As-0). Finally, cluster *c* represented a member of the Proteobacteria (Rhodospirillaceae, and Polyangiaceae), Bacteroidetes (Saprospiraceae), Planctomycetes, and Verrucomicrobia in 40 mg kg⁻¹ soil As treatment (As-40).

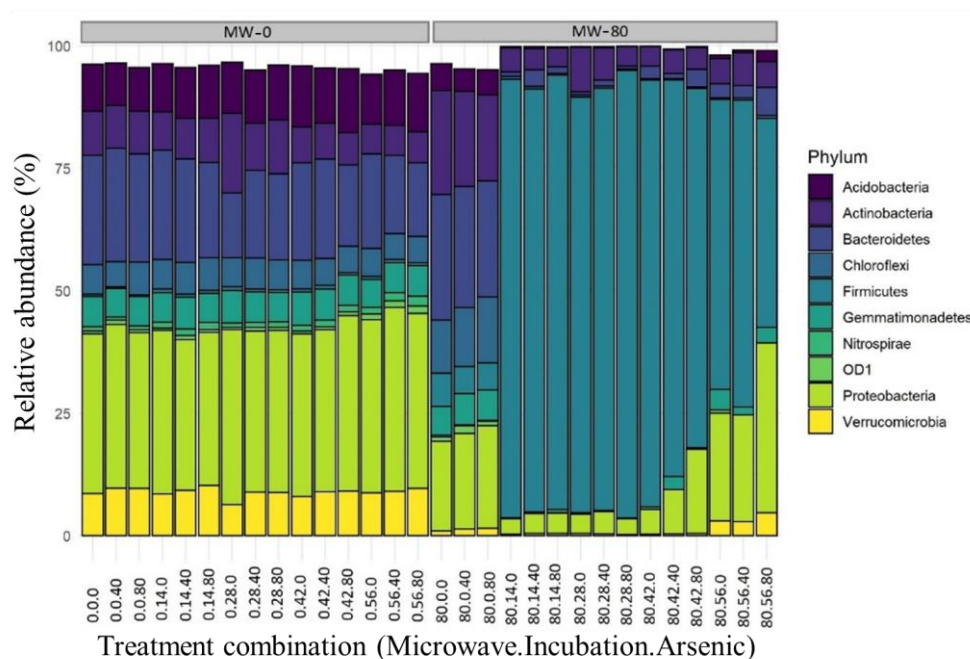


Figure 9.7. Relative abundance of the predominant bacterial taxa at phylum level that significantly changed over time in response to microwave soil heating. The bar graphs represent the replicated mean value at each time point

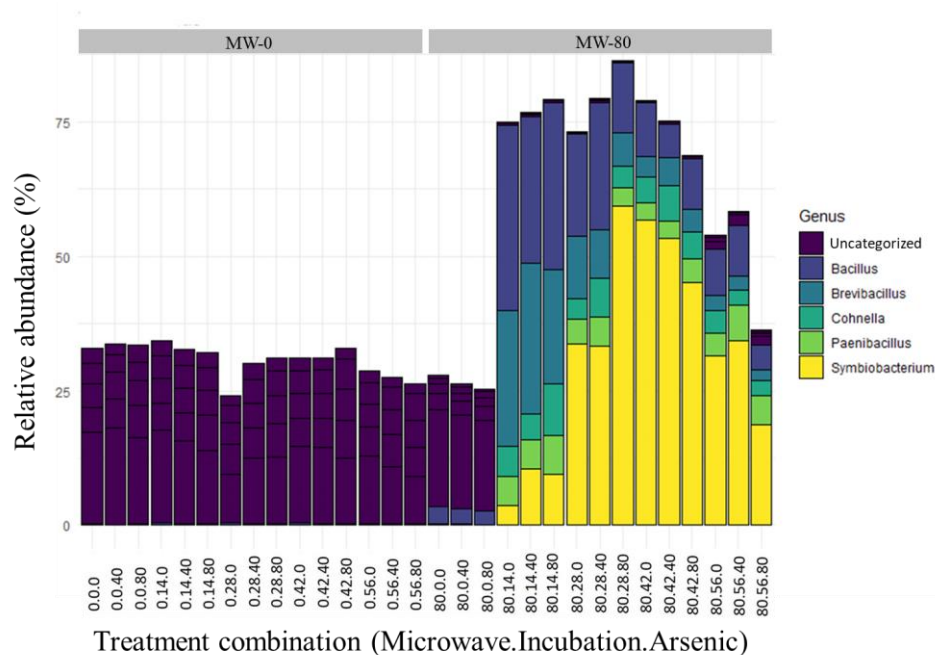


Figure 9.8. Relative abundance of the predominant bacterial taxa at genus level that significantly changed over time in response to microwave soil heating. The bar graphs represent the replicated mean value at each time point

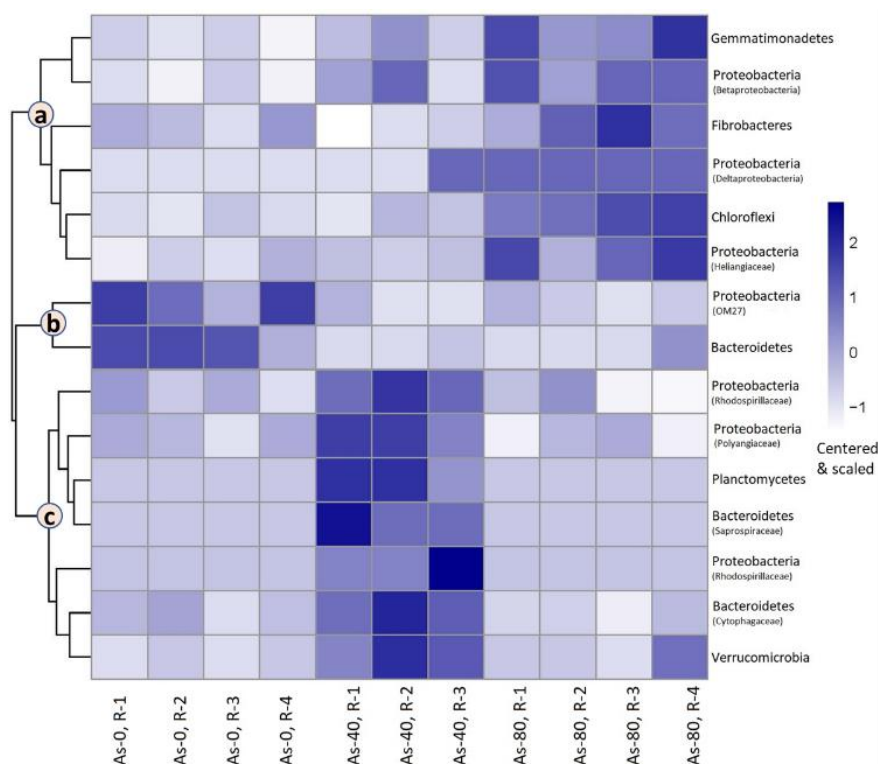


Figure 9.9. Relative abundance of significantly ($p < 0.001$) different OTUs across the soil arsenic (As) concentration after microwave treatment. Average relative abundances across the incubation time were calculated for each replication (R) and centered and scaled prior to plotting. The phylum of respective OTUs or shallower taxonomic level (in case of same phylum) are displayed in the right side. Taxa were clustered into three groups (a – c) in response to soil As concentrations after MW treatment

9.4.6 Soil arsenic speciation

Soil As speciation analysis was performed to observe any changes of the As species in response to MW soil heating treatment during the incubation time (days after MW heating). There was no significant effect of MW soil heating ($p = 0.053$) and incubation ($p = 0.396$) on the total As concentration. Throughout the incubation period, inorganic As species As(III) and As(V) were the dominant form of As irrespective of MW treatment (Figure 9.10). In contrast, among the organic forms of As, MMA was not detected in any of the samples, while trace amounts of DMA were detected in MW treated soil at highest soil As (80 mg kg^{-1} soil) treatment (Figure 9.10). Since As was applied in the soil as As(V) form (sodium arsenate heptahydrate; $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$), the portion of it converted to As(III) and DMA increased with incubation time. The concentration of As(III) in the MW treated soil was comparatively lower than the control (MW-0) soil.

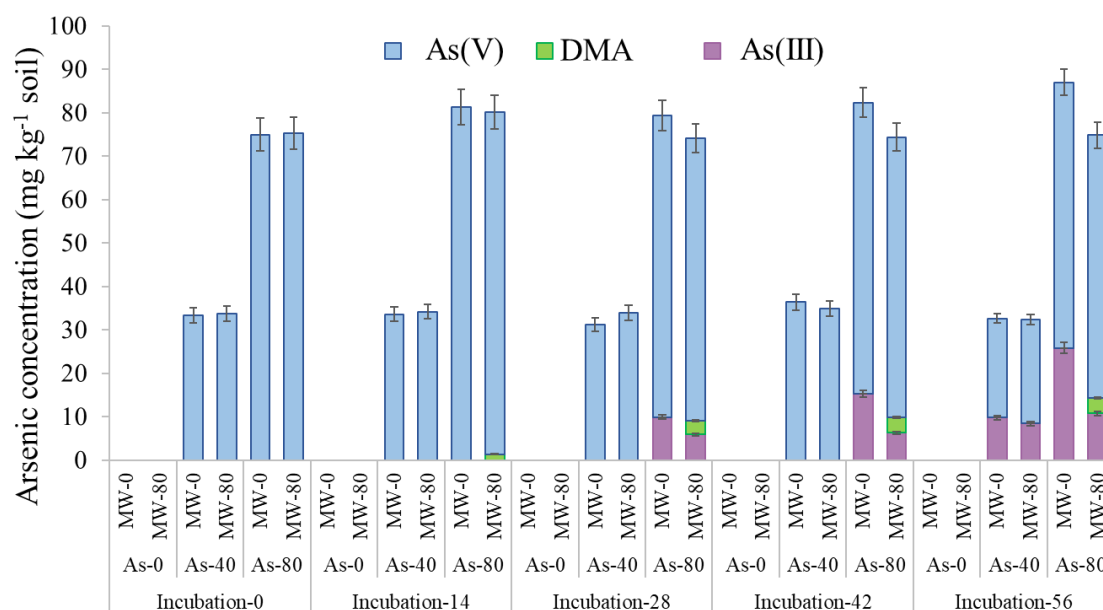


Figure 9.10. Concentration of soil arsenate [As(V)], arsenite [As(III)], and dimethyl arsinic acid (DMA) of arsenic (As) contaminated soil in response to microwave (MW) soil heating at different incubation time (days after MW heating)

9.5 Discussion

From Figure 9.1 it was evident that the total abundance of bacterial decreased significantly immediately after MW heating (MW-80) compared with the control (MW-0) treatments, which again started to increase and approached a similar abundance to control treatment after 42 days of incubation time. Microwave soil heating can reduce the total abundance of soil bacterial by directly destroying the bacterial cell (Shamis et al., 2012) as a consequence of dielectric heating of living cells in the soil (Falciglia et al., 2016). Maynaud et al., (Maynaud et al., 2019) reported a significant decrease in the total bacterial abundance with an increase of soil temperature to around 80 °C in MW treated soil (2 kw 8 min, and 4 kw 4 min) compared with the control soil. This result agrees with the findings of present study. Although soil microorganism can decline immediately after heating, they can recover rapidly a few days after heating (Moreno and Bååth, 2009). Likely, some bacteria, sometimes also known as thermo tolerant bacteria or spore forming high temperature resistance bacteria can survive even higher temperature (Moreno and Bååth, 2009). Hence, heat-tolerant, and fast growing bacterial will get more advantages in recolonizing after heat disturbance. Thus, recovery and rapid growth of bacterial communities after MW heating may happen, which ultimately could increase the bacterial abundance in MW treated soil. Several studies also

indicated that soil bacteria can recover rapidly even after higher levels heating (Guerrero et al., 2005; Mabuhay et al., 2006) which agrees with the findings of this current experiment.

