COMBINED EFFECTS OF THE BIOCHAR/BIOCHAR COMPOSITES AND WATER MANAGEMENT STRATEGIES ON THE PHYTO-AVAILABILITY OF ARSENIC IN PADDY RICE SOILS

A Thesis submitted by

Prasanna Kumarathilaka, M.Phil.

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Dedication

DEDICATED TO SRI LANKANS WHO CONTRIBUTED FOR

FREE EDUCATION

Abstract

Rice (*Oryza sativa* L.) is the main staple carbohydrate source for more than 50% of the world's population. Rice production needs to be increased by 70% by 2030 to meet the demand of an ever-increasing population worldwide. It is reported that millions of people around the world are at risk of health problems because of ingestion of arsenic (As) through rice consumption. The application of pristine/modified biochar (BC) would be a sustainable way of improving rice yield and decreasing the bioavailability of As in the rice rhizosphere. Taking these facts into account, this PhD investigated, for the first time, the integrated effects of pristine/modified BC-water management approaches (flooded and intermittent) on plant growth parameters and As phyto-availability in As-contaminated paddy rice soils with respect to potential mechanisms.

In the first phase, the effects of pristine rice hull BC supplementation to As-contaminated paddy soils under different water management practices were investigated. The incorporation of rice hull BC to As-contaminated paddy soils has increased rice yield by 11%-19% in rice hull BC-intermittent and -flooded treatments compared to the conventional flooded treatment. Inorganic As concentration in rice roots, shoots, husks, and unpolished rice grains and abundance of Fe(III) reducing bacteria in the rice rhizosphere decreased by 10%-83% and 40-70%, respectively, in rice hull BC-flooded, -intermittent, and intermittent treatments compared to the conventional flooded treatment. Concentrations of essential elements such as Fe, Mn, Zn, Mg, and Ca in unpolished rice grains increased by 45%-329% in rice hull BC-flooded and -intermittent treatments compared to flooded treatment. The steady release of Si, SO_4^{2-} and essential elements and the adsorption of As species, following the incorporation of rice hull BC in paddy soils could contribute to a decreased As accumulation in rice tissues, while increasing the concentration of essential elements in unpolished rice grains.

In the second phase, the effects of birnessite modified rice hull BC supplementation to As-contaminated paddy soils under different water management practices were evaluated. Rice yield in both selected rice varieties (*Jayanthi* and *Ishikari*) increased by 10%-34% under birnessite modified rice hull BC-flooded and birnessite modified rice hull BC-intermittent treatments compared to the conventional flooded treatment.

In most cases, inorganic As concentration in rice roots, shoots, husks, and unpolished grains in both rice varieties was significantly ($p \leq 0.05$) decreased by 20% - 81%, 6% - 81%, 30% - 75%, and 18% - 44%, respectively, under birnessite modified rice hull BC-flooded, birnessite modified rice hull BC-intermittent, and intermittent treatments over flooded treatment. Incremental lifetime cancer risks associated with consumption of both rice varieties were also decreased from 18% to 44% under birnessite modified rice hull BC-flooded, birnessite modified rice hull BC-intermittent, and intermittent treatments compared to the conventional flooded treatment. Formation of Mn plaque on rice roots, physi- and chemisorption of As species to birnessite modified rice hull BC, following the supplementation of birnessite modified rice hull BC in As-contaminated paddy soils could involve the reduction of bioavailability of As species in the rice rhizosphere.

In the third phase, the effects of Fe-modified rice hull BC addition to As-contaminated paddy soils under different water management practices were examined. Compared to conventional flooded water management, rice yield per pot under Fe-modified rice hull BC-intermittent and Fe-modified rice hull BC-flooded treatments increased by 24%-39%. The supplementation of Fe-modified rice hull BC has decreased the As/Fe ratio and the abundance of Fe(III) reducing bacteria by 57%-88% and 24%-64%, respectively, in Fe-modified rice hull BC-flooded and Fe-modified rice hull BC-RBC-intermittent treatments compared to the conventional flooded treatment. Fe-modified rice hull BC-intermittent treatment has also significantly ($p \leq 0.05$) reduced the accumulation of As in rice roots, shoots, husks, and unpolished rice grains by 62%, 37%, 79%, and 59%, respectively, compared to the conventional flooded treatment. The steady release of Si and Fe to paddy pore water, promoting Fe plaque formation, adsorption of As species onto Fe-modified rice hull BC could contribute to a decreased As accumulation in rice tissues under Fe-modified rice hull BC-intermittent treatment compared to other treatments.

Overall, this PhD research paves the way to a sustainable-integrated approach of pristine/modified rice hull BC-intermittent water supply management strategy that can be adapted for rice grown in As-contaminated paddy rice soils. The results of this approach would be to improve the quality of rice, to increase rice yield for the demand of ever-increasing populations worldwide.

Certification of Thesis

This Thesis is the work of Mr. Prasanna Kumarathilaka except where otherwise acknowledged, with the majority of the authorship of the papers presented as a Thesis by Publication undertaken by the Student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal Supervisor: Prof. Jochen Bundschuh

Associate Supervisor: Assoc. Prof. Saman Seneweera

Associate Supervisor: Prof. Andrew Meharg

Associate Supervisor: Prof. Yong Sik Ok

Associate Supervisor: Dr. Alla Marchuk

Student's and supervisors' signatures of endorsement are held at the University of Southern Queensland.

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The following detail is the agreed share of contribution for candidate and co-authors in the presented publications in this thesis:

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As	Arsenic
As(III)	Arsenite
As(V)	Arsenate
BC	Biochar
BET	Brunauer-Emmett-Teller
DCB	Dithionite-Citrate-Bicarbonate
DMA(V)	Dimethylarsinic acid
EDTA	Ethylene-diamine-tetra-acetic acid
Eh	Redox potential
FTIR	Fourier Transform Infrared Spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
MMA(V)	Monomethylarsonic acid
SEM	Scanning Electron Microscopy
TC	Total carbon
TOC	Total organic carbon
UHPLC-ICP-MS	Ultra-High Performance Liquid Chromatography - Inductively Coupled Plasma Mass Spectrometry
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Rationale of study

1.1.1 Background

Arsenic (As) ranks 20th in natural abundance of the earth's crust, 14th in seawater and 12th in the human body (Cheng et al., 2009). Arsenic is ubiquitous in the environment and it possesses both metallic and non-metallic properties. Arsenic occurs in both solid and liquid phases, however, it rarely exists in nature in the native state. It is reported that there are 150 species of As-bearing minerals in nature, including arsenic sulphide (As_2S_2), arsenic tri-sulphide (As_2S_3) and arsenopyrite ($FeAsS_2$). More precisely, all rocks, soils, water, air, and biological tissues contain As in varying degrees (Hossain, 2006, Kumarathilaka et al., 2018a).

Arsenic contamination in groundwater aquifers is a worldwide problem since As affects countries in all five continents. Previous studies revealed that over 200 million people across 70 countries suffer from As-contaminated drinking water (based on 10 $\mu g\ L^{-1}$ of As, which is the guideline value of the World Health Organisation, WHO). The WHO reported that approximately 130 million people around the world are exposed to an As level above 50 $\mu g\ L^{-1}$ through drinking water (Fatoki et al., 2013). Apart from drinking water sources, humans could be exposed to As through the consumption of foods, in particular, rice (*Oryza sativa* L.). Therefore, in recent years, significant attention has been paid to investigating As phyto-availability in paddy rice soils.

Rice is the staple food for approximately half of the global population, the majority in the Asian region, supplying 50% and 70% of their protein and energy needs, respectively. It is estimated that human beings consume more than 400 million metric tons of milled rice annually, which is approximately 50% of total cereal consumption globally (Bhattacharya et al., 2012). Laborte et al. (2017) have reported that nearly 115 countries worldwide produce rice, with about half of the countries in Asian region having up to three cropping seasons per annum. Countries in Latin American, Caribbean, and African regions have a maximum of two seasons per year. Since rice is a summer crop, rice cultivation in all temperate countries is restricted to one season per year (Laborte et al., 2017). China is the leading rice producer in the world, followed by India, Bangladesh, Indonesia and Vietnam. It is estimated that by 2030, world rice production will need to be increased by 70% to meet the demand of a growing population (Norton et al., 2017). Recent findings demonstrated that most of the rice producing countries are vulnerable to As accumulation in rice tissues. Therefore, urgent attention needs to be paid to the production of rice grains with safer As levels for human consumption.

Different geogenic and anthropogenic sources and processes are involved in As distribution in the paddy rice soil. Natural weathering of As-bearing minerals and alluvial deposits are geogenic sources of As in paddy paddy rice soils. Use of As-contaminated groundwater for irrigating rice is the main anthropogenic process of As build-up in paddy rice soils (Biswas et al., 2014, Dahal et al., 2008, Dittmar et al., 2007). The Food and Agricultural Organization (FAO) recommends that total As in irrigation water should be under 100 $\mu g\ L^{-1}$. Dry and wet depositions of As-bearing minerals from mining activities (Ag, Au, Cu, Pb, W, and Zn) have been found to increase As levels in paddy soil-water systems. Other anthropogenic sources of As in paddy rice soils are phosphate (PO_4^{3-}) based fertilizers, cattle manure, use of arsenical based pesticides on cotton-rice rotations, and leaching from hazardous waste dumps. The European Union (EU) has established the maximum limit for As in agricultural soils as 20 mg kg^{-1}.

Different physico-chemical and biological factors (i.e. pH, redox potential (Eh), organic matter, redox sensitive elements (i.e. Fe, Mn, and S), and availability of Si and PO_4^{3-} , and microbial community) affect As speciation in the paddy rice soil (Kumarathilaka et al., 2018a). Arsenite (As(III)) and arsenate (As(V)) are inorganic As species detected in the paddy soil-water system. Dimethylarsinic acid (DMA(V)) and monomethylarsonic acid (MMA(V)) are the commonly found organic As species in paddy soil-water systems. In terms of toxicity, the inorganic As species has been found to more toxic than the organic As species. In rice plants, As (III) is acquired by nodulin 26-like intrinsic proteins (NIPs) which are also responsible for Si uptake in rice plants. Phosphate transporters share the same uptake pathway for both PO_4^{3-} and As(V) in rice plants. It is suggested that organic As species are acquired by $Si(OH)_4$ transporters through rice roots (Kumarathilaka et al., 2018b).

International Agency for Research on Cancer (IARC) classifies inorganic As as a group A carcinogen (IARC, 2004). Long-term chronic exposure to As causes a range of ailments including skin lesions, nervous system impairment, irritation of respiratory organs and the gastrointestinal tract, anaemia, liver disorders, vascular illnesses and even diabetes mellitus, cancers (i.e. skin, lung, liver, kidney, and bladder) and also can affect the intellectual development of children (Florea et al., 2007, Melak et al., 2014). In addition, As could act as a catalyst in redox reactions, forming reactive oxygen species which damage DNA, proteins and lipids. As a result of the chronic toxicological effects of As, the European Union (EU) has recommended a maximum level of 100 µg kg^{-1} for inorganic As in rice grains intended for young children (EC, 2015). The WHO has set a permissible level of inorganic As in polished rice grains for adults consumption at 200 µg kg^{-1} (WHO, 2014).

Paddy rice soils under conventional paddy water management practices undergo a long-term flooded (reductive) and a short-term non-flooded (oxidative) conditions, during the initial and final stages of rice growth, respectively (Kögel-Knabner et al., 2010). Such conditions change the redox chemistry of the paddy soil-water system. The long-term reductive conditions increase the mobility and bioavailability of inorganic As in the paddy soil-water system. Therefore, rice plants acquire and accumulate higher inorganic As concentrations in their vegetative and reproductive tissues than other cereal crops (Bakhat et al., 2017). Straighthead disease, in which the panicles remain upright because of lack of grain filling and sterility, is prominent in rice plants due to the accumulation of As (X. Li et al., 2016). Moreover, accumulation of As in rice plants negatively affects plant growth parameters such as root length, shoot biomass, plant height, grain yield, and the number of filled grains. Furthermore, recent health risk assessment studies have demonstrated that the consumption of rice and rice-based food products derived from As-prone areas has increased the cancer risks, particularly in infants, children, and pregnant women and as well as in adults (González et al., 2020, Khan et al., 2020).

It is, therefore, timely to either remediate As-contaminated paddy rice soils or to adapt the rice plants to decrease or prevent As accumulation in the edible portions, thereby reducing human exposure to As through rice consumption as well as sustaining the rice yield to meet the demands of an ever-increasing population worldwide. In this sense, agronomic (alternative water management practices, application of plant nutrients and supplementation of soil amendments), introduction of microorganisms, and transgenic approaches have been investigated to reduce the As burden in paddy rice soils. However, there are limitations to the practical employability of the above mentioned methods.

Intermittent water supply management strategy has been found to decrease As accumulation in rice tissues, due to an increased Eh in the paddy rice soil (P. Hu et al., 2013, Mukherjee et al., 2017). During the intermittent water supply management strategy, the rice field is only flooded to desired levels (~3 - 5 cm) when the soil is dry and cracks are found. Even though intermittent water supply management strategy has decreased As concentration in rice grains, loss of rice yield is one of the major concerns to be addressed (Basu et al., 2015). The decreased rice yield could indicate a failure to meet the demand for rice by an ever-growing population worldwide. At the same time, it could also adversely affect farmers' incomes.

The incorporation of biochar (BC) to As-contaminated paddy rice soils might be a viable option for decreasing As levels in rice tissues and increasing the rice yield. Biochar could be produced through the process "pyrolysis" by using a range of organic feedstocks (i.e. forestry and agricultural crop residues, the organic portion of municipal solid wastes, invasive plant species, animal manures, and wood waste) (Jayawardhana et al., 2019, Kumarathilaka and Vithanage, 2017). The application of BC in agricultural soil systems has been found to have a wide assortment of benefits such as decreasing bioavailability of heavy metal(loids), increasing water holding capacity, improving soil nutrient levels, providing habitat for microorganisms, and minimizing the effect of greenhouse gases through carbon sequestration (Ghani et al., 2013, Kumarathilaka et al., 2018c). The pristine BC could be modified through chemical and physical activation processes to modify the characteristics of BC towards contaminant removal and nutrient supplementation.

Limited studies have focused on the role and potential application of pristine and modified BC in As-contaminated paddy rice soils. An integrated approach of pristine BC supplementation and alternative water management practices might sustain the rice yield and improve the rice quality for safer human consumption. So far, none of the studies have investigated the integrated effect of pristine and modified BC amendments with alternative water management approaches in As-contaminated paddy ecosystems. Taking these facts into account, this PhD research principally investigated the effect of pristine and modified BC amendments to As contaminated paddy soils under different water management practices (i.e. conventional flooded water management and intermittent water supply management strategy) on As accumulation in rice tissues and plant growth parameters with respect to the potential mechanisms.

1.1.2 Significance of study

Since rice consumption has recently been recognized as an important source of As exposure in humans around the world, development of scientifically sound management strategies is urgently required. This PhD research project provides a multifaceted and interdisciplinary understanding of the uptake, translocation, and accumulation of both inorganic and organic As species in rice tissues. Many studies have focused on decreasing As mobility and bioavailability in paddy rice soils by using different mitigation strategies such as supplementation of redox-sensitive nutrients and minerals, inoculation of microorganisms, phytoremediation approaches and introduction of transgenic plant species. However, cost, source scarcity, low predictability, secondary contamination, and requirement for technical skills have limited the practical applicability of those mitigation strategies in As-contaminated paddy rice soils.

Taking those facts into account, this PhD study has assessed and developed a cost-effective, scientific, technically and practically employable approach/s that can easily be adopted by paddy farmers in As-contaminated paddy rice soils around the world. Intermittent water supply management strategy could decrease the mobility of As species in the soil-water system due to changes in redox chemistry and reduce the water volume required for rice cultivation, thus substantially decreasing the cost for irrigation water. Even though several studies have demonstrated that intermittent water supply management strategy leads to reduced rice grain yield and rice quality, BC application under such conditions could sustain rice productivity for a growing population throughout the world since BC releases essential plant nutrients into the paddy soil-water system.