Result from this present study revealed that, the MW soil heating treatment significantly reduced the bacterial richness (α -diversity) compared with the control soils which did not recovered after 56 days of incubation (Figure 9.2). A similar finding was reported in a recent study where a significant reduction (on an average 58 %) in bacterial community richness was observed in high intensity MW treatments for 60 s and 90 s (2-kW MW generator; 2.45 GHz) as compared with low intensity (30 s) and control treatments. This reduction in richness did not increased after 28 days from MW heating (Khan et al., 2019d). Another study also reported the soil bacterial richness declined after 90 s of MW irradiation treatment (800 W; 2.45 GHz) which subsequently started to recover by 29 days after soil heating (Jurburg et al., 2017b).

From the PCoA of Bray-Curtis distances of the 16S rRNA gene sequences, significant differences were observed between the community structure in response to MW soil heating (Figure 9.4). Similar result was reported in a recent study where, the MW soil heating (2kw; 60 s and 80 s) significantly changed the bacterial community composition and reshaped the community compared with control treatments (Khan et al., 2019d). Mortality of some bacterial species, caused by the MW soil heating, could be an important driver of heat-mediated shifts in the reassembled microbial community composition, suggesting the reorganization of the community after MW heating during incubation time (Jiang and Patel, 2008). Although the ability of soil bacteria to recover and reorganize depends on the soil condition, such as soil organic matter, available nutrient, and water content, the observed pattern of bacterial community response is likely depended on temperature distribution and retention in the soil, and differential recolonization patterns, following heating (Neary et al., 1999).

The relative abundance of bacteria changed significantly after MW heat treatment while it was unchanged in control (MW-0) treatment. This result can be explained in two different stages. In the first stage, immediately after MW heating (0 – 28 days of incubation), the relative abundance of phylum Firmicutes increased significantly. Firmicutes was the top phylum in terms of relative abundance (32.10 %). All the top five abundant genera depicted in this study (Figure 9.8) viz. *Symbiobacterium*, *Bacillus*, *Brevibacillus*, *Paenibacillus*, and *Cohnella* were under the phylum Firmicutes. Relative abundance of all these genera increased after MW treatment. Similar results were found by Nunes et al., (Nunes et al., 2018), where Firmicutes group (FRG4) was reported as being the most abundant (15.4 %) of the total taxa and showed

more resistance to the highest (5 min) MW heating dose and increased significantly compared with other bacterial groups. Another study also reported the Firmicutes as most dominant phylum after MW soil heating (Khan et al., 2019d). Numerous members of Firmicutes can produce heat-resistance endospores, and some of these may be stirred to germinate by higher temperature (Galperin, 2013). Specifically, some species under the phyla Firmicutes have been reported to withstand temperatures of 80 °C for over 10 min and can produce profuse spores within a short time (3 – 4 day) after a heat shocked (Pregerson, 1973). Under the phylum Firmicutes, the most common genus is gram-positive endospore forming *Bacillus*, which is known for heat and desiccation resistance (Vos et al., 2011), increased in relative abundance in MW treated soil (Khan et al., 2019d). The germination of *Bacillus* spores may have been induced by MW heating (Aslan et al., 2008). Some member of the thermophilic genus *Symbiobacterium* (e.g. *Symbiobacterium thermophilum*) can tolerate a wide temperature range (60 – 80 °C) (Ohno et al., 2000). Other members of the phylum Firmicutes, *Brevibacillus*, *Paenibacillus*, and *Cohnella* are also endospore forming bacteria and can withstand a wide range of temperatures (Kämpfer et al., 2006). Thus, all these endospores forming, and heat resistant bacteria, were most abundant in this study after MW soil heating at 80 °C.

The second stage (42 – 56 days of incubation) is where a gradual increase in the relative abundance of some Proteobacteria and a decrease of Firmicutes was observed (Figure 9.7). Similar results were also reported in some other studies where there were increases in the relative abundance of Firmicutes and decreases in Proteobacteria early in the incubation time and the opposite later in the incubation time (Jurburg et al., 2017a; Jurburg et al., 2017b). In this study, among all samples, Proteobacteria was the second most abundant phylum (23 %) after Firmicutes. Within the phylum Proteobacteria, both the heat sensitive and resistance bacteria was observed in this study. Similar findings were reported by Nunes et al., (Nunes et al., 2018), where some Alphaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria increased in relative abundance in the lower doses of MW treatment in an 800W MW oven for 15 s – 2 min although they decreased at the highest MW dose (5min).

The relative abundance of Acidobacteria was decreased significantly when soil was exposed to MW heating and started to increase at 56 days of incubation time, while no changes were observed in the control soil (Figure 9.7). Similar findings were also reported by Jurburg et al., (Jurburg et al., 2017b) where a negative impact was found on the relative abundance of Acidobacteria, which showed conventional recovery thereafter and was approaching the relative abundance observed in the control soil. In general, this phylum is predominantly

abundant in soil habitats and can constitute up to 52 % of the total bacterial community (Dunbar et al., 2002). Some Acidobacteria are known to grow slowly but withstand heat (Hayakawa et al., 2010), which indicates their presence in soil after MW heating without showing any specific pattern in relative abundance.

The relative abundance of Bacteroidetes decreased significantly when soil was treated with MW, but this decline was not detected immediately after MW heating (0 days) and remain same later in the incubation period (Figure 9.7). Similar result was observed in previously, where a negative impact on Bacteroidetes was also revealed in MW heating, although increasing trend was observed with incubation time (Jurburg et al., 2017b).

Actinobacteria (8.40 %) was the fourth most abundant phylum across all the samples and Micromonosporaceae is an important family under this phylum. The relative abundance of Micromonosporaceae increased in the MW treated soil compared with the control. A similar result was also revealed in a recent study (Khan et al., 2019d), where an increase of Micromonosporaceae was observed in the high heating intensities of MW soil treatment (2kw; 60s and 90s) in the top soil level (0 – 5 cm). It is reported that, Micromonosporaceae is a gram-positive spore-forming bacterium and induced by soil heating of around 70 °C (Hoskisson et al., 2000) and dormant spores are resistant to high heat around 75 – 90 °C (Suarez et al., 1980).

Among all the most abundant bacteria described in this study those which survived and increased in relative abundance after MW soil heating, have some advantageous aspects. For example, some species of Micromonosporaceae (e.g *Micromonospora*) can facilitate crop protection by producing antifungal and antimicrobial compounds lethal to some pathogen (Hirsch and Valdés, 2010), help in root nodulation and nitrogen-fixing symbiotic processes, phytohormone formation (Solans et al., 2006), and carbon decomposition (Yeager et al., 2017). Some members of *Paenibacillus* (e.g *Paenibacillus polymyxa*) are capable of fixing nitrogen, and some *Paenibacillus spp* can produce antimicrobial substances that can affect a wide range of fungi and plant pathogenic bacteria (e.g *Clostridium botulinum*; responsible to produce neurotoxin botulinum) (Girardin et al., 2002). Some species of Rhodospirillaceae (e.g *Rhodopseudomonas capsulata*) are directly involve in nitrogen fixation (Madigan et al., 1984). A recent study reported that, there was no negative impact of MW soil heating on ammonia oxidising bacteria (Khan et al., 2019d) because of their resistant to a certain MW energy (Ferriss, 1984). Additionally, some key nutrient processes may be favored by a functionally altered less diverse community in MW treated soil (Boynton and Greig, 2016) since the

functionality of soil mostly depends on the soil bacterial community composition (Wagg et al., 2014). Thus, the positive response of these bacteria, after MW soil heating, could be responsible for better crop growth.

Furthermore, there was no significant effect of soil As concentration on the bacterial total abundance, community richness, and community composition. Das et al. reported no significant change in bacterial community in lower As contaminated soils (7 – 48 mg kg⁻¹ soil) while, significant change was observed at highly As contaminated soils (108 – 417 mg kg⁻¹ soil) (Das et al., 2014). In present study the highest soil As treatment was 80 mg kg⁻¹ soil, which might not be enough to show a significant effect on bacterial community composition, as shown by Das et al. However, the relative abundance of some bacteria shows different cluster in response to As treatment (Figure 9.9). In support of this result the variance partitioning analysis showed that the community variance increased with increasing soil As concentration (Figure 9.6). It seems that the bacterial community composition changed and shifted according to MW soil heating but not to soil As, since the degree of stress to bacteria could be more from MW than As.

The results reported here also showed that, there was no significant effect of MW soil heating on soil As speciation (Figure 9.10). A trace amount of DMA was detected only in MW treated soils with the highest soil As (80 mg kg⁻¹ soil) treatment (Figure 9.10) although it was not significant. This indicated that there was a slight methylation occurring in the MW treated soils. In this study, the most resilient and abundant bacteria after MW soil heating treatment was Firmicutes followed by Proteobacteria and Actinobacteria, and interestingly all these bacteria possess the As methylating gene (*arsM*), as reported previously (Jia et al., 2013). In addition, under the most abundant phylum Firmicutes, *Bacillus* was the utmost abundant genera after MW soil heating in this study, which was previously reported as an As methylating bacterial genera (Huang et al., 2018). Thus, the detection of methylated As species, only in MW treated soil, could be the consequence of the abundant of the above-mentioned bacteria.

9.6 Conclusions

Bacterial community composition changed significantly, and clear differences were observed between the community composition in response to MW soil heating. Bacterial richness decreased after MW soil heating, which did not recover to its original preincubation richness. Heat tolerant bacterial groups, such as Firmicutes, survived in MW heated soil and heat susceptible groups of Proteobacteria decreased after MW heating compared with the control (MW-0) soil. The presence of methylated As species, only in MW treated soil, indicates the more survival of methylating bacteria after MW heating than before. Thus, MW soil treatment can potentially be applied for As phytotoxicity alleviation without significantly destroying the ecologically important taxa. Nevertheless, future research should be performed for a better understanding of how bacterial communities reorganize and recover over time. It is also important to determine how often the MW soil treatment should be applied and whether there are long-term consequences on the soil biota. Moreover, MW soil heating in field conditions needs to be study because the bacteria can recolonize from the surrounding environment and can change the community composition and richness over time.

Chapter 10 – General discussion and future directions

This thesis investigated the potential impacts of microwave (MW) soil heating and biochar application in soil on the alleviation of arsenic (As) phytotoxicity and accumulation of As in wheat and rice grain as an alternative to already existing As remediation techniques. Before the application of MW soil heating treatment, it is crucial to know the dielectric properties of soil, since dielectric heating of a material is the main mechanism of MW heating. Therefore, dielectric properties of different types of soil were investigated (Chapter 3).

10.1 Determination of dielectric properties of soil

It was evident from the result that, with increasing soil moisture, dielectric properties increased. Thus, soil moisture was the major contributor to the dielectric behavior of soil. This increase in dielectric value with moisture was also observed by Wang (Wang, 1980) and Dobson, Ulaby (Dobson et al., 1985). When MW radiation is applied, it causes rotation of the dipoles of water molecules because of the oscillating electromagnetic field and produces heat by intermolecular friction and collision (Falciglia et al., 2016). Hence, soil with higher moisture content showed higher dielectric properties.

The dielectric properties of sandy soil were much lower than the other soils; however, the highest dielectric properties were observed in loam soil. Because sand has a much lower surface area per unit volume than the other mineral components of soil, which result in the lower moisture holding capacity, and consequently the field capacity of soils is inversely related to their sand content. Therefore, lower dielectric properties of sandy soil attributed to the lower field capacity. Models were developed to explain the dielectric properties of soils as a function of frequency and moisture content. Higher goodness of fit (r^2) for these models suggested the models were adequate to describe the dielectric properties of these soils over the range of frequencies and moisture contents assessed in this study. This model can be used in future studies of these soils.

Since MW heating depends on the soil dielectric properties, determination of the dielectric properties of the experimental soil and probable influencing factors of dielectric properties was important before the application of MW irradiation for the design and set-up of the experiment. As soil moisture found to be the main influencing factor of soil dielectric properties, a constant soil moisture was maintained for all the MW treatment to ensure the same treatment matrix. Also, different soil texture also showed a significant difference in response

to dielectric properties. Since different types of soil were used for wheat and rice cultivation, it was crucial to know the dielectric properties of different soils and how they differ at the same frequency and soil moisture. Furthermore, for the calibration of the MW treatment time, soil moisture and soil types were important factors to be considered.

10.2 Effect of soil arsenic concentration

It was evident from the results that, wheat and rice plant growth and grain yield reduced and grain As concentration increased significantly with increasing soil As concentration from 20 to 80 mg kg⁻¹ compared with the control treatment (Chapter 4, 5, and 6). This negative effect was observed due to the As induced phytotoxicity. This result was in agreement with previous studies where it was reported that the reduction of plant growth and grain yield was ultimately the result of As phytotoxicity when crops are grown in As contaminated soil (Rahman et al., 2004; Asaduzzman et al., 2010; Vromman et al., 2013). In the soil environment As is mainly present in inorganic [arsenate; As(V), and arsenite; As(III)] and organic [e.g. monomethyl arsonic acid (MMA) and dimethyl arsinic acid (DMA)] forms (Han et al., 2017). Inorganic As species are more toxic than the organic species and among the inorganic species As(III) is more toxic (about 60 times) than As(V), due to higher mobility and solubility in soil and plant (Cai et al., 2016). Plant uptake different essential nutrient elements from the soil through different transporters, present in plants' roots. Due to the imperfect selectivity of these transporters, plants also uptake nonessential and toxic elements like As. Since As(V) is phosphate (PO₄³⁻) analogous, it is taken up by the high-affinity phosphate transporters (PHTs) (e.g. OsPHT1;1 in rice) while, aquaglyceroporins, which are the more likely nodulin 26-like intrinsic protein (NIP) class of aquaporin channels (e.g. Lsi1; silicon transporter in rice), are reported for As(III) uptake (Zhao et al., 2010a; Mosa et al., 2012). Due to the lower affinity of transporters for organic As, the uptake rate of MMA and DMA is much slower than the inorganic forms (Raab et al., 2007b).

After plant uptake of As from the soil, it can exert its adverse effect to the plants by inactivating some of the key enzymes either by replacing the obligatory ions from their active site or through interaction with sulfhydryl groups (Finnegan and Chen, 2012). The phytotoxicity of As(V) mainly happens due to an antagonistic relationship with a PO₄³⁻, which is a consequence of a reaction with P or a P-ester as a substrate thereby disrupting vital biochemical processes including phospholipid metabolism, RNA/DNA synthesis, protein synthesis, phosphorylation, and glycolysis (Gresser, 1981; Finnegan and Chen, 2012). Arsenite

has a high affinity to react with sulfhydryl groups of enzymes and proteins, which leads to the deterioration of cell membranes, inhibition of cellular functions and subsequently cell death (Meharg and Hartley, 2002). Lower photosynthesis rate by decreasing the chlorophyll content was also reported due to As phytotoxicity (Rahman et al., 2007a). It was evident from the current study that, photosynthesis of wheat and rice plants reduced significantly with increasing soil As concentration, which could be attributed to the lower grain yield as well. Thus, the above-mentioned adverse effect of As on plants can cause the phytotoxicity and subsequent reduction in plant growth and grain yield. To alleviate this As induced phytotoxicity, MW soil heating treatment was applied.

10.3 Effect of microwave soil heating

It was evident from the current study that, pre-sowing MW soil heating, especially MW-6, had the potential to alleviate the As phytotoxicity as significantly increased plant growth and grain yield and significantly reduced grain As concentration of wheat (Chapter 4) and rice (Chapter 5) crops was observed irrespective of soil As concentrations. Earlier research also reported the increased plant growth and grain yield of wheat (Khan et al., 2016) and rice (Khan et al., 2018) in MW treated soils as an additional benefit besides the non-herbicidal weed control. Also, the results from the residual experiment (Chapter 6) revealed that MW soil heating had the potential to alleviate As phytotoxicity in both wheat and rice after one year of MW treatment. The positive residual effect of MW soil heating was also reported by Khan et al. (Khan et al., 2019b), where they reported that MW soil heating at 75 – 85 °C not only significantly increased wheat shoots biomass and grain yield in the first main treatment season but also in the first (440 days after MW treatment) and second residual season (740 days after MW treatment) of crop cultivation. However, no published study so far reported to alleviate As phytotoxicity by using MW soil heating.

The possible reasons for increased crop growth and grain yield in MW treated soils can be explained by a couple of changes in soil after MW heating. One of the possible reasons could be the higher availability of nutrients for the plants due to enhanced decomposition of SOM in MW treated soil. The increased concentration of soil N, P, and S was observed after MW soil heating in both wheat (Table 4.1) and rice (Table 5.1) soils. Similarly, the previous research findings demonstrate that MW treatment increased the availability of some soil nutrient e.g. N, S, and P (Gibson et al., 1988; Brodie et al., 2017; Khan and Brodie, 2018). The increased N content could be attributed to the mineralization of soil indigenous organic N (org-

N) after MW soil heating (O'Brien et al., 2018). Amino acids, amino sugars, peptides, ammonia, pyrenes, and proteins are the main source of soil indigenous N (Schulten and Schnitzer, 1997). Plants are unable to utilize this org-N unless it is converted into mineral forms. The conversion of org-N to mineral forms (mineralization) depends on the decomposition of SOM. Thus, MW heating could enhance the N mineralization process through the enhanced decomposition of SOM. This org-N mineralization process could sustain for more than one cropping season and be attributed to the positive residual effect of MW. In support of this, Khan et al. reported that the increased indigenous N supply in the residual cropping season was the main reason for the better crop production (Khan et al., 2019b). Additionally, the dead soil microorganisms, due to MW heating, could be another cause of increased soil nutrient (Zhou et al., 2010).

Furthermore, result from the wheat and rice grain As speciation analysis (Chapter 8) revealed that MW soil treatment, especially MW-6, significantly reduced the concentration of the more toxic form of As [As(III)] both in wheat and rice grain samples and As(V) in wheat grain sample compared with the control treatment, irrespective of soil As concentration. Increased concentration of P and Si was observed after MW soil heating in both wheat (Table 4.1) and rice (Table 5.1) soils. Relevant to this, the previous research findings demonstrate that, soil heat treatment can increase the availability of Si (Khanna and Raison, 1986). It is identified that P (PO_4^{3-}) and Si have an antagonistic effect on As(V) and As(III) respectively having a similar pathway for plant uptake (Ma et al., 2008; Zhao et al., 2010a; Mosa et al., 2012). Hence, increased P and Si concentration could compete with As(V) and As(III) respectively for plant uptake and ultimately reduce their concentration in grain by lowering the plant uptake. Also, S plays an important role in As tolerance through complexation of As(III) by thiol ligands, glutathione, and phytochelatins (Dixit et al., 2015) and subsequent sequestration to vacuoles as an As detoxification mechanism (Song et al., 2010). The increased S concentration in MW treated soil could enhance the rate of As(III)-thiol complexation and sequestered more As(III) into the vacuoles of the roots and restricted As(III) mobility to rice shoots and grain. Hence, more availability of soil nutrients and less percentage of more toxic As form could explain the reason of minimizing As phytotoxicity and subsequent lower As the concentration of wheat (Chapter 4) and rice (Chapter 5) grain in MW treatment.