Rice hull is the by-product of paddy milling process from the field. Rice milling industries in Australia and other rice growing countries generate an enormous amount of rice hull during the paddy milling process. It is estimated that >200 million tons of rice hulls are generated annually worldwide. Depending on the rice variety and the harvesting method, 1 kg of milled rice generates approximately 20 - 25 wt% (dry basis) of rice hulls as a by-product. A large quantity of rice hulls remains unutilized and/or is burnt in open environment, causing environmental and atmospheric pollution issues. Therefore, production of BC using rice hulls is an alternative waste management approach in the environment. Since rice hulls are a rich source of Si, the application of rice hulls into As-contaminated paddy rice soils could be a cost-effective way to mitigate highly toxic As(III) uptake in rice plants. Moreover, rice hull BC supplementation in As-contaminated paddy rice soils may act as an alternative fertilizer source for Si in the short- and long-term basis. In addition, application of rice hull BC is a sustainable method of Si fertilization in As-contaminated paddy rice soils since Si fertilization in As-contaminated paddy rice soils is limited due to its high cost and source scarcity.

Most rice producers export rice worldwide and therefore As ingestion through the consumption of rice and rice-based products could occur over a large geographical area. Data obtained from this PhD research project provides a cost effective, technically and practically feasible, and more science-based mechanistic understanding of As phyto-availability in paddy rice soils. Such an understanding provides avenues to sustain rice productivity in terms of rice yield and rice quality in As-contaminated paddy rice soils. In this regard, this PhD study fully contributes to minimizing As accumulation in rice tissues and increases rice yield for the demand of an ever-increasing population around the world.

1.2 Research gaps

1. None of the previous studies have investigated the integrated effect of rice hull BC amendments with alternative water management approaches in As-contaminated paddy rice soils with respect to the potential mechanisms.

2. No study has so far reported essential elemental levels in rice grains under the integrated rice hull BC-alternative water management conditions in As-contaminated paddy rice soils.

3. None of the previous studies have evaluated health risk assessments under the integrated approach of birnessite-modified rice hull BC-water management practices in As-contaminated paddy rice soils.

4. None of the studies have evaluated the integrated effect of iron-modified rice hull BC amendments-alternative water management techniques in As-contaminated paddy rice soils with respect to mechanistic interpretations.

1.3 Research questions

There are three main research questions in this study as follows.

1. How does an integrated approach of rice hull BC-alternative water management practices affect rice yield and accumulation of As in rice tissues, and sustain essential element contents in rice grains? This research question is subdivided as follows:

    (I) Can an integrated approach of rice hull BC-alternative water management increase plant growth parameters?
    (II) How does an integrated approach of rice hull BC-alternative water management affect physico-chemical characteristics in paddy pore water?
    (III) Can an integrated approach of rice hull BC-alternative water management significantly decrease As accumulation in rice grains?
    (IV) How does an integrated approach of rice hull BC-alternative water management affect microbial diversity and abundance which involves mobilization of As in the rice rhizosphere?
    (V) Can an integrated approach of rice hull BC-alternative water management improve essential elemental concentrations in rice grains?
    (VI) What are the potential mechanisms involving As phyto-availability in paddy soil-water systems under rice hull BC-alternative water management conditions?

2. How do different rice genotypes behave in relation to As stress and cancer risk through rice consumption under integrated birnessite-modified rice hull BC-alternative water management applications? This research question is subdivided as follows:

   (I) Can an integrated approach of birnessite-modified rice hull BC-alternative water management increase plant growth parameters in different rice genotypes?
   (II) How does an integrated approach of birnessite-modified rice hull BC-alternative water management affect As fractionation in paddy soils?
   (III) Can an integrated approach of birnessite-modified rice hull BC-alternative water management significantly decrease As accumulation in rice tissues in different rice genotypes?
   (IV) How does an integrated approach of birnessite-modified rice hull BC-alternative water management affect incremental lifetime cancer risks associated with rice consumption in different rice genotypes?
   (V) What are the potential mechanisms involved in As phyto-availability in paddy soil-water systems under birnessite-modified rice hull BC-alternative water management conditions?

3. How does an integrated approach of iron-modified rice hull BC-alternative water management practices affect rice yield, formation of iron plaque, and accumulation of As in rice grains? This research question is subdivided as follows:

   (I) Can an integrated approach of iron-modified rice hull BC-alternative water management increase plant growth parameters?
   (II) How does an integrated approach of iron-modified rice hull BC-alternative water management affect Fe, Si, total C, and total organic C concentrations in paddy pore water?
   (III) Can an integrated approach of iron-modified rice hull BC-alternative water management enhance Fe plaque formation on rice roots to sequestrate As in the rice rhizosphere?

(IV) Can an integrated approach of iron-modified rice hull BC-alternative water management significantly decrease As accumulation in rice grains?

(V) What are the potential mechanisms involving As phyto-availability in paddy soil-water systems under iron-modified rice hull BC-alternative water management conditions?

1.4 Research aim and objectives

The overall aim of this PhD project is to develop economically feasible and technically employable method/s to decrease As accumulation in rice plant tissues while sustaining the rice yield and quality that could be adaptable to As-contaminated paddy rice soils. The general objectives are listed below.

1. Quantitative analysis of inorganic and organic As species concentrations in rice tissues (roots, shoots, husks, and unpolished rice grains) under the integrated pristine/modified-rice hull BC-water management regimes.

2. Assessment of plant growth parameters under the integrated pristine/modified-rice hull BC-water management techniques.

3. Investigation of physico-chemical parameters in paddy soil solution under the integrated pristine/modified-rice hull BC-water management conditions.

4. Examination of microbial diversity and abundance, which could involve As mobilization in the rice rhizosphere, under the integrated pristine/modified-rice hull BC-water management conditions.

5. Investigation of the possible mechanisms involved in As mobilization/immobilization in the paddy agro eco-system under different integrated pristine/modified-rice hull BC-water management conditions.

1.5 Organization of the thesis

This PhD thesis consists of six chapters and the organization of the thesis is shown in the Fig. 1.1.

Chapter 1 describes the background and significance of the study, research gaps, research questions, and research aims and objectives.

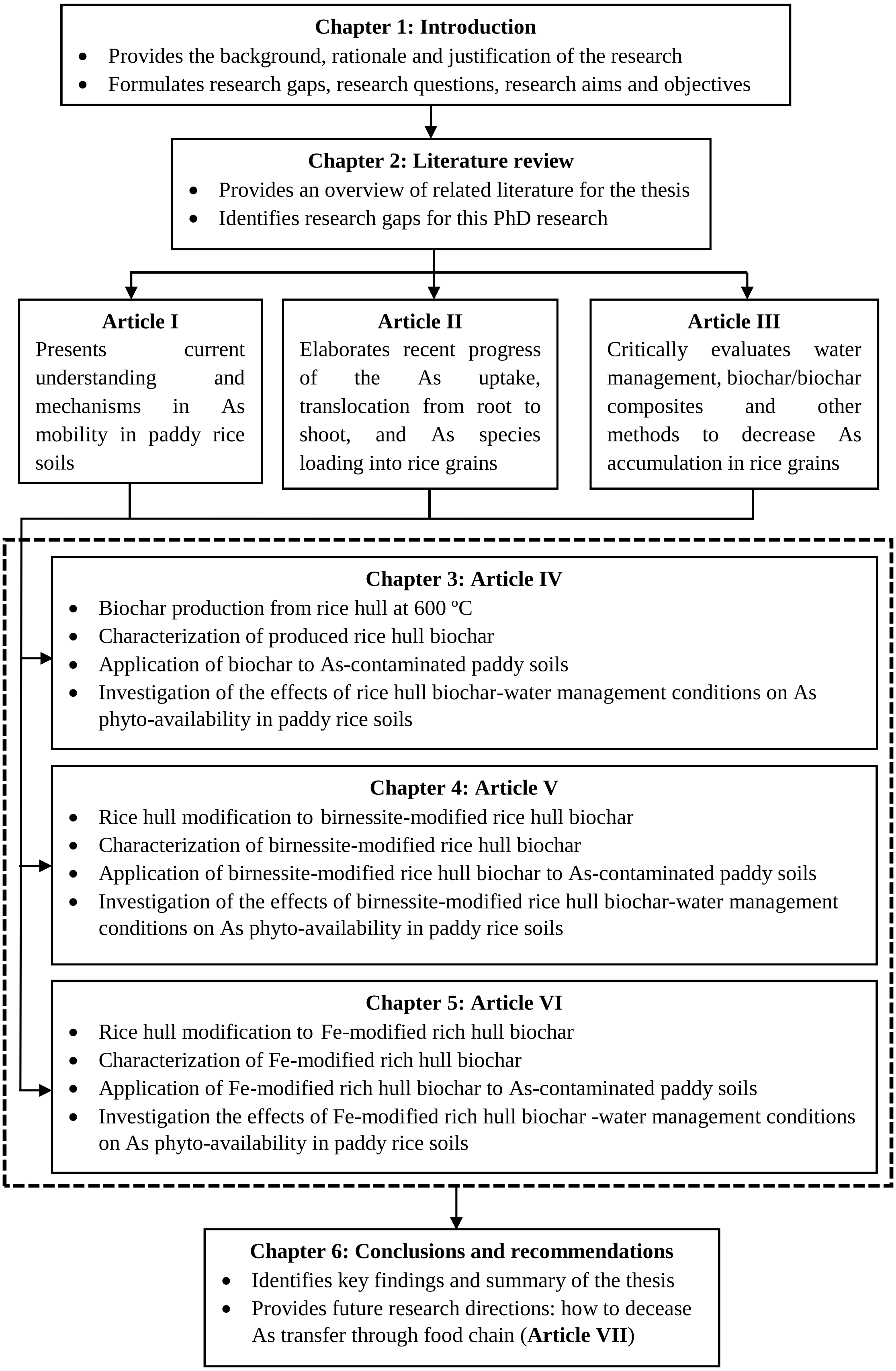
Chapter 2 presents current understanding and mechanisms in As phyto-availability in paddy rice soils, highlighting research gaps. This chapter comprises three Q1 ranked articles: **Article I** (Arsenic speciation dynamics in paddy rice soil-water environment: sources, physico-chemical, and biological factors - a review) presents the current understanding of As mobilization and speciation in paddy rice soils. **Article II** (Arsenic accumulation in rice (*Oryza sativa* L.) is influenced by environment and genetic factors) and describes recent progress of the As uptake, translocation from root to shoot, and As species loading into rice grains. **Article III** (Mitigation of arsenic accumulation in rice: an agronomical, physico-chemical, and biological approach - a critical review) critically evaluates efficiency, practical applicability, and technical feasibility of physico-chemical and biological approach to decrease As accumulation in rice grains. The new insights and research gaps highlighted in these articles were utilized for the development of research studies in **Chapters 3, 4, and 5**.

Chapter 3 presents **article IV** (An integrated approach of rice hull biochar-alternative water management as a promising tool to decrease inorganic arsenic levels and to sustain essential element contents in rice) which investigates the effects of rice hull biochar-alternative water management approaches on As phyto-availability in paddy rice soils, rice growth parameters, and essential elemental concentrations in rice grains.

Chapter 4 presents **article V** (Rice genotype's responses to arsenic stress and cancer risk: the effects of integrated birnessite-modified rice hull biochar-water management applications) that investigates the effects of integrated birnessite-modified rice hull biochar-water management approaches on As accumulation, rice yield and incremental lifetime cancer risks through rice consumption in different rice genotypes.

Chapter 5 presents **article VI** (Iron modification to silicon-rich biochar and alternative water management to decrease arsenic accumulation in rice (*Oryza sativa* L.) which investigates the application of Fe-modified rich hull BC-water management approaches on the accumulation of As in rice tissues, rice yield, and microbial diversity and abundance in the rice rhizosphere.

Chapter 6 provides the conclusions and recommendations of this PhD research project while also highlighting future research directions. The **article VII (**Arsenic in cooked rice foods: assessing health risks and mitigation options) presents comprehensive details of post-harvesting technologies and future research directions to decrease As transfer through food chain.



Chapter 1: Introduction

- Provides the background, rationale and justification of the research
- Formulates research gaps, research questions, research aims and objectives

Chapter 2: Literature review

- Provides an overview of related literature for the thesis
- Identifies research gaps for this PhD research

Article I

Presents current understanding and mechanisms in As mobility in paddy rice soils

Article II

Elaborates recent progress of the As uptake, translocation from root to shoot, and As species loading into rice grains

Article III

Critically evaluates water management, biochar/biochar composites and other methods to decrease As accumulation in rice grains

Chapter 3: Article IV

- Biochar production from rice hull at 600 ºC
- Characterization of produced rice hull biochar
- Application of biochar to As-contaminated paddy soils
- Investigation of the effects of rice hull biochar-water management conditions on As phyto-availability in paddy rice soils

Chapter 4: Article V

- Rice hull modification to birnessite-modified rice hull biochar
- Characterization of birnessite-modified rice hull biochar
- Application of birnessite-modified rice hull biochar to As-contaminated paddy soils
- Investigation of the effects of birnessite-modified rice hull biochar-water management conditions on As phyto-availability in paddy rice soils

Chapter 5: Article VI

- Rice hull modification to Fe-modified rich hull biochar
- Characterization of Fe-modified rich hull biochar
- Application of Fe-modified rich hull biochar to As-contaminated paddy soils
- Investigation the effects of Fe-modified rich hull biochar -water management conditions on As phyto-availability in paddy rice soils

Chapter 6: Conclusions and recommendations

- Identifies key findings and summary of the thesis
- Provides future research directions: how to decease As transfer through food chain (**Article VII**)

Fig. 1.1 Organization of the thesis

CHAPTER 2

LITERATURE REVIEW

This chapter is divided into three main subsections based on the review articles published during the PhD research. The first subsection describes the sources, physico-chemical and biological factors that govern the As mobilization and speciation in paddy rice soils. The second subsection provides an overview of the uptake, translocation, and grain filling of inorganic and organic As species in rice plants. The third subsection elaborates water management, physico-chemical, and biological methods to mitigate As accumulation in rice plants. Overall, this chapter represents comprehensive details regarding As phyto-availability in paddy rice soils, As metabolism in rice plants, and measures to mitigate As accumulation from paddy soils to rice grains, and highlighting future research directions.

2.1 Arsenic phyto-availability in paddy rice soils: sources and factors

Article I

Kumarathilaka, P., Seneweera, S., Meharg, A., Bundschuh, J., (2018). Arsenic speciation dynamics in paddy rice soil-water environment: sources, physico-chemical, and biological factors - a review. Water Research, 140, 403-414. (Published).

2.1.1 Introduction

This review describes the current understanding of As mobilization and speciation in paddy soil-water systems. Natural geogenic and anthropogenic sources (i.e. use of As-contaminated irrigation water and mining activities) lead to high As concentrations in paddy rice soils. The effects of physico-chemical properties such as pH and Eh, organic matter, clay mineralogy, redox sensitive ions (i.e. Fe, Mn, and S), and other competing ions and compounds (PO_4^{3-} and $Si(OH)_4$) on the determination of mobilization and speciation of As in the paddy rice soil have been discussed in detail in this review. The involvement of aerobic and anaerobic microorganisms in the paddy soils for the mobilization and speciation of As through different processes including oxidation, reduction, methylation, and volatilization is presented. Research gaps and future research directions have also been elaborated to understand the As phyto-availability in the paddy rice soil.