Moreover, it has been demonstrated that MW irradiation markedly altered the physical and chemical properties of soil organic matter (SOM), molecular composition (C, H, O and N), chemical structure, and enhanced humification of SOM (Hur et al., 2013; Kim and Kim, 2013).

It also increased the soil organic carbon (Zagal, 1989), macromolecular organic substances that possess a higher number of functional groups (Kim and Kim, 2013), and syntheses of organometallic and coordination compounds (Taylor et al., 2005). These organic substances could adsorb more As and reduce plant uptake which may facilitate the lower grain As concentration in MW treatments.

In addition to MW soil heating, saw dust biochar was used in rice experiment to alleviate the As phytotoxicity. It has been demonstrated that, rice can accumulate about 10 times more As than other cereal crops (e.g. Wheat and barley) due to genetic ability to accumulate more As and cultivated in flooded condition (Williams et al., 2007; Su et al., 2010). Therefore, biochar was also tested as other option to alleviate As toxicity in rice crop through experiment conducted on rice crop.

10.4 Effect of biochar application

The addition of saw dust biochar at 10 t ha⁻¹ soil (BC-10) had the potential to alleviate As phytotoxicity as increased plant growth and grain yield and reduced rice grain As concentration (at As-20 and As-40) was observed compared with the control treatment. Similar results were observed in the residual experiment (Chapter 6). The positive effect of biochar could be attributed to the adsorption of As on biochar surface with different functional groups and reduce their availability for plant uptake and direct nutrient supply (e.g. P, S, and Si) from biochar (Sohi et al., 2010; Zhang et al., 2012a). The beneficial effects of biochar could continue for a couple of cropping years (Nzediegwu et al., 2020). The recalcitrant nature of biochar and slow process of mineralization could explain the positive effect of biochar in the residual year. Nzediegwu et al. (Nzediegwu et al., 2020) identified identical functional groups in both original biochar treatment year and residual cropping year. Thus, organo-metallic complexation could also be observed in the residual year, thereby immobilizing As in the residual year. However, the beneficial effect of biochar was not observed when soil As concentration increase to As-60 and As-80. At As-60 and As-80 the soil As concentration could be high enough to outperform the positive effect of biochar treatment.

The application of saw dust biochar at 20 t ha⁻¹ soil (BC-20) had negative effect on rice plant growth and grain yield and increased grain As concentration was observed in both original treatment year (Chapter 5) and residual year (Chapter 6). The undesirable effect of biochar was also reported in some previous studies (Zheng et al., 2012; Beesley et al., 2013). The negative effect of biochar could be attributed to increase in reduction of As(V) to As(III)

due to transfer of π -electrons from DOC to As(V) (Choppala et al., 2016), increase of reductive resolution of FeOOH leading to the release of As(III) into the soil solution (Mladenov et al., 2015; Chen et al., 2016), and lower adsorption of As(III) due to anion exclusion resulted from more negatively charged functional groups on the biochar surface (Zhu et al., 2016). In support of this, it was evident from the rice grain As speciation analysis (Chapter 8) that, the As(III) concentration increased in the biochar treatment (BC-20) compared with the control (BC-0) treatment, which can be correlated with the negative effect of saw dust biochar application. However, positive impact of BC-10 at lower soil As concentration (As-20 and As-40) may attributed to decreased DMA concentration in the BC-10 treatment. Also, increased DMA concentration in BC-20 irrespective of soil As concentration may add more on negative effect of BC-20. It is important to note that, although, DMA is less toxic compare with inorganic forms, a higher concentration of DMA could exert substantial toxicity. Moreover, it is widely reported that the addition of biochar to soils has resulted in pH increases (Jones et al., 2012; Kim et al., 2018). Similarly, increase in soil pH after biochar application was observed (Chapter 5). During the pyrolysis process, at high temperature, cations are transformed into oxides, hydroxides or carbonates in the biochar and (Yuan et al., 2011) dissolution of these alkaline substances increase the soil pH (Houben et al., 2013). In the soil solution, As is mainly present as hydro anion and increased soil pH after biochar application can reduce As sorption capacity by decreasing the positively charged sites on soil minerals, which increases As mobilization and release from the soil (Wilson et al., 2010). These negative impacts of biochar could sustain for longer time which may explain the reduced plant growth and grain yield and increased grain As concentration in the residual year as well. Considering these negative impacts, application rate of saw dust biochar and method of biochar development needs to be compared and standardized for As phytotoxicity alleviation.

Based on the findings of Chapter 4, Chapter 5, and Chapter 6, MW soil heating could be proposed as a novel technique to alleviate As phytotoxicity and subsequent lower As accumulation in grain. However, it is reasonable of arising the question that, will it be the same response if soil heated from different sources since MW has a faster heating rate, a higher penetration, and an inside-out heating mechanism compared with conventional heating? Therefore, Chapter 7 investigated the potential of conventional electric oven (EO) soil heating to alleviate As phytotoxicity in comparison to MW soil heating at same soil temperature (80 – 90 °C) and As contaminated soils (0, 40 and 80 mg kg⁻¹ soil).

10.5 Comparison of microwave soil heating and conventional electric oven heating

The results showed that both the MW and EO soil heating alleviated As phytotoxicity as plant growth and grain yield increased significantly and facilitated less grain As accumulation, compared with the control treatment, across all the soil As concentrations and there was no significant difference between MW and EO heating. This result indicates that it was mainly the heating effect irrespective of the heating source. However, an increasing trend in plant growth and grain yield and a decreasing trend in grain As concentration was observed in the MW treatment compared with the EO treatment, which indicates that there might be some other effects of MW soil heating compare with conventional heating. This differential effect in MW compared with EO heating could be explained by the heating mechanism, changes in SOM, and effect on soil microorganisms since this would be the ultimate difference between MW and EO heating effect.

Dielectric heating mechanism of MW produces heat faster throughout the volume of the soil (Taylor et al., 2005) and not limited by exterior temperature, temperature gradients, or thermal conductivity which is the main mechanism of conventional heating (Li et al., 2016a). Thus, the changes in soil properties, especially SOM due to different heating mechanisms and the quick heating during MW treatment, might not be the same as in EO heating. In comparison with conventional heating, the differential effect of MW heating on soil microorganisms has been reported. For instance, different effects on structural and molecular components of *Bacillus subtilis* spores, different cell morphology of *Escherichia coli*, increased bacterial growth, increased enzyme activity of enterobacteria, and increase the synthesis of DNA and RNA of *Bacillus mucilaginous* were reported in MW treatment compared with conventional heat treatment (Spencer et al., 1985; Shamis et al., 2011; Kothari et al., 2012). Besides the thermal interaction, non-thermal interaction of MW has also been reported (Kothari et al., 2012; Cekmer and Davidson, 2017). For instance, Barnabas et al. reported the destruction of bacterial cells, without causing substrate heating, after MW irradiation (Barnabas et al., 2010). Thus, differential changes could happen in SOM and soil microorganisms after MW soil treatment compared with conventional soil heat treatment which could be attributed to the better plant growth and yield in MW treatment. Furthermore, it was evident from the result (Chapter 7) that, EO required more energy (4.59 times), time (2.5 times), and cost (4.59 times)

to apply heat treatment than the MW. Therefore, MW soil heating will be a better choice over conventional heating for As phytotoxicity alleviation.

Above and beyond the As phytotoxicity alleviation, MW soil heating could be detrimental to soil beneficial microorganisms particularly on bacteria. Therefore, Chapter 9 investigated the effect of MW soil heating on soil bacterial community response in As contaminated soil (0, 40, and 80 mg kg⁻¹ soil) over a period (0, 14, 28, 42, and 56 days after MW heating).

10.6 Bacterial community response to the microwave soil heating

Although, it was evident from the results that, MW soil heating at 80 – 90 °C had a negative impact on bacterial community richness (α -diversity) and composition (β -diversity), the total abundance of bacteria recovered after 42 days of MW treatment. This indicated that, 56 days after MW heating was not enough to recover community diversity, however, the community restructured after MW heating. In support of this, significantly increased relative abundance of Firmicutes was observed after MW heating which remain dominant throughout the incubation period. Numerous members of Firmicutes are able to produce heat-resistance endospores, and some of these may be stirred to germinate by higher temperature (Galperin, 2013). Specifically, some species under the phyla Firmicutes have been reported to withstand temperatures of 80 °C for over 10 min and can produce profuse spores within a short time (3 – 4 day) after a heat shocked (Pregerson, 1973). An increase of heat tolerant bacteria after MW irradiation could be attributed to the more availability of substrate. In this view, proteaceous compounds may release after the death of heat-sensitive microorganisms which can enhance the growth of the remaining heat-tolerant species (O'Brien et al., 2018). In addition, under the most abundant phylum Firmicutes, *Bacillus* was the utmost abundant genera after MW soil heating in this study, which was previously reported as an As methylating bacterial genera (Huang et al., 2018). *Bacillus* can replace the hydroxyl groups of inorganics As species by methyl groups at the time of As methylation process. Since As methylation is one of the ways of reducing As phytotoxicity, increased abundant of *Bacillus* after MW heating could be correlated to As phytotoxicity reduction by MW soil heating. Related to this, the methylated As species was found only in MW treated soil (Chapter 8).

Furthermore, the relative abundance of some beneficial bacteria such as *Paenibacillus* increased after MW soil heating. Previous research also reported the increase of some beneficial bacteria (e.g. *Paenibacillus*, *Micromonospora*, and *Rhodopseudomonas*) after MW

soil heating (Hirsch and Valdés, 2010; Khan et al., 2019d). These bacteria have diversified benefits. For example, some species of Micromonosporaceae (e.g. *Micromonospora*) can produce antifungal and antimicrobial compounds lethal to some pathogen (Hirsch and Valdés, 2010), help in root nodulation and nitrogen-fixing symbiotic processes (Solans et al., 2006), and organic matter decomposition (Yeager et al., 2017). Also, some members of *Paenibacillus* (e.g. *Paenibacillus polymyxa*) are capable of fixing nitrogen, and some *Paenibacillus* spp can produce antimicrobial substances that can affect a wide range of fungi and plant pathogenic bacteria (e.g. *Clostridium botulinum*; responsible to produce neurotoxin botulinum) (Girardin et al., 2002). Some species of Rhodospirillaceae (e.g. *Rhodopseudomonas capsulata*) are directly involve in nitrogen fixation (Madigan et al., 1984). A recent study reported that, there was no negative impact of MW soil heating on ammonia oxidizing bacteria, which is important to maintain the soil N cycling (Khan et al., 2019d), because of their resistant to MW soil heating at 80 – 85 °C (Ferriss, 1984). Additionally, some key nutrient processes may be favored by a functionally altered less diverse community in MW treated soil (Boynton and Greig, 2016) since the functionality of soil mostly depends on the soil bacterial community (Wagg et al., 2014). Thus, based on the positive impact of these bacteria it can be concluded that MW soil heating may brought a beneficial impact on soil health, which can explain the reason for the alleviation of As phytotoxicity for more than one cropping season.

10.7 Conclusions and future research directions

Elevated concentration of As in soil can significantly reduce the wheat and rice grain yield via inducing the As phytotoxicity. Which, in turn, can lead significant economic loss for the farmers of highly As contaminated area and ultimately can affect the national economy of a country. Also, accumulating a substantial amount of As in grain, when crop grown in As polluted soils, can be threat to global food security as a food chain contaminant and a potential risk to human health through soil-crop-food-human pathway as a carcinogenic element.

Pre-sowing MW soil heating at 80 – 90 °C, which is found as more energy, time, and cost efficient compared with conventional soil heating (e.g. electric oven heating), can be a novel technique for As phytotoxicity alleviation and subsequent lower grain As accumulation in wheat and rice grain of highly As polluted soil. Thus, MW technology can be applied in the As contaminated crop growing area to reduce the grain As concentration and subsequent less As accumulation in human body through dietary exposure. Primarily in the rice growing area, since rice can uptake more As than the other cereals due to cultivation in flooded condition and

rice grain As can play a leading role for contributing dietary As exposure to human as the staple food for more than half of the world population.

The beneficial effect of MW soil treatment can sustain more than one cropping year. Microwave soil heating can increase the availability of some key nutrients (e.g. N, P, Si, and S) which could be due to decomposition of SOM and from dead microorganisms. However, enhanced decomposition and humification of SOM can decline the soil nutrient stock in low OM soil which need to be monitored by regular checking of nutrient status after MW soil heating. Therefore, a reduced amount of nutrient application would be sensible for the subsequent cropping period if farmer go for residual year without further MW soil treatment. Nevertheless, the dose-response study would be crucial to adjust nutrient application rate without compromising with yield. Also, for the sustainability and determining the application frequency of MW, long-term residual effect need to be explored. Furthermore, the response of MW treatment may vary with soil type, soil pH, SOM and nutrient content, extent of As contamination, and intensity and frequency of MW irradiation.

Application of saw dust biochar at 10 t ha^{-1} soil can be effective to alleviate As phytotoxicity in rice for the soil with As contamination up to 40 mg kg^{-1} . Higher application rate (20 t ha^{-1}) of saw dust biochar can increase As concentration in rice grain. For effective use of saw dust biochar for As phytotoxicity alleviation, further research needed to correct the biochar application rate. Since, different types of biochar (e.g. rice husk, wheat and rice straw, sewage sludge, poultry litter, dairy manure etc.) has been reported for soil As immobilization as a means of lowering the grain As concentration, this biochar could also be used along with MW soil treatment especially for highly contaminated soils to get maximum As immobilization. However, appropriate application rate of biochar needs to be determined. Also, biomass type, pyrolysis temperature, and method of biochar preparation may affect the efficiency of biochar for As immobilization.

Besides the As phytotoxicity alleviation, in the question of MW heating impact on soil microorganisms, particularly on soil beneficial bacteria, MW soil heating can potentially be applied without significantly destroying the ecologically important taxa. However, research needs to be performed for a better understanding of MW soil heating effect on potentially active microbial communities, their recovery trajectories, and their functionality in field condition. In terms of species dependent As toxicity, the effect of MW soil heating on the As methylating soil microbes need to be studied, since methylation of the inorganic As species to organic form

is one of the ways of As detoxification and methylated As form was detected only in MW soil heating treatment in this present study (Chapter 8). Considering this, quantification of *arsM* gene, which is present in some soil microbes and responsible for microbial As methylation, after MW soil heating can be effective to give insight information on microbial As methylation. If methylating microbial community increase after a certain time of MW soil treatment, as it was evident from the current study (Chapter 9) that the relative abundant of some beneficial bacteria increased after MW soil heating, MW soil treatment will be a great benefit in terms of As detoxification.

In conclusion, MW could be applied in the field condition for As remediation. In view of this, MW technology could be more beneficial in the As contaminated area like India, China, Bangladesh, and Indonesia where most of the world's rice is grown and a noticeable As contaminated rice growing area with high inorganic As concentration in rice grain has been reported. In this context the major advantages of MW application will be on-site field application without disturbing the soil as compared with other remediation techniques where on-site field application is not feasible and requires further pre and post processing of soil. Also, in highly contaminated areas MW soil treatment could be a better choice to avoid the secondary pollution involved in the existing remediation techniques. However, there could be some challenges to apply the MW treatment in field condition. For example, further validation experiments needed for adopting the MW soil heating technology to apply in full scale for naturally As contaminated agricultural soils. Manufacturing of industrial scale MW applicators is required for treating a vast area of agricultural land. Also, for full-scale field application and industrialization of MW soil heating system, further characterization, scaling-up, design, modelling, and assessment of application cost of MW system is required. Furthermore, sustainability of the MW technology for As remediation in field condition needs to be considered.

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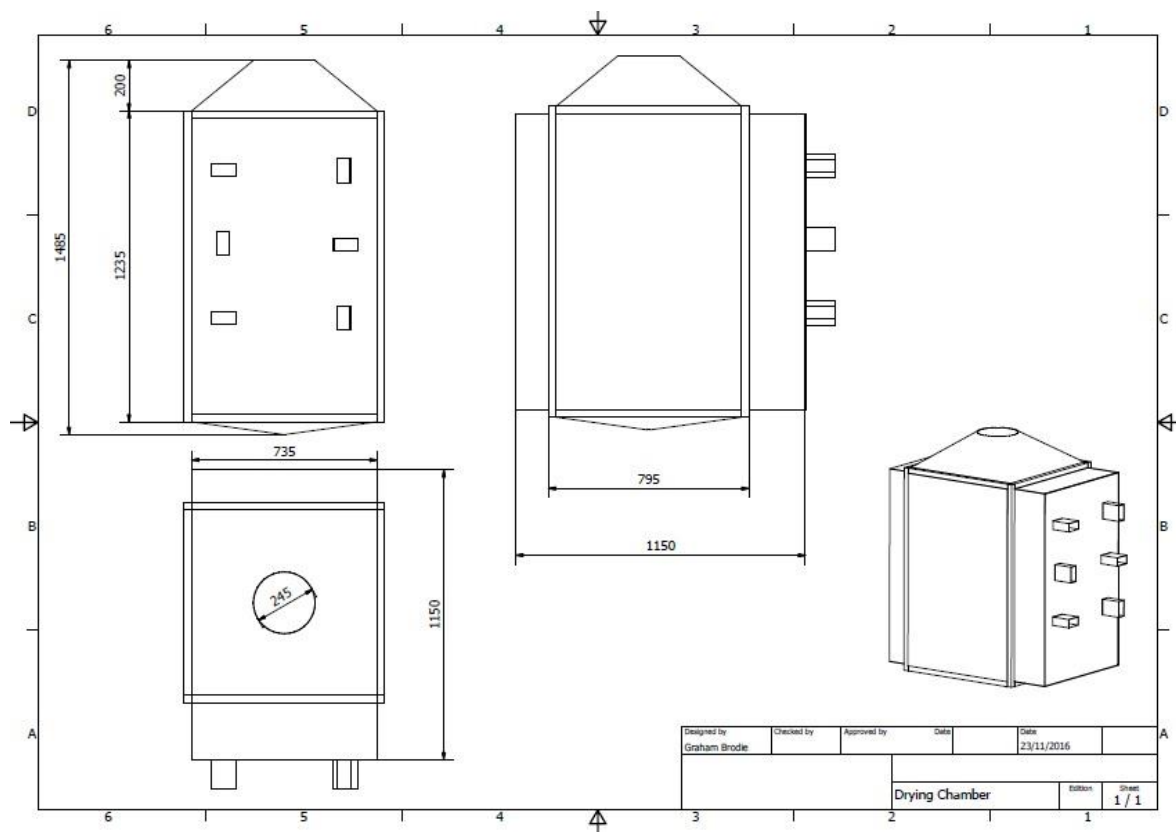
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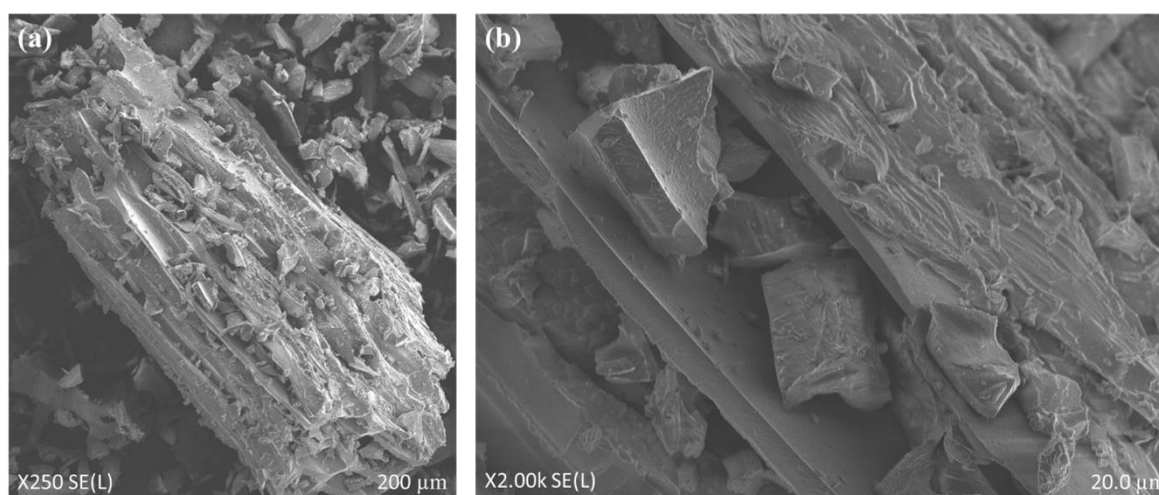
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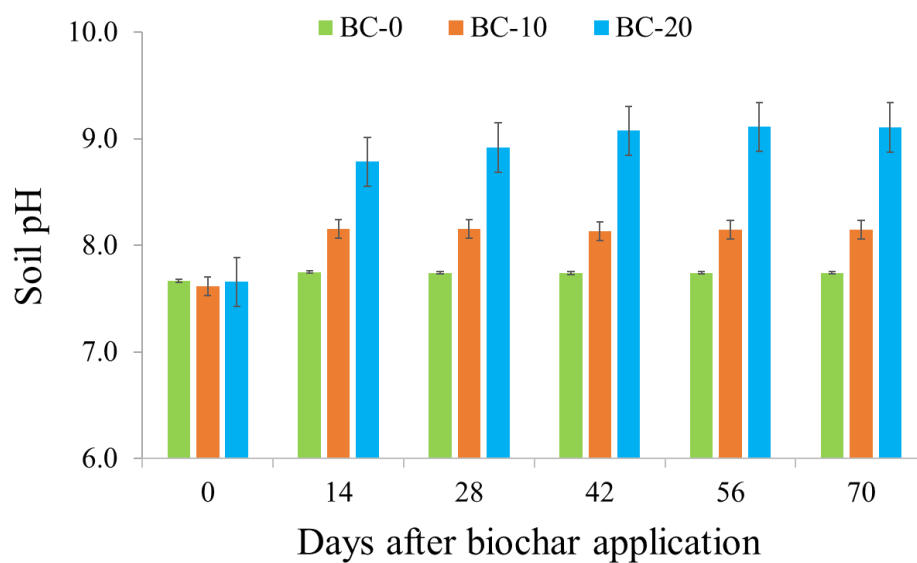
Appendices