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Arsenic speciation dynamics in paddy rice soil-water environment: sources, physico-chemical, and biological factors - A review

Prasanna Kumarathilaka [a], Saman Seneweera [b], Andrew Meharg [c], Jochen Bundschuh [a, d, *]

[a] School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350, Australia
[b] Center for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350, Australia
[c] Queen's University Belfast, Institute for Global Food Security, David Keir Building, Malone Road, Belfast, BT9 5BN, United Kingdom
[d] UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350, Australia

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ABSTRACT

Rice is the main staple carbohydrate source for billions of people worldwide. Natural geogenic and anthropogenic sources has led to high arsenic (As) concentrations in rice grains. This is because As is highly bioavailable to rice roots under conditions in which rice is cultivated. A multifaceted and interdisciplinary understanding, both of short-term and long-term effects, are required to identify spatial and temporal changes in As contamination levels in paddy soil-water systems. During flooding, soil pore waters are elevated in inorganic As compared to dryland cultivation systems, as anaerobism results in poorly mobile As(V), being reduced to highly mobile As(III). The formation of iron (Fe) plaque on roots, availability of metal (hydro)oxides (Fe and Mn), organic matter, clay mineralogy and competing ions and compounds (PO_4^{3-} and $Si(OH)_4$) are all known to influence As(V) and As(III) mobility in paddy soil-water environments. Microorganisms play a key role in As transformation through oxidation/reduction, and methylation/volatilization reactions, but transformation kinetics are poorly understood. Scientific-based optimization of all biogeochemical parameters may help to significantly reduce the bioavailability of inorganic As.

Contents

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* Corresponding author. UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, Queensland, Australia. Tel.: +61 7 4631 2694.
E-mail address: jochen.bundschuh@usq.edu.au (J. Bundschuh).

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1. Introduction

Around the world many countries have levels of Arsenic (As) present that are of concern to the environment (Abedin et al., 2002a; Bissen and Frimmel, 2003). The United States Environmental Protection Agency (USEPA) classifies inorganic As as a group A carcinogen (IARC, 2004). Long-term chronic exposure to inorganic As in humans causes cancers (i.e. skin, lung, kidney, liver, and bladder), along with cardiovascular, neurological, hematological, renal and respiratory diseases. Furthermore, it is a root cause of diabetes mellitus, damage to DNA, proteins and lipids, and as significant affects on the intellectual development of children (Johnson et al., 2010; Melak et al., 2014; Sharma et al., 2014). As a consequence of the chronic toxicological effects of As, recommended and regulatory limits established by various authorities have been revised. The World Health Organization's (WHO) permissible level for total As in drinking water was decreased in 1993 from 50 to 10 $\mu g\ L^{-1}$ (WHO, 1993), followed by the USEPA in 2001 (USEPA, 2001).

Arsenic tends to accumulate in higher concentrations in some food items, most notably rice, *Oryza sativa* L., (Lin et al., 2015; Rahman and Hasegawa, 2011). Rice is the staple food for approximately three billion people, predominantly in Asia, supplying 70% and 50% of their energy and protein needs, respectively. According to Laborte et al. (2017), 115 countries worldwide produce rice, with about half of the countries in Asia having 2–3 cropping seasons. Whereas African, Latin American and the Caribbean countries have a maximum of two seasons per annum. Rice production in all temperate regions is restricted to one season per year with peak rice planting months in each season differing between countries (Laborte et al., 2017).

Paddy rice is managed to ensure a standing head of water above the soil, and includes: (a) plowing to homogenize waterlogged top soils, (b) maintaining a standing head of water in the fields during the cropping period, and (c) water drained and soil allowed to dry out before harvest (Winkler et al., 2016). Flooding and draining are used to manage the redox chemistry of the paddy soil-water environment.

Arsenic builds up in paddy soil-water through natural (i.e. rock weathering and alluvial deposits) processes. The use of As-contaminated groundwater to irrigate rice additionally causes a build-up of As (Jia et al., 2015; Sofuoglu et al., 2014). The Food and Agricultural Organization (FAO) recommends that total As in irrigation water should be under 100 $\mu g\ L^{-1}$. Mining of Ag, Au, Cu, Pb, W, and Zn and associated activities can lead to As contamination of neighboring paddies (Buschmann et al., 2007; Fitzmaurice et al., 2009; Zhu et al., 2008). Other sources of As in paddy soils are from phosphate (PO_4^{3-}) based fertilizers, cattle manures, and use of arsenical based pesticides on cotton-rice rotations (Meharg and Zhao, 2012). The European Union (EU) has established the maximum limit for As in agricultural soils as 20 $mg\ kg^{-1}$.

In this review, we explore the current understanding of As dynamics in rice paddy ecosystems, discussing the temporal and spatial distributions of As in paddy soil-water environments with respect to physico-chemical properties of the soil. Furthermore, the role of microbiome on As mobility and transformation between different arsenic species in paddy soil-water will be assessed.

2. Arsenic speciation and its association with physico-chemical properties

2.1. pH and Eh

Arsenic exists in the environment in several oxidation states (i.e. −3, 0, +3, and +5), and occurs in both solid and liquid phases, however, it rarely exists in nature in its native state, As(0) or as As(-3) (Wang and Mulligan, 2006). In groundwater, with a typical pH range of 6.5–8.5, As tends to readily mobilize. In paddy soil pore waters, As is mainly present in its inorganic form as oxyanions of trivalent arsenite (As(III)) or pentavalent arsenate (As(V)), with the most common organic As species being monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)), which are generally present at lower concentrations (Campbell and Nordstrom, 2014; Keller et al., 2014). Ahmed et al. (2011) found that soil pH is positively correlated with total As concentration in rice grains. This is because high pH induces negative surface charges which may promote the desorption of As(III) and As(V) into the soil solution.

Redox potential (Eh) determines the speciation, mobility, and bioavailability of As species. Under oxidizing conditions, arsenic acid (H_3AsO_4) is a dominant species at low pH (<2), whereas $H_2AsO_4^-$ and $HAsO_4^{2-}$ are predominant at pH ranges of (2–11) (Bissen and Frimmel, 2003; Smedley and Kinniburgh, 2002). When reduction conditions develop, arsenious acid (H_3AsO_3) is prominent and is converted into $H_2AsO_3^-$ at lower pH and $HAsO_3^{2-}$ for higher pH levels (>12). Different mechanisms, such as the reductive dissolution of metal hydro(oxides), formation of root plaques, and microbial-mediated reduction and oxidation, are all factors in As speciation in the paddy environment (Smedley and Kinniburgh, 2002).

2.2. Organic matter

Organic carbon derived from decomposed plant and animal products plays an important role in As dynamics in the paddy environment (Tareq et al., 2013). In rice farming, organic matter is often added to soil, in the form of manure. In most cases, rice stubble and roots are incorporated back into paddy fields after harvest (Said-Pullicino et al., 2016). Such practice will lead to the formation of dissolved organic carbon (DOC) in paddy soils, accentuated under flooded conditions. Organic matter has a high affinity with As, leading to the formation of organo-As complexes (Paikaray et al., 2005; Radloff et al., 2007; Williams et al., 2011). Rahaman et al. (2011) demonstrated that the combined amendment of lathyrus, vermicompost, and poultry manure into As-contaminated paddy soils decreased total As concentration in rice tissues. Similarly, Fu et al. (2011) observed that soil organic matter levels exhibited a close negative correlation with total As concentration in rice grains. However, DOC can be adsorbed onto Fe(III) (hydro)oxides (FeOOH) via ligand exchange and subsequently, compete with both As(III) and As(V) for active adsorption sites. As a result, DOC found in the soil solution may enhance both As(III) and As(V) mobility and bioavailability (Mladenov et al., 2015). In addition, DOC stimulates Fe(III) reducing bacteria, promoting microbial FeOOH reduction and leading to the release of As(III) into the soil solution (Chen et al., 2016; Mladenov et al., 2009).

2.3. Clay mineralogy

Clay minerals such as kaolinite, montmorillonite, and illite, are primarily formed due to the weathering of rocks by physical, chemical, and biological factors (Regmi et al., 2013; Wimpenny et al., 2014). A lower Eh developed under flooded conditions leads to enhanced flocculation and dispersion of clay particles. In this regard, clay minerals migrate into the bottom of the ploughed layer and form a plough pan (Li et al., 1997). Mechanical manipulation by farmers, animals, and heavy machinery also promote the plough pan formation. Repeated tilling over many years increases the micropores (<0.2 μm), instead of mesopores, and macropores in the plough pan. Therefore, water retention capacity in ploughed layers is enhanced (Kögel-Knabner et al., 2010). Increased water retention is an important consideration in order to minimize irrigation water requirements, particularly those with As contamination. The presence of clay minerals leads to finer textured soils with a relatively high surface area. FeOOH is mainly incorporated in the clay size soil fraction and therefore, clay soils may promote both As(III) and As(V) retention in paddy soils (Sahoo and Kim, 2013). Surface runoff due to the heavy rainfall and the subsequent destruction of soil structure through ploughing is responsible for the loss of clay minerals during paddy cultivation (Kögel-Knabner et al., 2010), and has a negative impact on As immobilization.

2.4. Redox sensitive ions

2.4.1. Iron

Iron chemistry plays an important role in determining the As(III) and As(V) mobility in paddy soils (Eq. (1) and Eq. (2)) (Pan et al., 2014; Smedley and Kinniburgh, 2002). Paddy soils may contain FeOOH minerals such as ferrihydrite, goethite, lepidocrocite, and hematite. In addition, the formation of Fe plaque under flooded conditions leads to sequestered As species (discussed in detail in section 3.2). Both As(V) and As(III) can be adsorbed or can coprecipitate at the oxic/anoxic boundary of FeOOH, which has a large specific surface area (i.e. ferrihydrite: 100–700 $m^2\,g^{-1}$, goethite: 8–200 $m^2\,g^{-1}$, lepidocrocite: 15–260 $m^2\,g^{-1}$, hematite: 2–115 $m^2\,g^{-1}$). The ligand exchange between inorganic As species and OH_2 and OH^- in coordination spheres of Fe atoms, facilitates the sorption process (Jain et al., 1999). In this regard, FeOOH decreases As(III) and As(V) bioavailability for rice plants (Youngran et al., 2007).

$$\equiv FeOH_{(s)} + AsO^{3-}_{3(aq)} + 3H^{+}_{(aq)} \rightarrow FeH_2AsO_{3(s)} + H_2O_{(l)} \quad (1)$$

$$\equiv FeOH_{(s)} + AsO^{3-}_{4(aq)} + 3H^{+}_{(aq)} \rightarrow FeH_2AsO_{4(s)} + H_2O_{(l)} \quad (2)$$

As mentioned above, reductive dissolution of FeOOH increases As(III) mobility in paddy soil-water systems (Huang et al., 2012; Yamaguchi et al., 2011). Therefore, rice plants are more likely to be susceptible to enhanced As accumulation when compared to other cereal crops. Overall, agronomic practices lead to changes in the Fe chemistry in paddy soils and indirectly affect As mobility and distribution.

2.4.2. Manganese

Manganese oxide minerals interact with As speciation in paddy environments. Being an electron acceptor during the oxidation process, manganese oxides have the ability to decrease As mobility in paddy soil-water in two ways, oxidizing As(III) to As(V) and by adsorbing As(V) (Eq. (3) and Eq. (4)) (Lafferty et al., 2011). For example, phyllomanganates, one of the most notable layered Mn-oxides, can readily oxidize As(III) to As(V) (Manning et al., 2002). Tani et al. (2004) demonstrated that some microorganisms could act to catalyze the manganese oxide induction of As(III) oxidation. Manganese plaque in rice roots also sequesters As, therefore, there is often an inverse correlation between total As in rice tissue and the Mn level in paddy soils (Hossain et al., 2008).

$$MnO_{2(s)} + H_3AsO_{3(aq)} + 2H^{+}_{(aq)} \rightarrow Mn^{2+}_{(aq)} + H_3AsO_{4(aq)} + H_2O_{(l)} \quad (3)$$

$$2Mn - OH_{(s)} + H_3AsO_{4(aq)} \rightarrow (MnO)_2AsOOH_{(s)} + 2H_2O_{(l)} \quad (4)$$

2.4.3. Sulfur

Sulfate (SO_4^{2-}) in the soil matrix reduces into sulfide (S^{2-}) as the redox potential decreases (from −120 to −180 mV) during flooded conditions. Microbial SO_4^{2-} reduction also plays an important role in determining As mobility in paddy soils (Jia et al., 2015; Wilkin et al., 2003). Sulfate reduction to S^{2-} results in immobilization of As(III) due to the precipitation of As(III), as arsenic sulfide, and iron sulfide minerals (Burton et al., 2014; Fisher et al., 2007). However, only limited studies have focused on SO_4^{2-} and inorganic As interactions in paddy soils. Burton et al. (2014) demonstrated that microbial SO_4^{2-} reduction leads to lower concentrations of both Fe(II) and As(III) in flooded soil, owing to mackinawite formation and As(III) co-precipitation. Whereas As(III) sorption onto iron sulfide depends upon As speciation in aqueous media and iron sulfide mineralogy (Jönsson and Sherman, 2008). Moreover, under flooded conditions, As(III) can react with S^{2-} and form aqueous thioarsenite complexes, which may precipitate as As-S solid phases (i.e., amorphous As_2S_3 or AsS) (Bostick et al., 2005). Typically, thioarsenite complexes are formed under low Fe(II) and high organic matter environments, however, thioarsenite complexes are not commonly found in paddy soils.

2.5. Other competing ions and compounds

The presence of competing anions such as PO_4^{3-} influences As mobility and bioavailability in paddy soils (Sharma et al., 2014). Phosphate, an analogue to As(V), affects As(V) mobilization by desorbing As(V) associated with soil/mineral particles, or adsorbed onto soil surfaces (Cao et al., 2003; Fitz and Wenzel, 2002). Similarly, silicic acid ($Si(OH)_4$) competes with As(III) for binding sites on mineral phases in paddy soils and influences As(III) concentrations in soil pore water. However, silicon (Si) fertilization, as SiO_2, of As-contaminated paddy soil has shown both negative and positive effects on rice plants. Silicic acid can compete with As(III) for retention sites on soil mineral surfaces and consequently increase the As(III) concentration in the soil solution. As a result, rice plants may acquire high concentrations of As(III), however, Li et al. (2009) demonstrated that SiO_2 application greater than the recommended rate decreased the As(III) uptake in rice plants. This is because both $Si(OH)_4$ and As(III) are acquired by the same transporter, OsNIP2; 1 (Lsi1), in rice plant and higher SiO_2 application promotes the $Si(OH)_4$ uptake rather than As(III).

3. Arsenic dynamics in the paddy environment

Natural and anthropogenic sources contribute to As distribution in a paddy environment. Table 1 summarizes the main causes (i.e. As-contaminated irrigation water, industrial waste/wastewater discharges, mining activities, and natural geogenic sources) for As introduction into the paddy environment and total As concentrations in paddy soils in As-affected rice cultivation regions around the world. In the following subsections, the main causes that introduce As into paddy ecosystems are discussed in detail. Fig. 1 illustrates key features and reactions in As-contaminated paddy soil-water systems at different stages of the rice growth cycle.

3.1. *Geogenic As elevation*

Geogenic sources such as rock weathering and alluvial deposits lead to contaminate paddy soils with As. However, limited studies have focused on geogenic As accumulation in the paddy environment (Table 1). Weathering of As-bearing minerals such as bearsite ($Be_2(AsO_4)(OH).8H_2O$), wallisite ($(Cu,Ag)TlPbAs_2S_5$), and claudetite (As_2O_3) are largely responsible for As in paddy soils in Manipur, India (Eq. (5)) (Chandrashekhar et al., 2016). Such minerals lead to relatively high total concentrations of As in paddy soils. For example, total As concentrations in paddy soils in Manipur, India ranged between 50 and 90 mg kg^{-1}.

$$As_2O_{3(s)} + 3H_2O_{(l)} \rightarrow 2H_3AsO_{3(aq)} \tag{5}$$

It is therefore important to investigate the geological background in and around paddy environments to identify possible primary and secondary As-bearing minerals. The solubility constants for respective minerals different greatly from each other and are dependent upon various physico-chemical and biological factors. Arsenic in loosely bound fractions (primarily in the bioavailable fraction and redox sensitive phases) are vulnerable to release As into the paddy soil solution (Fu et al., 2011; Hsu et al., 2012). Alluvial deposition of sediments is also responsible for long-distance transport of As from point sources to floodplain paddy fields. Hundal et al. (2013) found that alluvial soils collected from North-West India released As(III) from a soil-water suspension under flooded conditions. Therefore, paddy environments located along the floodplains and near As-bearing mineral deposits are required to be investigated for possible As accumulation affects.

3.2. *Effect of As contaminated irrigation water*

Rice plant yield is mostly dependent on the water type used for irrigation over the respective growth cycle. To prepare the land for rice planting, approximately 150–200 mm of water is added, however, this figure may extend up to 900 mm of water in some instances (Bouman and Tuong, 2001). Throughout the growing season (~3 months), a volume of between 500 and 3000 mm of water is needed, and this figure differs with climatic conditions, soil type, and rice genotypes (Abedin et al., 2002b). When dry conditions exist, water losses are relatively high and paddy soils become partially or completely dry.

Rice cultivation in dryer periods is dependent on the available surface water, i.e. river, pond, lake, dam, or groundwater sources. Surface water sources are largely safe from As contamination (Sharma et al., 2014). However, mineral weathering and anthropogenic activities such as industrial discharge and municipal solid waste dump sites, may add inorganic and organic As species into surface water (Liu et al., 2007; Zheng et al., 2015). Due to a lack of accessibility and poor infrastructure development, most farmers in rice-producing localities have to depend on the readily available groundwater sources (Brammer, 2009). In this regard, As contaminated groundwater is extensively used in the form of irrigation water in many rice-producing countries (Table 2). As a result, paddy soils can be easily contaminated with As over time and in some instances, the total As level in soil exceeds the recommended level for agricultural purposes (Table 1). Therefore, most researchers have mainly paid attention to As accumulation in paddy soils with As-contaminated irrigation water (Dittmar et al., 2007; Huang et al., 2016; Rahman et al., 2010).

Aquifer chemistry is an important aspect in determining As concentrations in groundwater, especially for As impacted aquifer regions (Table 2). Well depth differs from region to region and does not necessarily show a close correlation with As concentrations in groundwater (Table 2). Groundwater tables tend to fluctuate during the year due to the large differences between rainfall and extensive withdrawal. As a result, oxygen reacts with As-bearing minerals (i.e., pyrite (FeS_2) and arsenopyrite ($FeAsS$)) to release As (Eqs. (6) and (7)) (Fakhreddine et al., 2015; Sun et al., 2016). The reduction of FeOOH under reducing conditions also releases As(III) into the groundwater (Eq. (8)) (Xie et al., 2015). Iron(III)-reducing microorganisms also enhance the reduction of FeOOH to release As(III). Therefore, the combination of natural processes, together with human involvement, lead to the introduction of inorganic As species into rice cultivating areas through groundwater irrigation.

$$2FeS_{2(s)} + O_{2(g)} + 2H_2O_{(l)} \rightarrow 2Fe^{2+}_{(aq)} + 4SO^{2-}_{4(aq)} + 4H^{+}_{(aq)} \tag{6}$$

$$4FeAsS_{(s)} + 11O_{2(g)} + 6H_2O_{(l)} \rightarrow 4Fe^{2+}_{(aq)} + 4H_3AsO_{3(aq)} + 4SO^{2-}_{4(aq)} \tag{7}$$

$$FeOAs(OH)_{2(s)} \rightarrow Fe^{2+}_{(aq)} + AsO^{3-}_{3(aq)} + 2H^{+}_{(aq)} \tag{8}$$

Irrigated groundwater is usually fed by channels into paddy fields (Kabir et al., 2016; Polizzotto et al., 2013). When As-contaminated groundwater is used for irrigation, typically, a gradient in total As concentration in paddy soil-water is observed. High concentrations found near the channel inlet tapering down to relatively low levels at significant distances (Dittmar et al., 2007; Panaullah et al., 2009). However, a number of studies have demonstrated that flow velocity has a close relationship with As concentrations in the irrigation water along the irrigation channel. At a slower flow velocity, aggregation and subsequent settling of As-bearing colloids occur due to the long residence time. Therefore, slower flow velocity leads to a gradual decrease in total As concentrations observed along the irrigation channel (Fig. 2) (Dittmar et al., 2007; Roberts et al., 2007). In contrast, a faster flow velocity has a short residence time which restricts the aggregation and subsequent settling down of As-bearing colloids along the irrigation channels. In this regard, total As concentrations in irrigation water along the irrigation channel remains relatively unchanged at higher operating flow velocities (Dittmar et al., 2007; Roberts et al., 2007). The type of irrigation channel (i.e. man-made concrete channel or soil/clay ditch channel) also change the total As concentration found in irrigation water along the channel length to a greater extent (Neumann et al., 2014).

Chemical changes can also lead to decreased total As concentrations observed along irrigation channels. Most studies have focused on the total As concentration rather than As speciation (As(III) and As(V)) in irrigation water. However, limited studies revealed that As(III) which represents the majority of total As in groundwater, gradually decreases as it travels along irrigation channels. The rationale for this is that diffusion of atmospheric O_2 into the irrigation water may oxidize As(III) into As(V). Reactive

Table 1
Key causes of As introduction to paddy environments and total As concentration in paddy soils in As-contaminated rice growing localities in the world.

Cause	Country	Number of samples	Sampling depth (cm)	Concentration (mg kg^{-1})	Reference
Groundwater irrigation	Bangladesh	113	0–60	4.5–68	Rahman et al. (2010)
		56	0–15	10–70	Panaullah et al. (2009)
		71	1–15	3.1–42.5	Meharg and Rahman (2003)
		224	0–15	11.41–61.04	Hossain et al. (2008)
		–	0–1	149	Dittmar et al. (2007)
			5–10	54	
			>30	4–11	
	India	–	0–15	2.41–14.09	Biswas et al. (2014)
				1.64–8.75	
		6	0–5	51.5	Shrivastava et al. (2017)
			5–10	37.7	
			10–15	21.1	
		–	0–5	38.2	Norra et al. (2005)
			50–60	20.4	
			100–110	11.1	
		–	10–15	4.6–9.72	Bhattacharya et al. (2010)
		21	0–15	0.68–2.33	Sidhu et al. (2012)
	Cambodia	16	–	0.68–26.36	Phan et al. (2013)
		23	0–20	0.8–18	Seyfferth et al. (2014)
	Vietnam	16	–	6–20	Huang et al. (2016)
	Nepal	10	0–10	6.1–12.5	Dahal et al. (2008)
			10–20	6.1–11.3	
			0–10	5.6–9.9	
			10–20	5.8–10.0	
	Taiwan	11	0–20	11.8–112	Hsu et al. (2012)
Surface water	Vietnam	2	–	9.5–11.0	Huang et al. (2016)
Industrial waste/wastewater	China	–	–	34	Zheng et al. (2015)
		6	0–20	3.05–5.58	Liu et al. (2007)
Mining deposition	South Korea	135	0–30	2.97–172.07	Yun et al. (2016a)
		40	0–15	64.4	Kwon et al. (2017)
	China	27	–	9.14–25.84	Ma et al. (2017)
		19	0–15	97–709	Liu et al. (2005a)
		–	0–20	2.5–19.2	Jiang et al. (2014)
		–	–	0.1–45.9	Fu et al. (2011)
	Bangladesh	15	0–15	14.83–41.97	Halim et al. (2015)
Natural origin	India	–	0–100	13.9–24.1	Chandrashekhar et al. (2016)
		–	–	50–90	Hundal et al. (2013)
	France	23	0–10	5–10	Williams et al. (2007)
	Spain	25	0–10	4–11	
	USA	6	0–10	4–7	

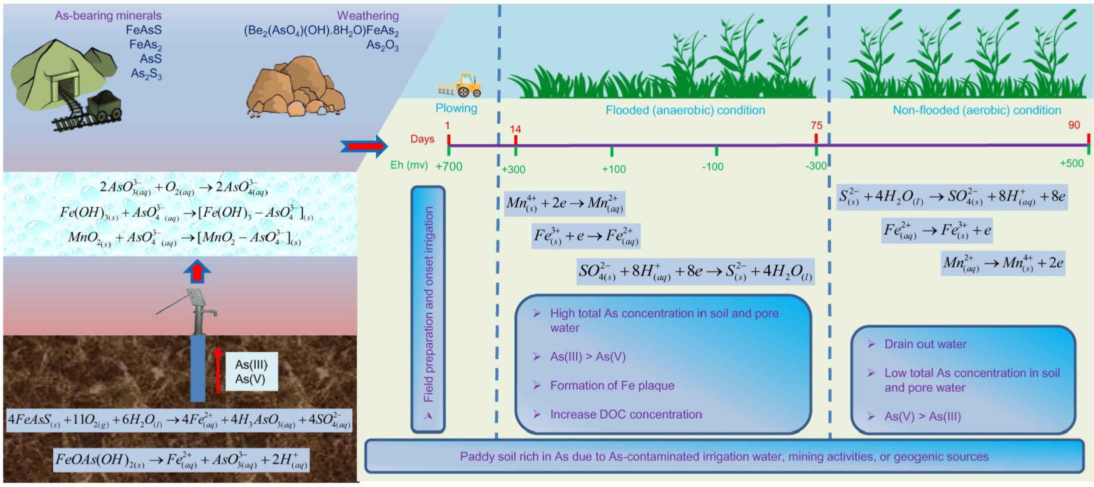

Fig. 1. Key sources, features and reactions at different stage of rice growth on As contaminated paddy soils.

intermediates (i.e. Fe(IV) species) formed during Fe(II) oxidation by O_2 may also partly oxidize As(III) into As(V) (Roberts et al., 2004). As the irrigation water flows through the irrigation channel, dissolved Fe(II) and Mn(II) are oxidized by diffused atmospheric O_2 and consequently precipitated as $Fe(OH)_3$ and MnO_2 (Dittmar et al., 2007). The complexation and co-precipitation of As(V) with metal hydro-oxides (i.e. Fe and Mn) further reduce the total As concentrations found along irrigation channels (Fig. 2).

Table 2
Rice growing regions which use As-contaminated groundwater in the form of irrigation water for rice cultivation.

Country	Extracting depth (m)	pH	Eh (mV)	Conductivity (μS cm^{-1})	As concentration (mg L^{-1})	Other metal concentrations (mg L^{-1})			Reference
						Fe	Mn	P	
Bangladesh	–	–	–	–	<0.01–0.55	–	–	–	Rahman et al. (2010)
	6–20	–	–	–	0.05–0.20	–	–	–	Van Geen et al. (2006)
	30–60	7.0	–	–	0.40 (~84% of As existed as As(III))	11.0	–	1.97	Dittmar et al. (2007)
	69	7.2		863	0.30	18.1	0.1	1.2	Martin et al. (2007)
	35	–	−70	–	0.10–0.13	1.97–2.87	0.76–0.79	0.49–0.67	Panaullah et al. (2009)
	–	–	–	–	0.40	9.2	–	1.8	Polizzotto et al. (2013)
India	–	–	–	–	0.40–0.70 (December–June; summer) 0.09–0.55 (June–December; winter)	–	–	–	Biswas et al. (2014)
	–	–	–	–	0.00–0.02	–	–	–	Sidhu et al. (2012)
	–	–	–	–	0.11–0.76	–	–	–	Bhattacharya et al. (2009)
	–	7.2–7.3	–	690–800	0.52 (~83% of As existed as As(III))	4.62–7.42	0.77–0.94	–	Norra et al. (2005)
Vietnam	–	6.94–7.12	–	770–1310	0.06–0.92 (~80% of As existed as As(III))	20.6	–	–	Huang et al. (2016)
Nepal	5–22	–	–	–	<0.00–1.01	–	–	–	Dahal et al. (2008)
Taiwan	20	–	–	–	0.03–0.07	2.0–4.2	–	–	Hsu et al. (2012)

Total As concentration further decreases once the irrigation water enters the paddy field for a range of different reasons. It is obvious that once irrigation water comes into the paddy field, that water flow rate across the paddy field will significantly decrease. As a result, colloid settling and As adsorption onto mineral particles takes place. Polizzotto et al. (2013) demonstrated that total As concentration dropped by almost 50% of initial well-water concentrations once irrigation water first entered the fields, located only 40 m from the irrigation well. When paddy fields were in static or standing conditions after being completely filled with irrigation water, dissolved total As concentrations declined across the fields exhibiting little lateral variation within 4 h after irrigation (Polizzotto et al., 2013). Moreover, As input with irrigation water mainly accumulates on top soil (i.e. the root zone of the rice plants) (Table 1) possibly due mainly to the oxidation, adsorption, and co-precipitation of As species.

The paddy soil is submerged (flooded) for a long period of time out of the growing cycle and consequently, undergoes a number of physico-chemical reactions. The water replaces the gaseous phase in the soil pores, resulting in a decrease of Eh for a flooded environment. A number of redox reactions (from high Eh (+700 mV) to low Eh (−300 mV): aerobic respiration, nitrification, denitrification, Mn(IV) reduction, Fe(III) reduction, SO_4^{2-} reduction, and methanogenesis) mainly governed by microorganisms occurs either sequentially or simultaneously during this period (Sahrawat, 2015). If the paddy soil is initially acidic, the pH of the soil tends to increase during the flooded conditions as reduction processes consume protons. The reverse is true for alkaline paddy soils since CO_2 induces mineralization triggers of acidification (Sahrawat, 2015).

Arsenic speciation and distribution in flooded and non-flooded conditions during rice growth is a completely different process. Soil pore water contains higher concentrations of total As, Fe, and Mn than the irrigation water under flooded conditions. In addition, As(III)/As(V) ratios in the soil solution and submerged soil increase over time during the flooded period (Takahashi et al., 2004). As stated above, the development of a lower Eh value in submerged paddy soils is the main reason for such results. Garnier et al. (2010) assessed the changes in Eh in paddy soil profile (0–20 cm) during the rice growth cycle over 3 months, from paddy soil preparation through to rice harvesting. They found that the Eh value had decreased along the soil profile, with only slight variations except for a depth of 7 cm where less reduced conditions were observed, possibly due to micro-aeration by rice plant roots.

Formation of root plaques greatly influence As mobility and bioavailability in paddy soil-water systems, with Fe and Mn co-existing in rice root plaques (Liu et al., 2005b). However, Fe is the main element followed by Mn in the plaques since FeOOH precipitates at lower Eh levels than Mn oxides. Therefore, most of the previous studies have mainly focused on As species sequestration onto Fe plaques in rice roots. Iron plaque mainly consists of ferrihydrite, goethite, and lepidocrocite (Violante et al., 2003). Radial oxygen loss (ROL) which is the phenomenon releasing O_2 into the rhizosphere through the root aerenchyma, promotes Fe plaque formation even though flooded conditions exist (Eq. (9)) (Colmer, 2002).

$$4Fe^{2+}_{(aq)} + 3O_{2(g)} + 6H_2O(l) \rightarrow 4Fe(OH)_{3(s)} \tag{9}$$

Since ROL depends on plant anatomy (i.e. root porosity and root density) and growth stage, the amount of Fe plaque on rice roots varies among rice genotypes (Liu et al., 2004; Mei et al., 2009; Violante et al., 2003). Indigenous Fe levels in paddy soil systems also plays a key role in determining the amount of root plaque to be formed. Therefore, a rice genotype grown in paddy soils with high Fe concentration could have higher amounts of Fe plaque than the same rice genotype grown in paddy soils with low Fe concentrations. Iron plaque has high affinity with both As(V) and As(III), hence, Fe plaque directly reduces the total As concentration in the paddy soil-water systems and subsequently in rice plants. External supplementation of FOOH to paddy soils has been found to have positive effects on Fe plaque formation (Suda et al., 2016; Yun et al., 2016b). One of the main drawbacks related to Fe plaque is that the high density of Fe plaque on rice roots may restrict the diffusion of O_2 into the rhizosphere. For this reason, rhizospheric microorganisms associated with rice plant growth and health promotion may be adversely affected, Fe precipitation could also further hinder the nutrient uptake by rice roots (Ultra et al., 2009). Therefore, a detailed investigation is required to further assess the role of Fe plaque on the immobilization of As species in the rice plant growth cycle.