Appendix 4.1. Design of a microwave chamber (Harris et al., 2011) used to treat the soil sample and for biochar preparation.



Appendix 5.1. Scanning electron microscopic (SEM) study showing the porous structure of sawdust biochar; (a) magnification at 200 μm and (b) magnification at 20 μm



Appendix 5.2. Influence of sawdust biochar application on soil pH during incubation time (days after biochar application). The mean values are shown in bars along with standard error. BC-0, BC-10, and BC-20 indicates the biochar application rate of 0, 10, and 20 t ha⁻¹ soil.

Appendix 5.3. Effect of soil arsenic on mean plant height in microwave and biochar treated soil

As (mg kg ⁻¹ soil)	MW (minutes)	Mean plant height (cm)								
		30 DAS			60 DAS			90 DAS		
		BC (t ha ⁻¹)								
		0	10	20	0	10	20	0	10	20
0	0	25.55	28.40	27.60	49.80	57.30	54.40	70.05	70.35	68.55
	3	29.85	30.94	30.54	57.15	58.80	60.70	80.25	79.20	70.15
	6	31.15	34.16	33.69	63.15	66.30	63.30	76.50	79.05	75.00
20	0	25.50	27.27	27.76	52.25	54.95	54.60	71.40	71.15	67.25
	3	30.25	31.67	33.38	58.25	59.60	61.40	72.15	79.10	71.80
	6	31.35	32.53	35.83	61.05	61.25	64.50	76.40	81.75	71.85
40	0	25.85	28.43	29.43	52.90	56.25	54.65	65.00	71.75	65.50
	3	31.25	32.96	31.87	56.55	60.60	56.50	73.70	69.15	70.40
	6	32.30	31.49	33.55	59.75	58.10	61.05	75.45	74.65	68.00
60	0	26.90	28.76	28.50	52.00	57.05	55.40	63.05	66.65	61.00
	3	31.15	30.86	31.30	57.85	56.20	55.80	68.10	67.35	63.75
	6	30.25	30.49	31.21	59.75	59.05	59.20	73.00	72.70	71.65
80	0	23.05	24.03	25.58	50.15	50.10	52.70	61.25	67.20	59.40
	3	27.70	27.92	29.13	54.55	54.70	55.60	65.80	65.20	61.85
	6	29.35	28.43	30.21	58.20	54.10	57.95	71.15	71.30	71.40
LSD _{0.05}		4.023			5.477			7.565		
p value										
As		< 0.001			< 0.001			< 0.001		
MW		< 0.001			< 0.001			< 0.001		
BC		0.002			0.051			< 0.001		
As.MW		0.470			0.196			0.447		
As.BC		0.793			0.404			0.940		
MW.BC		0.696			0.181			0.721		
As.MW.BC		1.000			0.942			0.540		

DAS (Days after sowing) indicates the sampling time. As.MW.BC indicates the interaction of arsenic (As), microwave (MW) and biochar (BC)

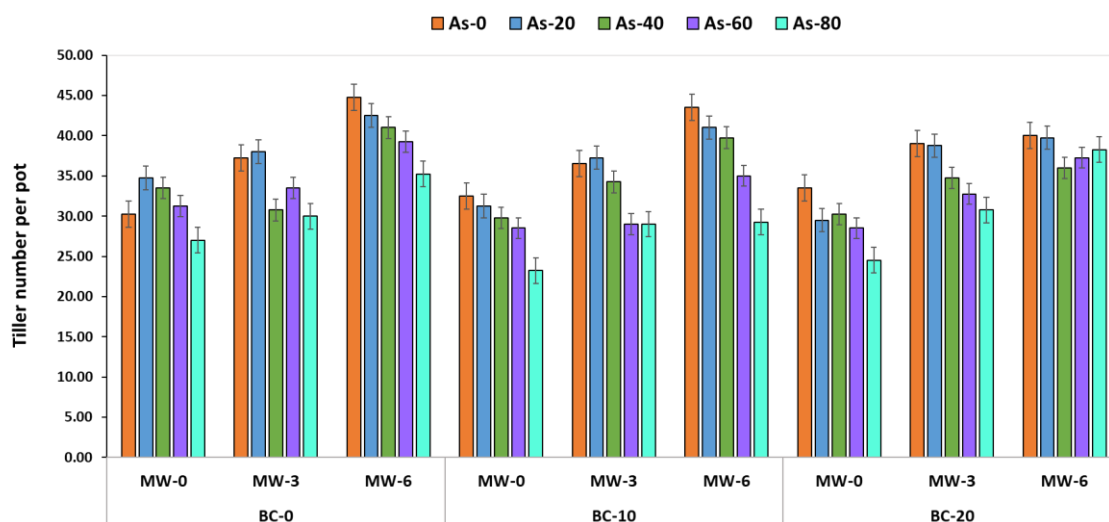
Appendix 5.4. Leaf chlorophyll content at different soil arsenic level in microwave and biochar treatments

As (mg kg ⁻¹ soil)	MW (minutes)	SPAD value		
		Biochar (t ha ⁻¹)		
		0	10	20
0	0	36.45	33.52	36.32
	3	38.35	36.83	38.75
	6	39.50	38.85	39.43
20	0	34.92	36.05	35.30
	3	36.20	36.52	37.35
	6	39.35	38.20	39.90
40	0	34.45	33.68	34.25
	3	36.83	35.73	35.30
	6	39.00	37.60	39.52
60	0	32.30	34.60	31.95
	3	35.35	36.17	36.97
	6	38.38	37.10	38.90
80	0	32.20	36.23	36.27
	3	35.87	37.02	36.05
	6	39.23	38.08	39.48
LSD _{0.05}			2.783	
			p value	
As			0.002	
MW			< 0.001	
BC			0.188	
As.MW			0.687	
As.BC			0.258	
MW.BC			0.284	
As.MW.BC			0.473	
DAS (Days after sowing) indicates the sampling time. As.MW.BC indicates the interaction of arsenic (As), microwave (MW) and biochar (BC)				

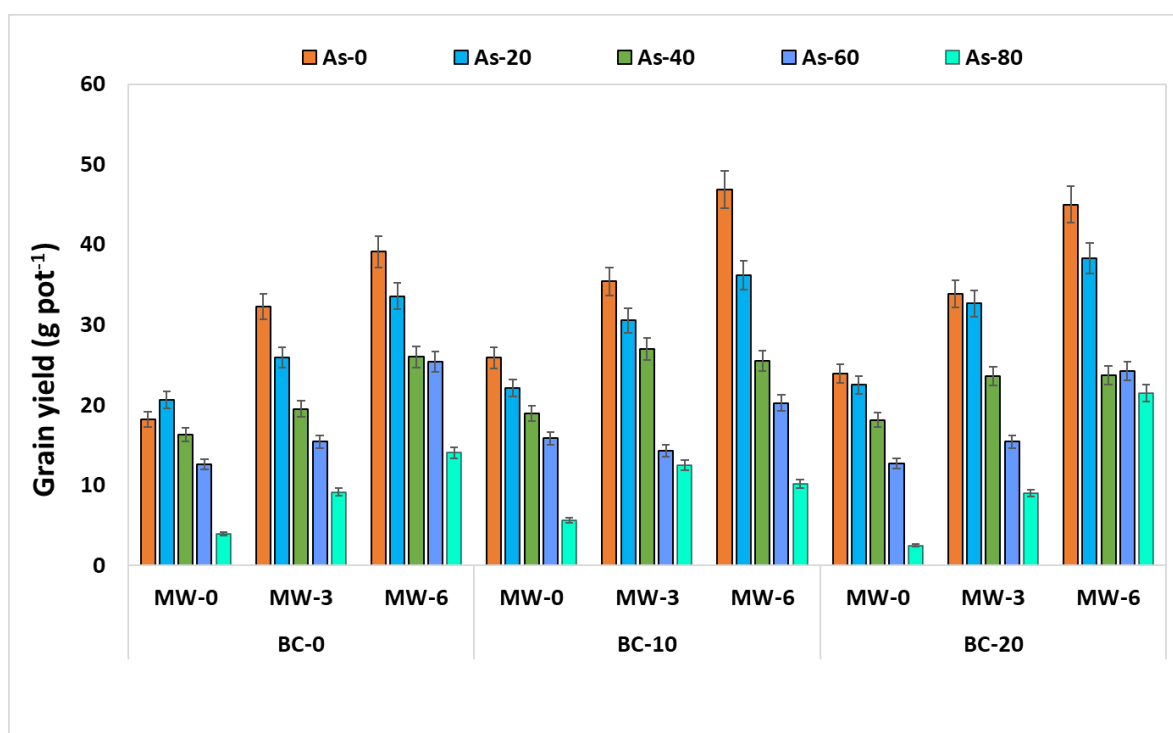
Appendix 5.5. Influence of soil arsenic on leaf length, width, area and perimeter in microwave and biochar treated soil