Another important concern regarding the total As accumulation

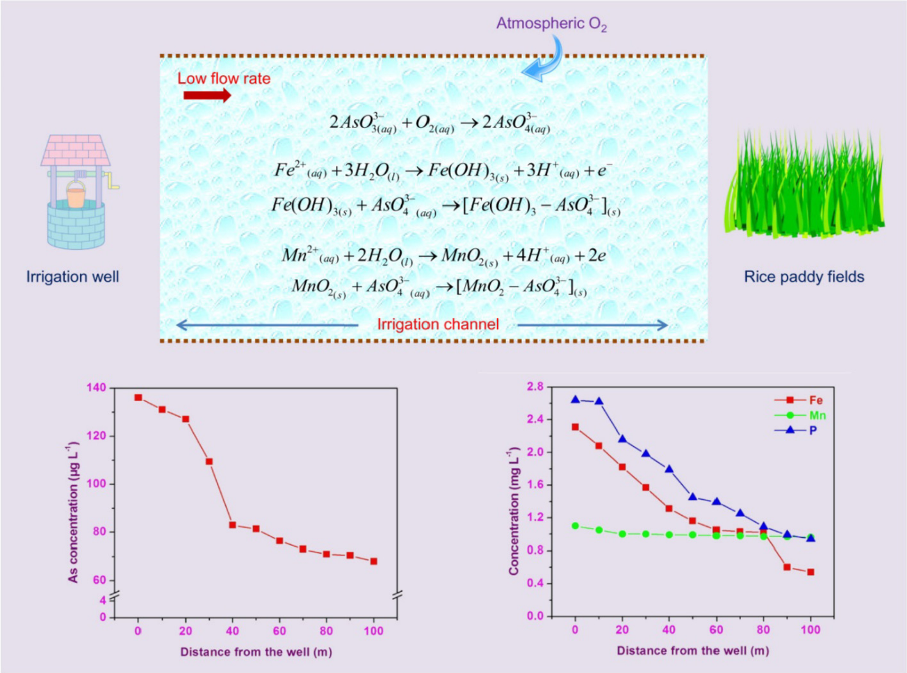

Fig. 2. Variation of As and other elemental concentrations and related reactions along the irrigation channel. Data was adapted from Hossain et al. (2008).

in paddy soils is interpretation of the total As content extent with respect to irrigation water, i.e. mass balance calculations. Different studies have revealed that usage of As-contaminated irrigation water in the concentration range of (100–400 µg L^{-1}) leads to accumulation of total As in top paddy soil at a rate of 1.0–1.6 mg kg^{-1} y^{-1} (Dittmar et al., 2007; Martin et al., 2007; Meharg and Rahman, 2003). Therefore, long-term studies are required to assess how loosely bound As fractions behave over time. In addition, As accumulation predictions can vary for many different reasons. Arsenic in paddy soil can also be removed through various different processes. As mentioned earlier, paddy fields which are subjected to seasonal flooding due to high rainfall remove a large amount of As. Roberts et al. (2010) estimated that seasonal flooding removes 13–62% of As added to the paddy soils through irrigation each year. In addition, rapid As transfer below the plow pan, i.e. a depth of approximately 20–25 cm, may enhance preferential flow paths e.g. soil cracks formed under dry condition and mouse burrows (Dittmar et al., 2007). Evapotranspiration might also concentrate As in the soil solution on a short-term basis, but the link between evapotranspiration and As concentrations in paddy soil-water system has not been comprehensively evaluated.

3.3. *Effect of mining activities*

Paddy soils can be contaminated by As if mining activities are active in close proximity to paddy fields (Table 1). So far, a limited number of studies has reported the effects of mining on As contamination in paddy soils and there is no reporting thus far on As speciation dynamics in mining-impacted paddy soils throughout the growth stage of rice plants. Mining of As-bearing minerals (i.e. arsenopyrite, pyrite, realgar (AsS), and orpiment (As_2S_3)) and accompanied secondary As minerals are responsible for As contamination in paddy soils. Tailings and waste rocks, acid mine drainage and wet and dry deposition of dust due to high rainfall and strong winds are responsible for As accumulation in rice fields in the vicinity of mining areas (Kwon et al., 2017; Yun et al., 2016a). China is the world's largest rice producer and is the best example for mining-related As in paddy fields. For instance, long-term mining activities in Hunan province, one of the largest rice producing regions in China led to high total As concentrations in adjacent paddy soils (Table 1). The pattern of the total As distribution in rice soils through mining operations varies due to different parent materials (Patel et al., 2005). Total As concentrations in paddy soils in Hainan Island, China, ranged between 0.1 and 45.9 mg kg^{-1}, depending on the different rock types (metamorphic rocks: 18.9–45.9 mg kg^{-1}, acid igneous rocks and basalt: 0.1–5.9 and 0.2–4.2 mg kg^{-1}, clastic sediments: 0.4–10.9 mg kg^{-1}) (Fu et al., 2011). However, total As concentrations in mining-impacted paddy soils cannot provide sufficient information regarding the mobility and bioavailability of As. Therefore, the associated environmental risks are to be required to be accessed, focusing on speciation levels.

Chemical fractionation through sequential extraction methods provides detailed information on As in paddy soils (Farooq et al., 2010; Fu et al., 2011; Hsu et al., 2012; Norra et al., 2005). Paddy soils affected by mining activities can have a higher percentage of residual phase As than the easily mobile phase and redox sensitive phases of As (Fig. 3). Arsenic binding to residual phases can only be released by strong environmental changes (i.e. strong acids). Therefore, the residual bound fraction is more stable than those loosely bound phases and redox sensitive phases. However, paddy soils contaminated with As-contaminated irrigation water have a different story on chemical fractionation. Those soils have a higher concentration of As retained in loosely bound phases and redox sensitive phases whereas only the lowest concentration of As is associated with residual phases (Fig. 3). Accumulation of As in paddy soils due to As rich groundwater is primarily due to its co-precipitation with or sorption onto poorly crystalline metal hydro(oxides) or other components such as crystalline metal hydro(oxides), clay minerals, and organic matter of the soil matrix. In contrast, paddy soils contaminated with mining operations mainly contain residual bound As which limits both the mobility and bioavailability of As.

4. Role of microorganisms on As dynamics in paddy soil-water systems

Aerobic and anaerobic microorganisms are in paddy soils involved in the As speciation through different processes (Zhang et al., 2015b). Fig. 4 illustrates the variety of direct As transformation pathways governed by microorganisms in paddy soil-water systems.

4.1. Arsenic oxidation and reduction

Microorganisms inhabiting in the rhizosphere and bulk paddy soil are responsible for both As oxidation and reduction. A range of genes present in microorganisms are encoded for As-transformation enzymes and transporters (Zecchin et al., 2017b). Microbial oxidation of As(III) to As(V), which is less toxic and less mobile than As(III), is considered as a detoxification mechanism of microorganisms, coded for by the *aioA* gene (Zhang et al., 2015b). Microbial oxidation of As(III) takes place in both flooded and non-flooded conditions in paddy soils. *Paracoccus* species can oxidize As(III) under both aerobic and anaerobic conditions in paddy soil by using O_2 and NO_3^- as the respective electron acceptor (Zhang et al., 2015a). In addition, As(III) acts as an electron donor during the bacterial metabolism (Zheng et al., 2013). Once abiotic oxidation of As(III) ceases due to passivation of reactive sites on the mineral surfaces, microbiologically-mediated As(III) oxidation becomes prevalent in non-flooded paddy soil even though the rate of conversion of As(III) to As(V) is a slow process, around 2.5–2.8% per day (Dong et al., 2014). As(III) oxidizing bacteria enhance plant growth promoting (PGP) substances. For example, a gram-positive bacterium, *Bacillus flexus* ASO-6, isolated from the rhizosphere of rice paddy plants showed elevated rates of As(III) oxidation ($V_{max} = 1.34\ \mu M\ min^{-1}\ 10^{-7}$ cell) and had secreted plant growth promoting substances such as siderophores, indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Das et al., 2016b). Therefore, inoculation of As(III) oxidizing bacteria is beneficial to not only minimize the As burden but also to promote growth in rice plants.

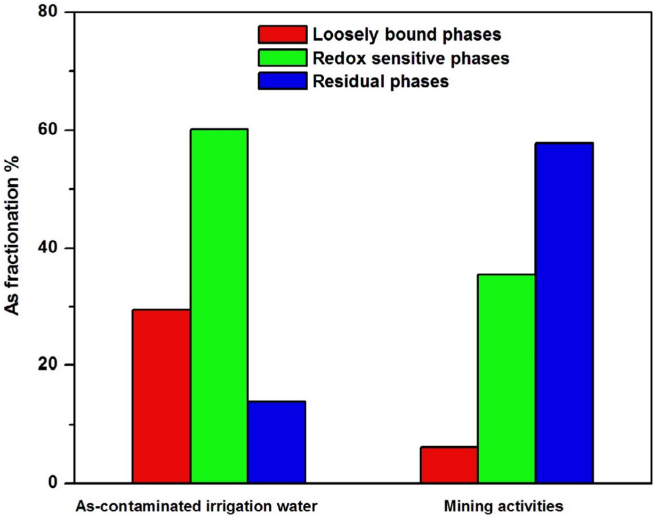

Fig. 3. Arsenic in different fractions in paddy soils (0–5 cm). Paddy soils are contaminated with As rich irrigation water (Farooq et al., 2010; Hsu et al., 2012; Norra et al., 2005) or mining activities (Fu et al., 2011). Loosely bound phases may consist of the mobile fraction, Mn-O bound fraction and an organically bound fraction. Redox sensitive phases may comprise of the humic acid bound fraction, amorphous Fe-O and crystalline Fe-O bound fractions.

Microorganisms are able to reduce As(V) to As(III) in paddy soils, mainly under flooded conditions. Two different pathways, namely As detoxification and the respiratory pathway, coded for by the *arsC and arrA* genes respectively, are involved in As(V) reduction (Malasarn et al., 2004; Villegas-Torres et al., 2011). Phylogenetically diverse bacteria can reduce As(V) to As(III) even under non-flooded conditions (Bachate et al., 2009). Microbial reduction of As(V) to more mobile As(III), under both flooded and non-flooded conditions, may also enhance the As mobility and bioavailability in the paddy environment.

Apart from direct As transformations, microorganisms could indirectly govern the As speciation in paddy soil-water systems. For instance, Fe(III)-reducing and Fe(II)-oxidizing bacteria play an important role in As transformation (Zecchin et al., 2017b). The relative abundance of Fe(III)-reducing bacteria is high in continuous flooded conditions. Therefore, the reduction of Fe(III) minerals and consequent release of As(III) is expected to be high under flooded conditions (Ohtsuka et al., 2013). The application of soil amendments such as biochar may influence As(V) reduction (Wang et al., 2017). The biochar can stimulate the growth and abundance of Fe(III) reducing bacterial growth (i.e. *Clostridium*, *Bacillus,* and *Caloramator*) to facilitate As(V) reduction (Wang et al., 2017). Unfavorable (low) concentrations of Fe(II) and high levels of Fe(III) may also decrease the activity and abundance of Fe(III)-reducing bacteria in paddy soils. However, little is known about the microbial community responsible for Fe(II) oxidation. Nitrate-dependent Fe(II)-oxidizing bacteria in flooded paddy soils can oxidize Fe(II) to Fe(III). Arsenic species may precipitate or adsorb onto Fe(III) minerals in root plaque and bulk soils under such conditions (Dong et al., 2016). As already stated in section 2.4.3, SO_4^{2-} reducing and SO_4^{2-}-oxidizing bacteria could also influence As speciation in paddy soil-water systems.

Overall, water management practices (i.e. flooded and non-flooded conditions) together with physico-chemical and environmental factors greatly determine the abundance and diversity of microorganisms in paddy soil systems (Zecchin et al., 2017a, 2017b). For example, the relative abundance of As-, Fe-, and S-reducing bacteria is increased under flooded conditions and the reverse is true under non-flooded conditions (Das et al., 2016a; Zecchin et al., 2017a). However, there is no detailed report on microbial mediated-As oxidation/ reduction kinetics and related rate limiting factors which may also slow down the reactions.

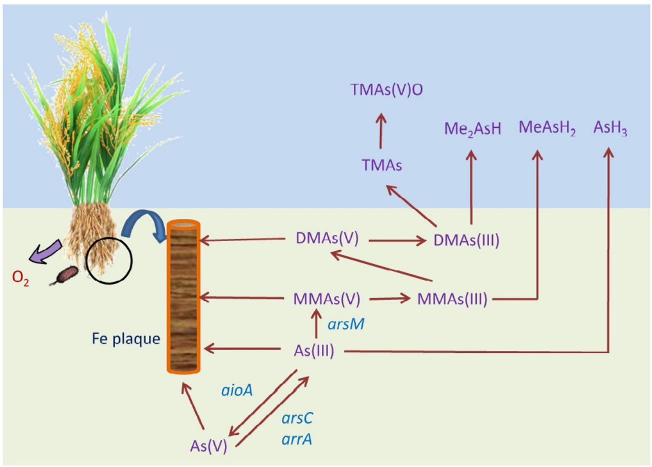

Fig. 4. Microbial mediated As methylation and volatilization, As(III) oxidation, As(V) reduction and dissimilative reduction in paddy soil-water environment.

4.2. Arsenic methylation and volatilization

Microorganisms, including bacteria, fungi, eukaryotic algae, and archaea are mainly responsible for the conversion of inorganic As(III) into methylated As species. The microbial gene for As(III) methylation (*arsM*) that encodes for As-processing leads to the formation of mono-, di-, tri-, and tetra-methyl As species (Wang et al., 2014; Zhao et al., 2013). Several factors contribute to the As methylation process under flooded conditions. First, increased diversity and activities of anaerobic microorganisms. Second, extended flooded conditions for As(III) transformation and increased substrate availability for As methylation. Third, the reductive dissolution of metal hydro(oxides) desorb already sorbed methylated As species (i.e. MMA(V) and DMA(V)) into the soil solution. Rice plants are unable to methylate inorganic As *in vivo*, therefore, methylated As species present in rice may come through microbial-mediated methylation in the rhizosphere (Lomax et al., 2012; Zhao et al., 2013).

Arsenic volatilization from paddy environments accounted for 0.9–2.6% of global As emissions into the atmosphere (Mestrot et al., 2011a). It has been estimated that paddy soils contribute 419–1252 t of As emissions annually, which is about 15–45 and 3–10 fold greater than the As emissions from sea spray and forest fires, respectively (Mestrot et al., 2011b; Zheng et al., 2013). Microorganisms are capable of volatilizing inorganic As to arsines (Fig. 4), primarily as trimethylarsines [TMAs ($(CH_3)_3As$)], with low quantities of arsine (AsH_3), monomethylarsine [$MeAsH_2$ ($(CH_3)AsH_2$)], dimethylarsine [Me_2AsH ($(CH_3)_2AsH$)] (Jia et al., 2012; Mestrot et al., 2011a). After volatilization, TMAs is rapidly oxidized by atmospheric O_2 into trimethylarsine oxide (TMAs(V)O) (Bhattacharya et al., 2007). Since As volatilization is strictly governed by microbial activities, the rate and efficiency of As volatilization can be induced through the biostimulation (promoting microbial activities) and bioaugmentation (inoculation of microbes) processes (Chen et al., 2017). Total As concentration in soil pore water showed a positive correlation with total released arsines (Jia et al., 2012; Mestrot et al., 2011a). Initial conversion of As(III) to MMA(V) determines the rate of arsines' volatilization from paddy soils. In this respect, MMA(V) and DMA(V) application into paddy soil have shown an increase in yearly arsines' volatilization rate of 3.5% (Mestrot et al., 2011a).