As (mg kg ⁻¹ soil)	MW (minutes)	Leaf length (cm)			Leaf width (cm)			Leaf area (cm ²)			Leaf perimeter (cm)		
		0	10	20	0	10	20	BC (t ha ⁻¹)			0	10	20
								0	10	20			
0	0	46.08	47.50	44.58	1.13	1.24	1.13	36.66	38.74	36.56	92.02	95.94	91.39
	3	49.83	49.75	41.75	1.25	1.29	1.01	38.89	39.79	34.23	97.97	98.75	85.59
	6	49.11	50.09	49.75	1.40	1.41	1.34	41.95	42.17	40.80	102.64	103.57	101.99
20	0	42.92	46.50	39.84	1.03	1.25	0.94	34.53	38.88	32.66	86.25	93.92	81.66
	3	45.25	49.67	42.42	1.11	1.34	1.04	36.12	40.73	34.78	90.30	101.82	86.95
	6	46.92	53.33	44.84	1.32	1.49	1.14	40.43	43.73	36.77	98.97	109.33	91.91
40	0	41.33	45.83	40.67	0.99	1.11	0.97	33.89	36.11	33.35	84.73	90.96	83.37
	3	45.17	45.00	43.08	1.15	1.19	1.05	37.04	37.79	35.01	92.59	93.08	86.62
	6	48.42	49.00	41.33	1.26	1.33	1.04	39.17	40.65	34.76	97.57	99.64	85.28
60	0	38.92	44.91	39.50	0.90	0.93	0.92	31.91	32.62	32.39	79.78	81.55	80.98
	3	42.66	43.17	41.25	1.02	1.11	0.99	34.32	36.05	33.82	85.80	89.41	84.56
	6	44.75	47.50	43.33	1.14	1.25	1.08	36.70	38.95	35.53	91.74	97.38	88.83
80	0	36.83	39.08	35.08	0.75	0.90	0.74	28.86	32.05	28.77	72.72	80.12	71.92
	3	42.09	41.33	37.08	0.94	1.00	0.82	32.78	33.89	30.41	82.28	84.73	76.02
	6	42.66	44.00	43.17	1.07	1.12	1.07	35.32	36.41	35.40	87.47	90.20	88.49
LSD _{0.05}		5.174			0.173			3.463			9.175		
p value													
As		< 0.001			< 0.001			< 0.001			< 0.001		
MW		< 0.001			< 0.001			< 0.001			< 0.001		
BC		< 0.001			< 0.001			< 0.001			< 0.001		
As.MW		0.942			0.649			0.66			0.713		
As.BC		0.432			0.106			0.106			0.181		
MW.BC		0.443			0.517			0.529			0.620		
As.MW.BC		0.595			0.866			0.857			0.812		

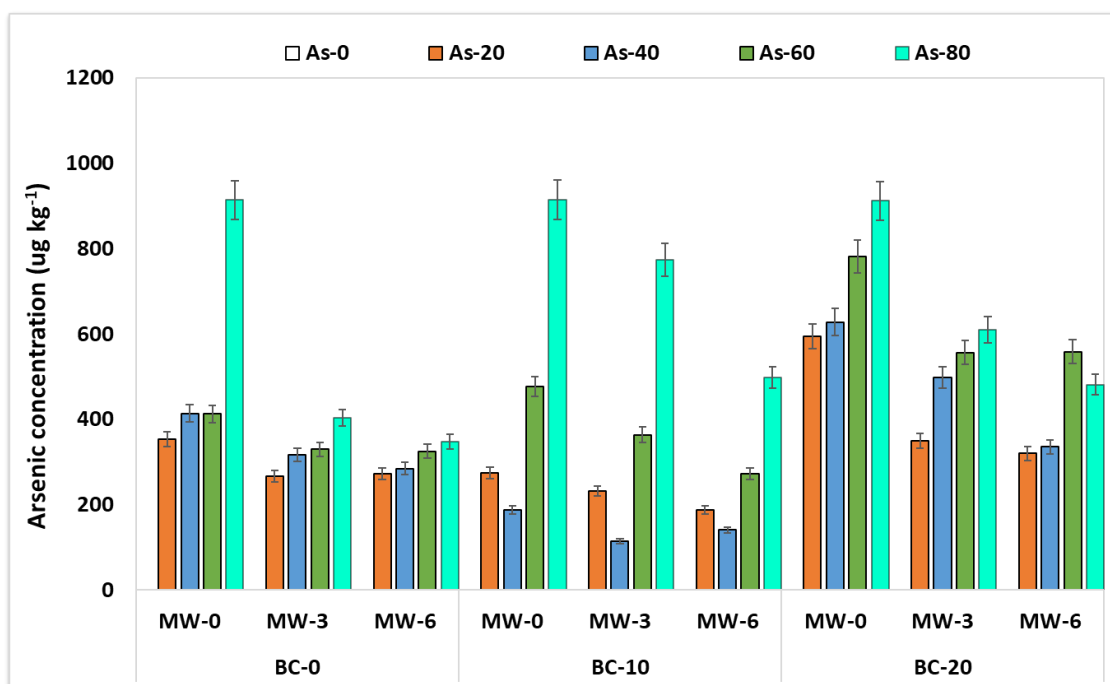
Data recorded at maximum tillering stage of crop growth. As.MW.BC indicates the interaction of arsenic (As), microwave (MW) and biochar (BC)



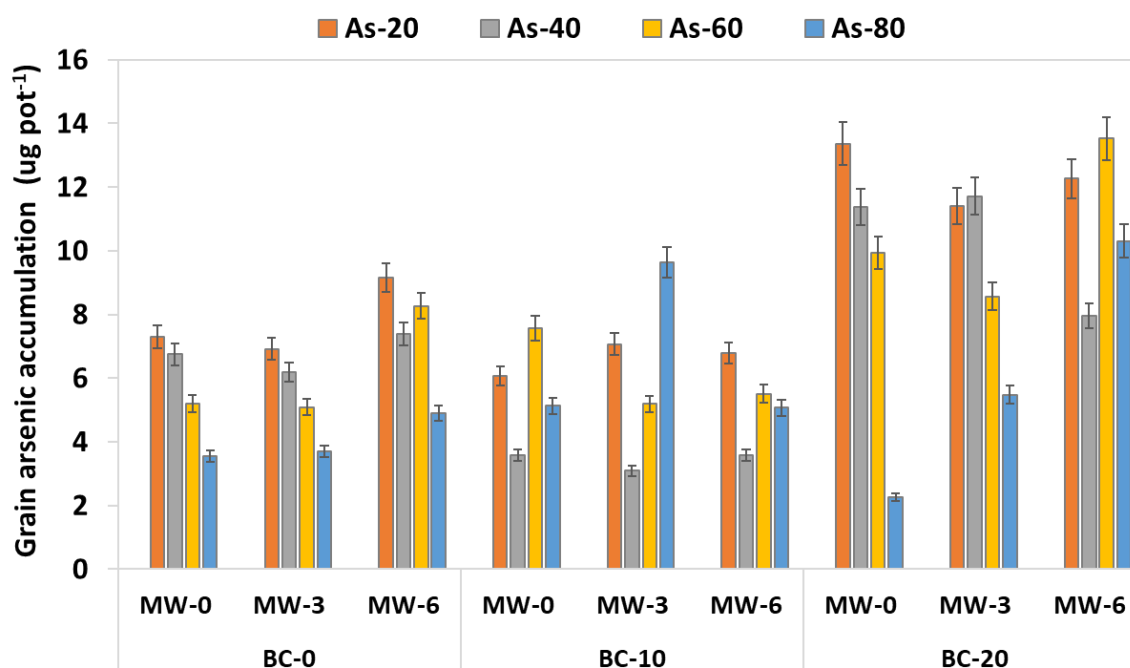
Appendix 5.6. Mean tiller number per pot at different arsenic, microwave (MW) and biochar (BC) treatments. Replicated mean value are shown in bars with standard error.



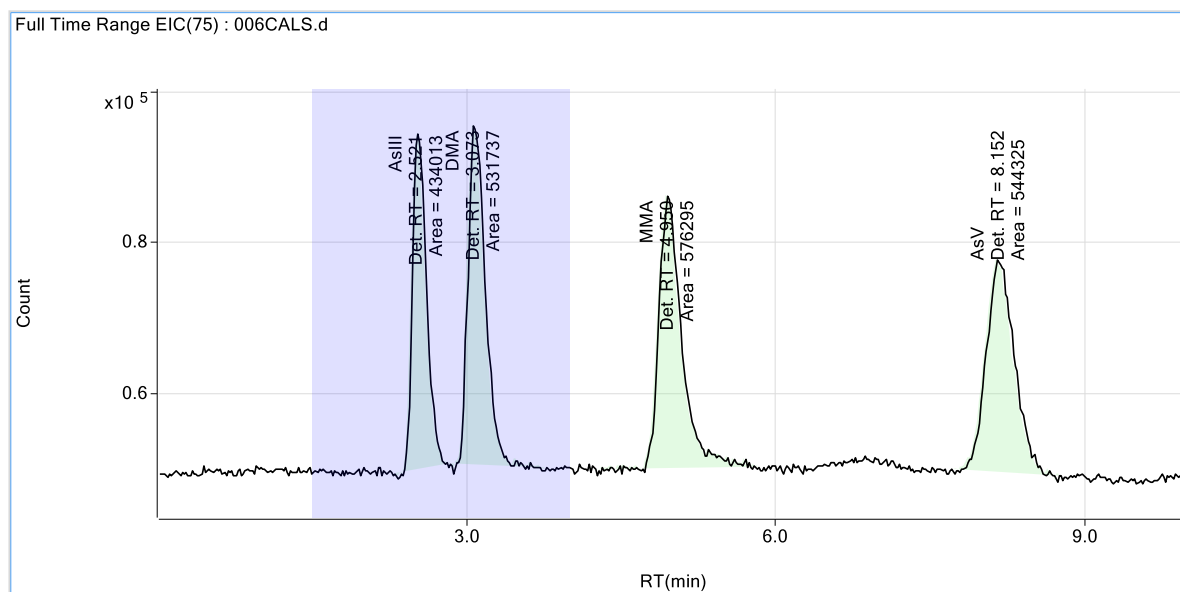
Appendix 5.7. Effect of microwave (MW) soil treatment and biochar (BC) application on grain yield at different soil arsenic concentration level. The mean grain yield values are shown in bar along with standard error.