5. Conclusions

The distribution of As species in paddy soil-water is obviously heterogeneous in space (both laterally and vertically) and varies over time. It is clear that total As concentration in paddy soil-water does not reflect the measurement from a single point and, therefore, an accurate understanding of spatial and temporal distribution of As species is essential. The key knowledge gaps have been identified and are required to be addressed in future studies. More importantly, the consequence of natural geogenic sources (i.e. weathering of As-bearing minerals and alluvial deposits) with respect to paddy soil As loadings needs more consideration. A detailed investigation of soil mineralogy in areas under rice cultivation is essential. The following aspects are also important: (1) assessing the rate limiting factors (i.e. physico-chemical parameters) on As speciation, mobility, and bioavailability, (2) investigation of element levels for Fe, Mn, and S which are direct influences on As speciation and mobility, at paddy soil profile levels, (3) study the levels of ions and compounds (PO_4^{3-} and $Si(OH)_4$)) that compete with inorganic As species for adsorption sites, and influenced As species uptake by rice roots, (4) identify key microorganisms and their mechanisms with regards to As speciation in paddy soil-water, (5) determination of how As biotransformation kinetics are regulated by different microorganisms, (6) investigation of rice genotype variations in root plaque formation and As speciation in the rhizosphere. Moreover, perturbed environmental conditions, such as more frequent flooding, acid rain, and global warming may change the As dynamics in the paddy environment. Therefore, impacts of extreme environmental conditions on As speciation and the mobility of rice ecosystems should be further explored.

Scientific-based knowledge on As dynamics in paddy ecosystems is important to design management practices to decrease As uptake into rice grain. Since water management practices mainly change the biogeochemical processes in paddy soil-water systems, aerobic and intermittent irrigation water management practices, rather than the conventional irrigation management (long period of flooded condition and short period of non-flooded condition), may decrease As levels in paddy soil pore water. External supplementation of Fe, Mn, and S into the As-contaminated paddy environments could immobilize inorganic As species through promotion of root plaque formation, complexation, and precipitation. Similarly, incorporation of PO_4^{3-} and $Si(OH)_4$) into the As-contaminated rice fields may reduce As species uptake by rice roots. However, secondary contamination could be a major drawback related to the above amendments. Biostimulation, by providing nutrients required for microbial growth, may decrease As toxicity in paddy environments due to the enhancement of microbial-mediated processes including As oxidation, methylation, and volatilization. Breeding and introduction of rice cultivars that decrease grain As accumulation would be another option, and this area of research may prove fruitful.

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2.1.3 Concluding remarks

A multifaceted and interdisciplinary understanding is needed to access the As phyto-availability in paddy rice soils since physico-chemical and biological factors directly or indirectly involve the mobilization and speciation of As in paddy soil-water systems. The distribution of As species in the paddy soil-water varies over time and is heterogeneous both laterally and vertically. In comparison to the dryland cultivation systems, flooded cultivation of rice contains higher concentrations of inorganic As in soil pore waters. In flooded systems, poorly mobile As(V) reduces into As(III) which is highly mobile and of higher toxicity than As(V). The formation of Fe plaque on rice roots may sequestrate both As(III) and As(V) species. Manganese oxides present in the paddy soils decrease the mobility of As though oxidizing As(III) to As(V) and by complexing with As(V). The knowledge of pH and Eh, organic matter, clay mineralogy, redox sensitive ions (i.e. Fe, Mn, and S), other competing ions and compounds (PO_4^{3-} and $Si(OH)_4$), and aerobic and anaerobic microorganisms on As phyto-availability in paddy rice soils is therefore important to design measures to reduce bioavailability of As in the paddy pore water for uptake by rice plants in this research project.

2.2 Arsenic metabolism in rice plants

Article II

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2.2.1 Introduction

This review identifies the major factors that lead to high As bioavailability in paddy rice soils. The bioavailable As in the rhizosphere is acquired by rice plants. Urgent attention is therefore required to understand how rice plants acquire and metabolize both inorganic and organic As species, in order to develop mitigation measures against As accumulation in rice plants. The recent progress of the As uptake, translocation from root to shoot, and As species loading into rice grains have been discussed in detail in this review. The translocation efficiency for different As species from root to shoot has been explored with respect to mechanisms. The impact of As on the quality and quantity of rice yield were also presented to understand the detrimental effects of As in paddy rice soils. The major knowledge gaps which need to be addressed in in the field of As metabolism in rice plants have been highlighted.

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Review

Arsenic accumulation in rice (*Oryza sativa* L.) is influenced by environment and genetic factors

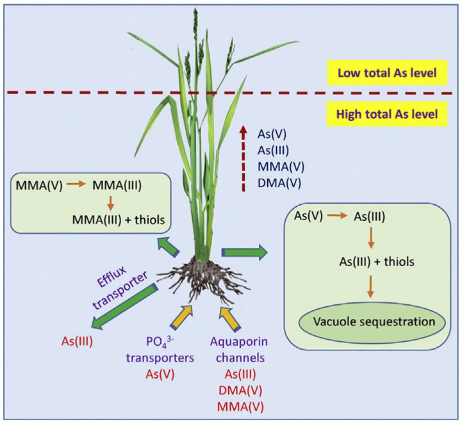
Prasanna Kumarathilaka [a], Saman Seneweera [b], Andrew Meharg [c], Jochen Bundschuh [a,d,*]

[a] *School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[b] *Center for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[c] *Queen's University Belfast, Institute for Global Food Security, David Keir Building, Malone Road, Belfast BT9 5BN, United Kingdom*
[d] *UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*

HIGHLIGHTS

- Biogeochemical factors govern As speciation in paddy soil-water systems.
- PO$_4^{3-}$ and Si transporters involve As(III), As(V), MMA(V), and DMA(V) uptake.
- As(III) efflux and complexation with thiols limit As(III) translocation.
- DMA(V) possesses the highest translocation efficiency (grain-to-root).

GRAPHICAL ABSTRACT



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ABSTRACT

Arsenic (As) elevation in paddy soils will have a negative impact on both the yield and grain quality of rice (*Oryza sativa* L.). The mechanistic understanding of As uptake, translocation, and grain filling is an important aspect to produce rice grains with low As concentrations through agronomical, physico-chemical, and breeding approaches. A range of factors (i.e. physico-chemical, biological, and environmental) govern the speciation and mobility of As in paddy soil-water systems. Major As uptake transporters in rice roots, such as phosphate and aquaglyceroporins, assimilate both inorganic (As(III) and As(V)) and organic As (DMA(V) and MMA(V)) species from the rice rhizosphere. A number of metabolic pathways (i.e. As (V) reduction, As(III) efflux, and As(III)-thiol complexation and subsequent sequestration) are likely to play a key role in determining the translocation and substantial accumulation of As species in rice tissues. The order of translocation efficiency (caryopsis-to-root) for different As species in rice plants is comprehensively evaluated as follows: DMA(V) > MMA(V) > inorganic As species. The loading patterns of both inorganic and organic As species into the rice grains are largely dependent on the genetic makeup and maturity stage of the rice plants together with environmental interactions. The knowledge of As metabolism in rice plants and how it is affected by plant genetics and environmental factors would pave the way to develop adaptive strategies to minimize the accumulation of As in rice grains.

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* Corresponding author.
E-mail address: jochen.bundschuh@usq.edu.au (J. Bundschuh).

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Contents

1. Introduction

Rice (*Oryza sativa*) consumption is a dominant dietary exposure route to arsenic (As), a non-threshold carcinogen. Rice grains can contain approximately 10-times as much as the baseline total As level when compared to other cereal grains (Williams et al., 2007). Rice and rice-based products can lead to an intake of excessive amounts of inorganic As, particularly in the populations in South and South-East Asia (Kile et al., 2007; Meharg et al., 2009). Rice is the primary source of As in a non-seafood diet in Europe and the United States (Fu et al., 2011). Ohno et al. (2007) demonstrated that the consumption of rice has contributed 56% of the total As intake in humans; the corresponding figure for drinking water, solid food, liquid food, and cereals were 13%, 11%, 4%, and 16%, respectively. Contrasting results have also been reported elsewhere suggesting that drinking water has mainly contributed to total As intake (93.5%), and the consumption of rice and vegetables has only contributed to 6.2% and 0.3% of total As intake in humans, respectively, in a groundwater contaminated Bangladeshi setting (Rahman et al., 2013).

Arsenic in the geological environment is mainly associated with sulfur (S) rich minerals such as arsenopyrite (FeAsS), realgar (As_4S_4), and orpiment (As_2S_3) (Majzlan et al., 2014). Natural processes such as rock weathering and alluvial deposits may contribute As release into the paddy environment (Bundschuh and Maity, 2015; Herath et al., 2016). Anthropogenic processes (i.e. mining activities and use of As-contaminated groundwater in the form of irrigation water) promote the accumulation of natural As in paddy ecosystems. Moreover, As-containing insecticides, herbicides, feed additives, and wood preservatives are the potential anthropogenic sources of As in rice ecosystems (W.Q. Chen et al., 2016; Zhao et al., 2009).

Natural soils can typically contain the level range of 0.1–10 mg kg^{-1} of total As (Zhao et al., 2010a). European Community (EU) recommends that soils must contain <20 mg kg^{-1} of total As to be used for agricultural purposes (Bhattacharya et al., 2009; Shrivastava et al., 2017). Arsenite (As(III)) and arsenate (As(V)) are the most commonly found inorganic As species whereas monomethylarsonoic acid (MMA(V)) and dimethylarsinic acid (DMA(V)) are the frequently reported organic As species in paddy soil-water systems (Honma et al., 2016; Jia et al., 2012). Both inorganic and organic As species in the rice rhizosphere are acquired by rice roots through the various nutrient assimilatory pathways and they are translocated via a variety of mechanisms (Ma et al., 2008; P. Wang et al., 2016).

Attention is therefore required to understand how rice plants acquire and metabolize As species so as to develop mitigation measures against this global contamination in the food chain. Taking these demands into account, this review presents recent progress in As dynamics in the rice ecosystem, and its uptake by rice roots and translocation to the rice grains. Further, mechanisms (i.e. metabolism/detoxification) that have evolved to mitigate the accumulation of As in rice tissues are discussed highlighting the major knowledge gaps that need to be addressed in future research.

2. Arsenic phytoavailability in paddy soil-water system

Paddy soils under conventional paddy management practices are subjected to flooded and non-flooded conditions, during initial and final stages of the growth of rice, respectively. The changes in water management regimes may alter the redox potential (Eh) of paddy soil-water systems (Kumarathilaka et al., 2018; Pan et al., 2014). During flooded conditions, water replaces the gaseous phase in the soil matrix. A number of redox reactions (from high Eh (~+700 mV) to low Eh (~−300 mV): reduction of Mn(IV), Fe(III), and SO_4^{2-}, and methanogenesis) take place either sequentially or simultaneously during the flooded conditions (Sahrawat, 2015). Arsenate in the soil matrix is converted to As(III), which has a higher mobility than As(V), when Eh turns more negative. The As(III)/As(V) ratio is high in the soil solution during flooded conditions and reverses for non-flooded conditions (Yamaguchi et al., 2014).

Iron (III) plaque, consisting of amorphous or crystalline Fe (hydro)oxides, was found to have a significant effect on the sequestration of both As(V) and As(III) (Eqs. (1) and (2)) (Liu et al., 2004). A higher ratio of As/Fe in Fe(III) plaque around rice roots compared to that of Fe minerals in the soil matrix indicates the high capacity of Fe(III) plaque for retention of As in the rhizosphere (Yamaguchi et al., 2014). Radial oxygen loss (ROL), the process that diffuses O_2 into the rhizosphere through root aerenchyma, can promote the formation of Fe(III) plaque even under flooded conditions (Mei et al., 2009). The root anatomy (i.e. root porosity) in different rice genotypes strongly correlates with ROL and substantial formation of Fe(III) plaque (Mei et al., 2009; C. Wu et al., 2011). In addition, the growth stage of rice plants and background Fe concentrations in paddy soils have effects on the quantities of Fe(III) plaque in rice roots (Li et al., 2015). Therefore, As retention by Fe(III) plaque and subsequent accumulation of As in rice plant tissues (i.e. root, shoot, husk, and grain) of different rice genotypes are remarkably varied (Lee et al., 2013; Liu et al., 2006).

$$\equiv FeOH_{(s)} + AsO_4^{3-}{}_{(aq)} + 3H^+{}_{(aq)} \rightarrow FeH_2AsO_{4(s)} + H_2O_{(l)} \tag{1}$$

$$\equiv FeOH_{(s)} + AsO_3^{3-}{}_{(aq)} + 3H^+{}_{(aq)} \rightarrow FeH_2AsO_{3(s)} + H_2O_{(l)} \tag{2}$$

Paddy soils consist of indigenous Fe (hydro)oxides such as ferrihydrite, goethite, lepidocrocite, and hematite. Iron redox cycling (Fe(II) oxidation and Fe(III) reduction) has a significant impact on the mobility and bioavailability of As in paddy ecosystems (Yu et al., 2016b). Iron-reducing bacteria can convert Fe(III) into Fe(II) (Eq. (3)) through two different pathways; dissimilatory reduction and assimilation reduction (Das et al., 2016; Qiao et al., 2017).

$$\mathrm{FeOAs(OH)}_{2(s)}\rightarrow \mathrm{Fe}^{2+}{}_{(aq)}+\mathrm{AsO}_3^{3-}{}_{(aq)}+2\mathrm{H}^{+}{}_{(aq)} \tag{3}$$

In contrast, Fe(II)-oxidizing bacteria may re-oxidize Fe(II) into Fe(III), particularly under non-flooded conditions. Since Fe (hydro)oxides have a strong affinity with both As(V) and As(III), they significantly reduce the mobility and bioavailability of As in paddy soil-water systems, and the subsequent uptake and accumulation of As in rice tissues (Yu et al., 2016b).

Recent studies have demonstrated that the amorphous Fe oxide-bound As can play an important role in the sinking of As (Liu et al., 2015; Yu et al., 2016a). The conversion of poorly crystalline ferrihydrite (surface area: 100–700 $m^2\ g^{-1}$) to other crystalline Fe oxides phases such as goethite (surface area: 8–200 $m^2\ g^{-1}$) and hematite (surface area: 2–115 $m^2\ g^{-1}$) may significantly decrease the sorption capacity for As due to the reduction of adsorption sites. External supplementation of Fe in the form of Fe oxides, mixed Fe sources, and Fe-rich industrial byproducts can lead to reducing As burden in rice ecosystems for different reasons (Farrow et al., 2015; Yu et al., 2017). Firstly, Fe amendments may increase the percentage of amorphous Fe fraction in paddy soils. Secondly, Fe supplementation could increase the quantity of Fe(III) plaque deposited on the root apoplast. Yu et al. (2017) assessed the effects of Fe compound supplementation on As accumulation in rice tissues throughout the whole growth stages of rice plants and found that Fe amendments can significantly reduce the accumulation of As in rice tissues at the grain-filling stage.

Nitrogen is one of the key nutrients enhancing the growth of rice plants and is supplied to paddy fields as N fertilizers (Ata-Ul-Karim et al., 2017). The process of Fe-redox cycling could be affected by nitrogen (N) cycling (i.e. nitrification, denitrification, and ammonification) (Yu et al., 2016b). The coupled NO_3^- reduction and Fe(II) oxidation may reduce the fate of As in the paddy environment (Li et al., 2012). Nitrate (NO_3^-)-dependent Fe(II)-oxidizing bacteria can promote the oxidation of Fe(II) under flooded conditions as shown in Eq. (4) (W. Sun et al., 2009). As described above, the higher the levels of Fe(III), the lower the concentration of bioavailable As in paddy soil-water systems. The reduction of Fe(III) could be inhibited by the process of NO_3^- reduction because of the competition for electrons (Yu et al., 2016b). Therefore, high levels of NO_3^- in paddy soils may decrease the reduction of Fe(III) and subsequent uptake and accumulation of As in rice tissues.

$$\mathrm{Fe}^{2+}{}_{(s)}+5\mathrm{H_2O}_{(l)}+\mathrm{NO}_3^-{}_{(aq)}\rightarrow 2\mathrm{Fe(OH)}_{3(s)}+4\mathrm{H}^{+}{}_{(aq)}+\mathrm{NO}_2^-{}_{(aq)} \tag{4}$$

Anammox-bacteria can drive the process of ammonia (NH_3) oxidation under anaerobic conditions. Ammonia oxidation is coupled with Fe(III) reduction, as shown in Eq. (5) (Shrestha et al., 2009). However, the impacts of NH_3 oxidation on the Fe-redox cycling in the paddy environment remain unclear.

$$\mathrm{NH}_4^+{}_{(aq)}+6\mathrm{FeOOH}_{(s)}+10\mathrm{H}^{+}{}_{(aq)}\rightarrow \mathrm{NO}_2^-{}_{(aq)}+6\mathrm{Fe}^{2+}{}_{(s)}+10\mathrm{H_2O}_{(l)} \tag{5}$$

Organic fertilizer, added to keep the soil fertility, may influence As dynamics in the paddy environment. Humic substances (i.e. humin, humic acid, and fulvic acid), which are the key components of organic matter, have positive and negative effects on the mobility and bioavailability of As (Jiang et al., 2009; Yu et al., 2016b). Arsenic species can be adsorbed onto the humic substances through organo-As complexes (Williams et al., 2011). In contrast, the process of adsorption of humic substances onto sorption sites in the soil matrix may increase the bioavailability of As in the paddy soil solution due to the site and electrostatic competitions (Mladenov et al., 2015). Humic substances could stimulate the microbe-driven Fe(III) reduction by facilitating electron shuttling (Newman and Kolter, 2000). As a result, As(V), sequestrated in Fe (hydro)oxides, may reduce to highly mobile As(III).

Sulfate (SO_4^{2-}) in the soil matrix is reduced to sulfide (S^{2-}) when decreasing the redox potential from −120 to −180 mV (Herath et al., 2018; Sahrawat, 2015). Sulfate-reducing bacteria can promote the reduction of SO_4^{2-} in the paddy environment (Das et al., 2016). The reduction of SO_4^{2-} into S^{2-}, particularly under flooded conditions, leads to immobilization of As(III) due to the precipitation of As(III), as arsenic sulfide and iron sulfide minerals (Burton et al., 2014). External supplementation of sulfur (S) into paddy soils may enhance the formation of low molecular weight thiol-rich peptides in rice tissues, which have a high affinity with As(III), and therefore, may reduce As translocation from root to grain (Zhang et al., 2011).

Manganese oxides (MnOH) play an important role in immobilizing As, through oxidation of As(III) into As(V), and substantial complexation of As(V) (Eqs. (6) and (7)) (Lafferty et al., 2011). X. Xu et al. (2017) demonstrated that MnOH, as a soil amendment (1200 mg kg^{-1}), has decreased the accumulation of As in rice straw and grains by 30–40%.

$$\mathrm{MnO}_{2(s)}+\mathrm{H_3AsO}_{3(aq)}+2\mathrm{H}^{+}{}_{(aq)}\rightarrow \mathrm{Mn}^{2+}{}_{(aq)}+\mathrm{H_3AsO}_{4(aq)}+\mathrm{H_2O}_{(l)} \tag{6}$$

$$2\mathrm{Mn{-}OH}_{(s)}+\mathrm{H_3AsO}_{4(aq)}\rightarrow \mathrm{(MnO)_2AsOOH}_{(s)}+2\mathrm{H_2O}_{(l)} \tag{7}$$

Microorganisms inhabiting the rhizosphere can influence the speciation of As through different biotransformation pathways: oxidation, reduction, methylation, and volatilization (Jia et al., 2012). A variety of genes in microorganisms encode for As-transformation enzymes and transporters. The process of microbial oxidation of As(III), coded for by *aioA* gene, can lead to producing As(V) (Zhang et al., 2015). In contrast, microbial As(V) reduction, coded for by *arsC* and *arrA* genes, may increase the mobility of As in the paddy environment (Malasarn et al., 2004; Qiao et al., 2017; Villegas-Torres et al., 2011). Inorganic As species in paddy ecosystems can be transformed into organic As species such as MMA(V), monomethylarsonous acid (MMA(III)), DMA(V), and dimethylarsinous acid (DMA(III)), and the process is coded for by the *arsM* gene. For this reason, organic As species found in paddy soil-water are likely to be a microbe-mediated product. Microorganisms can volatilize organic As to arsines, primarily as trimethylarsines ($(CH_3)_3As$), with a smaller quantity of arsine (AsH_3), monomethylarsine (CH_3AsH_2) and dimethylarsine ($(CH_3)_2AsH$) (Hayat et al., 2017; Jia et al., 2012). As a result, microbe-driven As transformation may decrease the phytoavailability of As in paddy soil-water systems. Moreover, microorganisms can indirectly affect the speciation and mobility of As by influencing Fe, N, and S cycling, as discussed above. Recent findings have further revealed that soil amendments such as biochar may significantly affect the abundance and diversity of microorganisms, and the consequent transformation of As in the paddy environment (Qiao et al., 2017; Qiao et al., 2018).

3. Arsenic metabolism in rice plants

Elucidating arsenic metabolism in different rice tissues is crucial for understanding the uptake of inorganic and organic As species, their translocation, and grain filling. There are different genes involving in As metabolism including reduction, efflux, transformation, complexation, and sequestration (Table 1). Fig. 1 illustrates the respective genes associated with the uptake, translocation, and grain filling of both inorganic and organic As species in the rice plant.

3.1. Uptake of inorganic arsenic species

3.1.1. Arsenite

Arsenite, the most prevalent As species under flooded conditions, is acquired dominantly through nodulin 26-like intrinsic proteins (NIPs), collectively termed as aquaporin channels. In rice roots, OsNIP2;1 (Lsi1), which is highly expressed in the distal side of the plasma membranes of the exodermis and endodermis cells where Casparian strips exist, governs the influx of both silicic acid ($Si(OH)_4$) and As(III) (as arsenous acid, $As(OH)_3$) into rice roots (Fleck et al., 2013; Ma et al., 2008). This could be attributed to the similar sizes of both $Si(OH)_4$ and $As(OH)_3$ with tetrahedral orientation along with more or less similar dissociation constants (pK_a) (9.2 and 9.3, respectively). Ma et al. (2008) demonstrated that NIP genes (OsNIP1;1 and OsNIP3;1) in oocytes mediate As(III) uptake but the $Si(OH)_4$ pathway was unaffected. Rice OsNIP3;3 and OsNIP3;2 are highly expressed in the lateral roots and stele region of the primary roots suggesting that As(III) is taken up by the highly active part of the root system (Chen et al., 2017b; Katsuhara et al., 2014). In addition, plasma membrane intrinsic proteins (PIPs) such as OsPIP2;4, OsPIP2;6 and OsPIP2;7 are associated with the uptake of As(III) (Mosa et al., 2012), but their functional role is not well studied.

Aquaporin channel OsNIP2;2 (Lsi2), being a $Si(OH)_4$ efflux transporter, localized on the plasma membrane of cells in both exodermis and endodermis of rice roots; however, contrary to Lsi1, Lsi2 is localized on the proximal side of the same cells. Preloading of the *Xenopus oocytes* with Si resulted in a release of Si back into the external environment, demonstrating that Lsi2 is a Si efflux transporter (Ma et al., 2007). Having an influx transporter on one side and an efflux transporter on the other side of the cell, As(III) could not pass freely towards the stele (Zhao et al., 2010b). In addition to Lsi2, As(III) efflux transporters are found in other organisms. For instance, Arsenic Compounds Resistance protein 3 (ACR3) in yeast extrudes As(III), however, ACR3 does not exist in flowering plants like rice. Meng et al. (2011) found that the introduction of ACR3 (ScACR3) into rice plant decreased the total As concentrations in rice grains by nearly 20% suggesting that the transgenic rice plant extruded As(III) back into the external environment. In this aspect, As(III) efflux is a key mechanism which releases a portion of As(III) back into the external environment, minimizing cellular As accumulation.

In some plant species, As(III) uptake is a bi-directional process; the direction is determined by the concentration gradient. For instance, aquaporins from different plant species such as *Pteris vittata* (PvTIP4;1), *Lotus japonicas* (LjNIP5;1 and LjNIP6;1), and *Arabidopsis thaliana* (AtNIP1;1, AtNIP1;2, AtNIP3;1, AtNIP5;1, AtNIP6;1, and AtNIP7;1) are known to be associated with As(III) efflux back into the external environment (Bienert et al., 2008; He et al., 2016; Kamiya and Fujiwara, 2009; Xu et al., 2015). In this sense, a transgenic approach (i.e. overexpression of As(III) efflux transporters) is likely to mitigate the accumulation of As in rice grains.

Following uptake, besides the As(III) efflux, As(III) has a high affinity for thiol-rich peptides such as glutathione (GSH) and phytochelatins (PCs) that are derived from GSH with the general structure of (γ-Glu-Cys)n-Gly (n = 2–11). Overexpression of the PCs leads to increased levels of As in roots, but a remarkably low level of As in rice grains in transgenic rice plants (Shri et al., 2014). After being formed, As(III)-PC complexes are transported into vacuoles for the sequestration by C-type ATP-binding cassette transporter (OsABCC1) which is expressed in the exodermis and pericle. Similarly, it has been reported that AtABCC1 and AtABCC2 in *Arabidopsis thaliana* mediate the transportation of As(III)-PC complexes into the vacuole (Song et al., 2010). The favorable pH (approximately 5.5) in vacuoles may provide an ideal condition for the stability of As(III)-PC complexes (Zhao et al., 2009). Therefore, vacuole sequestration of As(III)-PC complexes plays an important role in alleviating the translocation of As(III) into the rice grain.

Gene expression associated with As(III) assimilation (As(III) influx and efflux) varies largely with the developmental stages of the rice plants and with the type of cultivar. A recent study by Wu et al. (2017) demonstrated that the expression of Lsi1 and Lsi2 genes was significantly down-regulated under non-flooded conditions compared to flooded conditions. Lsi1 and Lsi2 genes are strongly associated with As assimilation and it might be one of the major factors that suppress the accumulation of As under non-flooded conditions. It has been found that a mutation in Lsi1 remarkably reduced the uptake and accumulation of As(III) in rice shoots and grains (Ma et al., 2008). Similarly, Guo et al. (2009) demonstrated that a Si-defective mutant in rice plants showed a decrease in As(III) uptake. Therefore, understanding the temporal distribution of As species and the expression of genes across As efficient and inefficient cultivars is paramount for developing mitigation measures of As in rice plants.

Rice plants are an efficient Si accumulator and can contain Si in shoots at about 10% of the shoot dry weight (Meharg and Meharg, 2015). In this regard, supplementation of Si fertilizers into As-contaminated paddy soils may provide an effective strategy to minimize As(III) uptake by rice roots (Guo et al., 2007; Seyfferth and Fendorf, 2012). It has been reported that Si application into the paddy soil decreased the total As levels by 33% in rice grains (Fleck et al., 2013). A similar study by H.Y. Wang et al. (2016) showed that high Si application rates (9000 kg ha^{-1}) decreased the total As levels in rice grains by 20%.

Table 1
Key representative genes involved in As uptake, transport, and metabolism in rice plants.

Function	Gene category	Gene name	Reference
As(V) uptake by root, loading into the xylem, phloem, and grain	Phosphate transporters	OsPHT1;8 (OsPT8)	P. Wang et al. (2016)
		OsPHT1;1	Kamiya et al. (2013)
As(III) uptake by root	Nodulin 26-like intrinsic proteins (NIPs)	OsNIP2;1 (Lsi1)	Ma et al. (2008)
		OsNIP2;2 (Lsi2)	
		OsNIP1;1	
		OsNIP3;1	
		OsNIP3;2	
		OsNIP3;3	
	Plasma membrane intrinsic proteins (PIPs)	OsPIP2;4	Chen et al. (2017a)
		OsPIP2;6	Katsuhara et al. (2014)
		OsPIP2;7	Mosa et al. (2012)
DMA(V) and MMA(V) uptake by root	Nodulin 26-like intrinsic proteins (NIPs)	OsNIP2;1 (Lsi1)	Li et al. (2009)
As(V) reduction to As(III) in root	As(V) reductase	OsHAC1;1	Shi et al. (2016)
		OsHAC1;2	
		OsHAC4	J. Xu et al. (2017)
As(III) efflux from root to the external environment	Nodulin 26-like intrinsic proteins (NIPs)	OsNIP2;2 (Lsi2)	J. Xu et al. (2017)
As(III) complexation in root and phloem	ATP-binding cassette transporter	OsABCC1	Song et al. (2014)
DMA(V) loading into the xylem, phloem, leaves, and grain	Putative peptide transporter	OsPTR7	Tang et al. (2017)
As(III) loading into the xylem	Natural Resistance-Associated Macrophage Protein (NRAMP) transporter	OsNRAMP1	Tiwari et al. (2014)

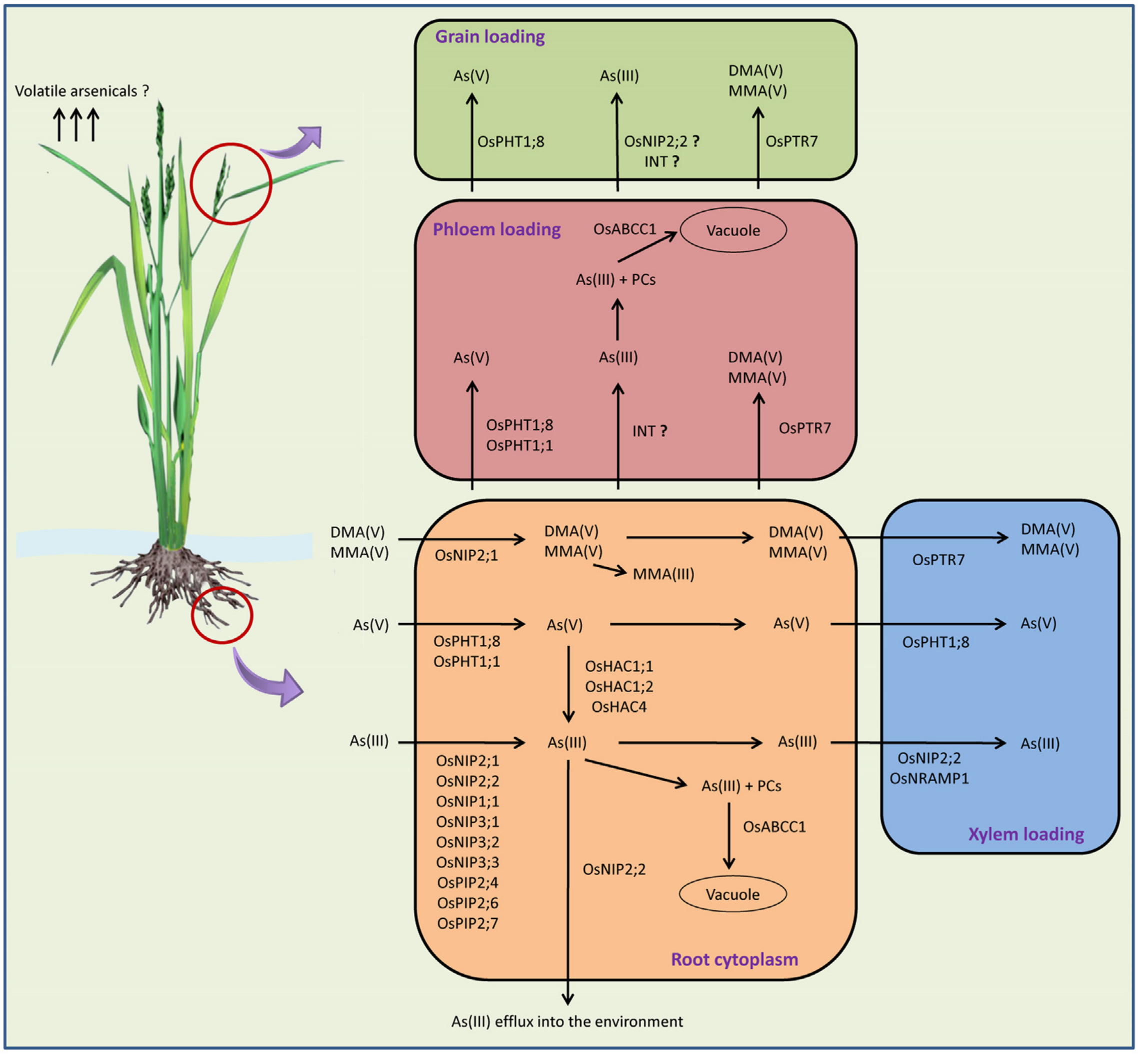

Fig. 1. Genes involved with inorganic and methylated As species uptake, transformation, and localization in rice plant.