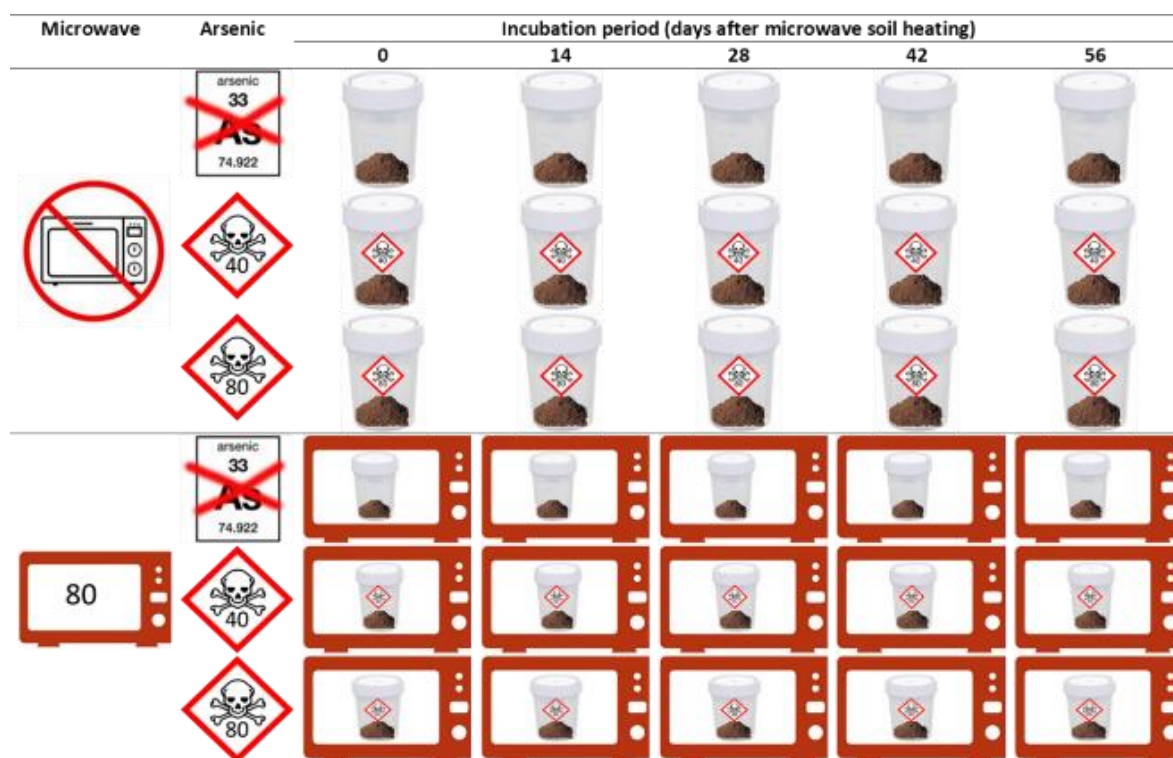
Appendix 5.8. Total arsenic concentration ($\mu\text{g kg}^{-1}$) in rice grain in response to microwave (MW) and biochar (BC) treatments at different level of soil arsenic (As) treatments. Replicated mean value are shown in bars with standard error.



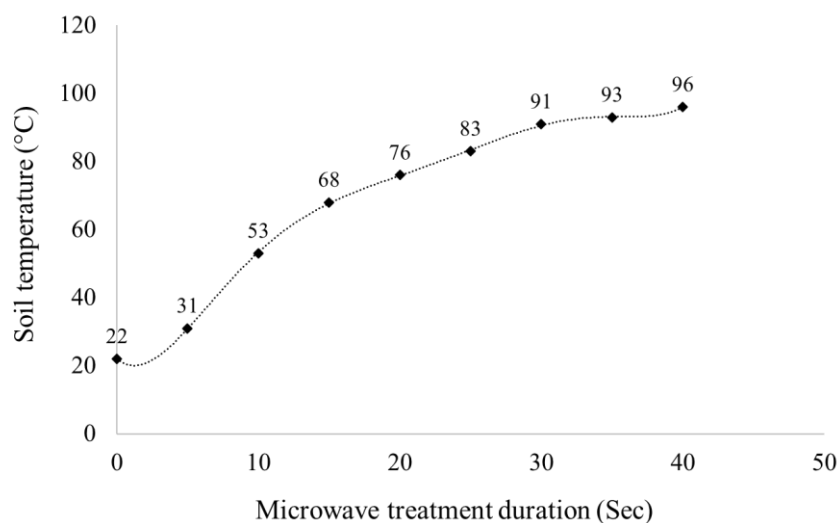
Appendix 5.9. Total amount of arsenic accumulation ($\mu\text{g pot}^{-1}$) in rice grain in response to microwave (MW) and biochar (BC) treatments at different level of soil arsenic (As) treatments. Replicated mean value are shown in bars with standard error.



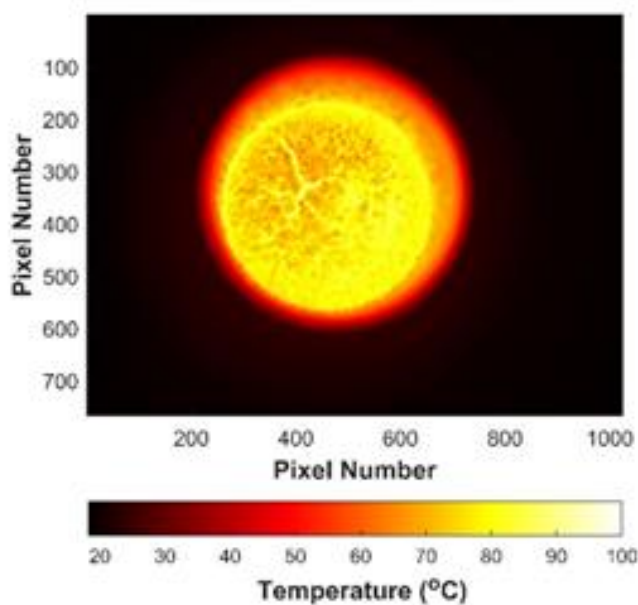
Appendix 8.1. Typical chromatogram of $100 \mu\text{g l}^{-1}$ of the four arsenic species [As(III), DMA, MMA and As(V)]. Peak represent the area of different arsenic species at specific retention time (RT).



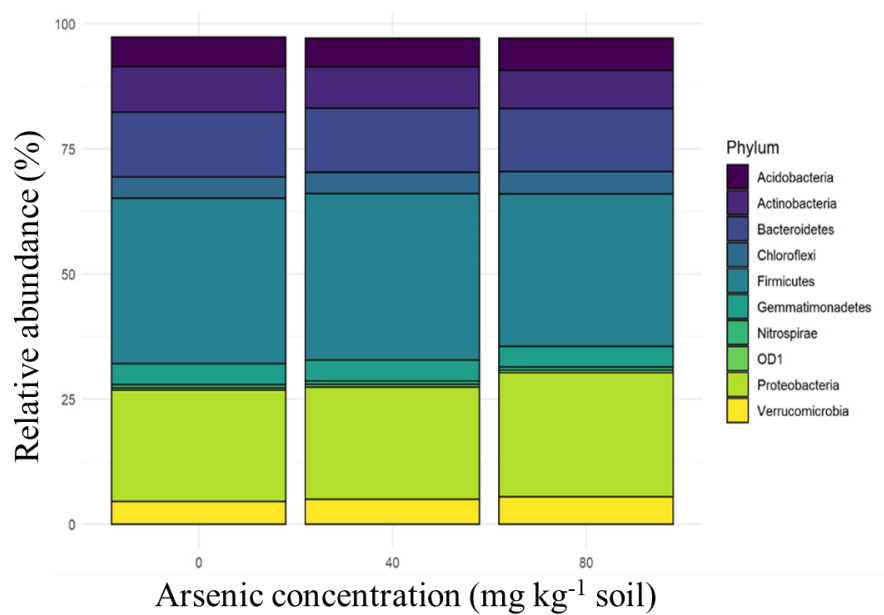
Appendix 9.1. Experimental design of Chapter 9 (Bacterial community response to the microwave soil heating in arsenic contaminated soils).



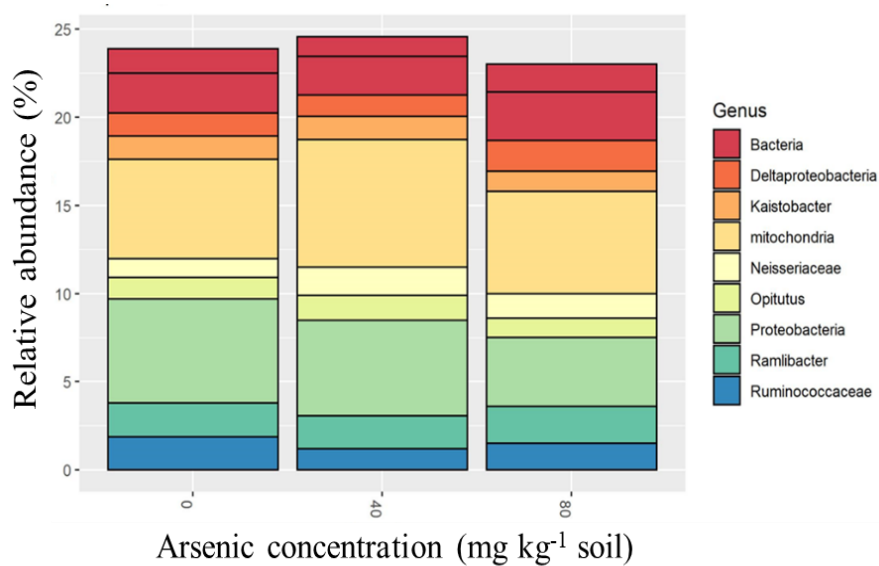
Appendix 9.2. Rise of soil temperature after microwave irradiation. A 1100 W microwave oven was used for microwave soil treatment.



Appendix 9.3. Infrared thermal images of the microcosm treated through microwave oven showing the soil temperature around 85 ± 5 °C. The raw image was post-processed in MATLAB® (The Mathworks Inc., Natick, MA, USA) to assess the soil temperature distribution in the microcosm.



Appendix 9.4. Relative abundance of soil bacteria at phylum level (top 10 phylum) in control soil (without microwave heat treatment).



Appendix 9.5. Relative abundance of soil bacteria at genus level (top 10 genus) in control soil (without microwave heat treatment).