Thus, Si application into As-contaminated paddy soils is beneficial for rice plants, partly because of the decreased uptake of As(III) by rice roots, and partly because of the increased plant resistance to biotic and abiotic stresses.

3.1.2. Arsenate

Arsenate is the most prevalent As species under non-flooded conditions. Physiological and electrophysiological studies have revealed that As(V) and PO_4^{3-} shared the same uptake pathway (Muehe et al., 2014). In the rice genome, 13 PO_4^{3-} transporter genes (OsPHTs) have been identified; however, their response to As(V) and contribution to As(V) uptake in rice plants has not been studied in detail yet.

Phosphate transporter, OsPHT1;8 (OsPT8), was found to have a high affinity for both PO_4^{3-} and As(V) uptake in rice plants. The overexpression of OsPT8 has increased the maximum influx of As(V) by 3–5 times in rice plants (P. Wang et al., 2016; Z. Wu et al., 2011). The PO_4^{3-} transporter gene, OsPHT1;1, also mediates the uptake of As(V) into the rice roots (Kamiya et al., 2013). Other plant species also contain PO_4^{3-} transporters which mediate the uptake of As(V). For example, PO_4^{3-} transporters such as PvPht1;3 in *Pteris vittata* and AtPHT1;1, AtPHT1;4, AtPHT1;5, AtPHT1;7, AtPHT1;8, and AtPHT1;9 from *Arabidopsis thaliana* acquire As(V) from the rhizosphere (DiTusa et al., 2016; Fontenot et al., 2015; Shin et al., 2004).

Following uptake, As(V) is readily reduced to As(III) in rice roots. As(V) reductase enzymes play a key role in controlling the conversion of As(V) into As(III) (Xu et al., 2007). Efficient As(V) reduction takes place in all tissues of the root apex which was supported by fluorescence-X-ray absorption near-edge spectroscopy (XANES) analysis (Batista et al., 2014). Recently, Shi et al. (2016) identified two As(V) reductases, OsHAC1;1 (abundant in the epidermis, root hairs, and pericycle cells) and OsHAC1;2 (abundant in the epidermis, outer layers of cortex, and endodermis cells) which play an important role in the reduction of As(V) into As(III) in rice roots.

Interestingly, As(III) is found in considerable concentrations in plant roots exposed to As(V). However, after the reduction of As(V), As(III) can be released back into the external environment via As(III) efflux system or can be complexed with thiol compounds as mentioned earlier. A new As(V) reductase, OsHAC4, has been identified and expressed highly in the epidermis and exodermis of rice roots, a location which is ideal for the efflux of As(III) into the external environment (J. Xu et al., 2017). Similar As(V) reductases, namely As(V) tolerance QTL1 (ATQ1) and High Arsenic Content 1 (HAC1), were identified in *Arabidopsis thaliana* (Chao et al., 2014). Therefore, reduction of As(V) into As(III) and the subsequent As(III) efflux or As(III)-thiol complexation in rice roots are likely to play major roles in determining the levels of total As in rice grains.

Since As(V) is an analogue for PO_4^{3-} in the plant uptake process, the application of external PO_4^{3-} into As-contaminated paddy soils could alleviate the accumulation of As(V) in rice tissues (Lihong and Guilan, 2009). Studies have clearly demonstrated that the supplementation of PO_4^{3-} fertilizers into As-contaminated paddy soils has largely decreased the total As concentrations in rice grains (Geng et al., 2005). An excess supplementation of PO_4^{3-} fertilizers may lead to eutrophication in surface water bodies. Therefore, a close monitoring must be carried out under excess usage of PO_4^{3-} fertilizers.

3.2. Uptake of organic As species

Rice plants are not capable of methylating inorganic As in vivo. Therefore, organic As species (DMA(V) and MMA(V)) in rice plants may come from the rhizosphere in which microorganisms mediate the methylation processes (Jia et al., 2012; Zhao et al., 2013a). The exact mechanisms that drive the uptake of organic As species in rice plants are largely unknown. It has been suggested that aquaporin Lsi1 mediates the uptake of MMA(V) and DMA(V) in rice plants (Li et al., 2009). The pH of the medium has a great effect on the uptake of MMA (V) and DMA(V). More precisely, pH can alter the equilibrium between protonation and dissociation that eventually influences the availability of MMA(V) and DMA(V) for the membrane transporter, Lsi1 (Li et al., 2009). Therefore, Lsi1 plays a key role in acquiring both inorganic As (III) and organic As species from the rhizosphere.

There is no detailed report on the metabolism of organic As species in rice roots. A recent study by Mishra et al. (2017) revealed that MMA(V) is readily reduced to MMA(III) which is then bound with thiols in rice roots. In addition, MMA(V) itself can complex with various thiol groups in rice plants. Mishra et al. (2017) found that 16 different MMA (V)-thiol complexes were present in the rice roots and shoots. However, DMA(V) is known to neither complex with thiols or convert into the reduced forms. The direct determination of organic As species - thiol complexation in various rice genotypes is an important step for understanding As metabolism in rice plants.

3.3. Arsenic species translocation from root to shoot

It is likely that both active and passive transporters are associated with the uptake and translocation of As species in rice plants. Active transportation is the process of passing materials against a concentration gradient by using the energy of metabolism (Liang et al., 2006). Passive transport is the movement of water and materials without using the energy of metabolism and mainly transport through transpiration flow. For example, a large number of nutrients such as potassium (K), nitrogen (N) and Si, are transported through both active and passive processes in rice plants (Bao et al., 2015; Yang et al., 2014). Following uptake by rice roots, various transporters and compounds are involved in the translocation of As species from root to shoot.

The translocation of inorganic As species from root to shoot is dependent on various processes. Lsi2 mediates the efflux of uncomplexed As (III) in the direction of xylem. The Natural Resistance-Associated Macrophage Protein (NRAMP) transporter, OsNRAMP1, may also facilitate the xylem loading of As(III) (Tiwari et al., 2014). Different metabolic activities such as As(III) efflux back into the external environment and the formation of As(III)-thiol complexes and consequent sequestration in vacuoles, limit the translocation of As(III) from root to shoot. Studies have revealed that rice cultivars with low levels of total As in their grains had remarkably higher concentrations of PCs in roots compared to the cultivars with high total As levels in their grains (Duan et al., 2011). In contrast, Batista et al. (2014) found that PCs produced in rice cultivars in response to the elevated levels of As exposure did not necessarily reduced the total As concentrations in rice grains.

The translocation efficiency of organic As species from root to shoot in rice plants is much higher than that of inorganic As species (Raab et al., 2007). It could be attributed to the favorable dissociation of MMA(V) and DMA(V) at the cytoplasmic pH. Since MMA(V) is partly reduced to MMA(III), only untransformed MMA(V) is transported to the shoots (Li et al., 2009). In addition, the lack of DMA(V)-PC complexation enhances DMA(V) to be readily moved between root and shoot (Zhao et al., 2013b). The putative peptide transporter in rice, OsPTR7, involves in the long-distance (root to grain) translocation of organic As species in rice. The OsPTR7 transcript is more prevalent in leaves, nodes, and roots at the flowering and grain filling stages; however, during the seedling stage, OsPTR7 is more abundant in shoots than in roots (Tang et al., 2017).

There have been no reports of volatile As species released by rice plants under the typical rice growing conditions. However, a large amount of volatile As is releasing from paddy soils due to microbial activities. The volatilization of $(CH_3)_3As$ from rice plants grown in an axenic system was detected when rice plants were treated with trimethylarsine oxide ($(CH_3)_3AsO$) but not with other inorganic or organic As species (Jia et al., 2012). A soil bacterium, *Rhodopseudomonas palustris*, is found to have a direct influence on As volatilization. When an *arsM* gene from *Rhodopseudomonas palustris* was expressed in rice, transgenic rice produced volatile arsenicals (i.e. $(CH_3)_3As$) which were 10 times greater than that of the control (Meng et al., 2011). Therefore, producing transgenic rice plants with the *arsM* gene could mitigate the As burden in rice plants by achieving As volatilization out of the rice crops.

3.4. Phloem and xylem-derived pathways of As species transport

A recent study by Ye et al. (2017) revealed that As(III) was the prevalent As species (69%) in the phloem exudates of As(III)-exposed rice, whereas As(V) dominated (58%) in As(V)-exposed rice and the remainder was As(III). When rice plants were treated with MMA(V) and DMA (V), the major As species in phloem exudates were 55% and 59% MMA (V) and DMA(V), respectively. Interestingly, a large proportion of As (V) (45% and 41%, respectively) was detected in the phloem exudates when the rice plant was treated with MMA(V) and DMA(V). This observation could correspond to the demethylation process, however, the mechanisms underlying demethylation remain unclear.

Phloem transportation is likely to be responsible for 54% As(III), 56% As(V), 100% MMA(V), and 89% DMA(V) transportation into the rice grains, respectively. In the phloem, organic As species are more mobile than that of inorganic As species as described by Ye et al. (2017). This finding is similar to those of the study reported by Carey et al. (2010). However, Carey et al. (2010) accounted the phloem transport to be responsible for 90% and 55% of transportation of As(III) and DMA(V) to the grain, respectively. The difference in the above two studies could be attributed to the experimental methods used. Stem girding, which blocks phloem transportation, has reduced grain ^{73}As radioactivity by 97% compared to the ungirdled control (Zhao et al., 2012). Therefore, it can be suggested that phloem acts as the primary route for both inorganic and organic As species transportation into rice grains. Transporters responsible for As species loading into the vascular tissues in rice plants need to be investigated in detail. Inositol transporters (INT) such as AtINT2 and AtINT4 are associated with phloem loading of As (III) in *Arabidopsis thaliana* (Duan et al., 2016). Similar transporters might be responsible for the phloem loading of As(III) in rice plants.

Synchrotron μX-ray fluorescence (μ-XRF) mapping has indicated that As(III) was sequestrated in the phloem, top node, and internode, limiting As(III) loading into the rice grains. The OsABCC transporter, which is expressed in the tonoplast of phloem in nodes, promotes the transportation of As(III)-PC complexes into vacuoles (Song et al., 2014). The knockout mutant of OsABCC has decreased the levels of total As in nodes but has increased the levels of total As in grains. Therefore, it is likely that rice plants have evolved a mechanism to minimize As loading into their grains.

There are only a few reports discussing the transportation of As species through xylem in rice plants. Arsenic(III) is the prevalent form of As present in the xylem of *Pteris vittata* and *Cucumis sativus* (Mihucz et al., 2005; Su et al., 2008). The predominant As species in the xylem sap of rice varies with water management practices. Ye et al. (2015) found that As(V), accounting for 64–88%, was the dominant form of As in the xylem sap of rice plants under aerobic treatment. In contrast, in the flooded treatment, the prevalent species in the xylem sap in rice was As(III), accounting for 26–77%, followed by As(V) (12–54%) and DMA (V) (11–20%) (Ye et al., 2015). A highly expressed Si pathway in rice plants may cause more loading of As(III) into the xylem, in particular, under the flooded cultivation of rice.

3.5. Arsenic species loading into the grain

Arsenic species loading into rice grain is particularly important since it leads to the primary pathway of As exposure in humans. From the available data, it is clear that there are significant site and genotype interactions on the distribution and concentration of As species in rice grains (Norton et al., 2009; Sommella et al., 2013). DMA (V) translocates from root to rice grain more efficiently (approximately 10-fold greater) compared to inorganic As species (Carey et al., 2010). As mentioned earlier, lack of complexation of DMA(V) may promote the efficient movement of DMA(V) in rice plants. The re-translocation of DMA(V) and MMA(V) from the flag leaf into the filling grain is more efficient than that of As(V) and As(III), which are not detectable even when leaves were fed with As(III). The formation and subsequent sequestration of As(III)-PC complexes in rice leaves may restrict the As (III) from loading into the phloem and subsequent translocation to the rice grains (Zheng et al., 2011).

Synchrotron analysis has revealed a marked difference in the pattern of inorganic and organic As species loading into the grain. As(III) is mostly retained in the ovular vascular trace (OVT) region, the vascular entry point into the rice grain and comprises of phloem and xylem cells, whereas DMA(V) disperses throughout the outer layers and into the endosperm (Carey et al., 2010). It is suggested that DMA(V) in rice grains is derived from the re-translocation of DMA(V) accumulated before flowering. In contrast, inorganic As species mainly transport to rice grains during flowering. In this respect, DMA(V) may accumulate at considerable concentrations in rice grains during the initial stage of grain filling and later is diluted by carbohydrate filling to the grain (Zheng et al., 2011). Relatively higher As concentrations in bran than in polished grain could be due to following reasons: firstly, the bran acts as a physiological barrier for As translocation into the rice grain; secondly, As is likely to be concentrated in the protein-rich tissues including embryo and aleurone layer. So far, none of the studies have shown any effort towards the quantitative determination of As species in different layers of the rice grain under different growing stages of rice plants, water management regimes, and with supplementation of nutrients and amendments in As-contaminated paddy environments.

3.6. Translocation efficiency of arsenic species

The highest translocation efficiency (caryopsis-to-root) in rice plants was reported for DMA(V) followed by MMA(V) and inorganic As species (Geng et al., 2017; Jia et al., 2012). The order of translocation efficiency differs in various parts of the rice tissues. Dittmar et al. (2010) showed that the straw to grain transfer factor declined exponentially with increasing total As concentrations in straws and eventually reached a constant value (~0.04). High yielding rice cultivars tend to have a high efficiency for As translocation than rice cultivars with a low yielding capacity (Bhattacharya et al., 2010; Zheng et al., 2011). Regardless of the location and season, total As concentrations in rice tissues decreased in the following order: root > shoot > straw > husk > grain (Tables 2 and 3).

Agronomic practices greatly influence the speciation and accumulation of As in rice grains. The dynamics of total As in rice grains under both flooded and non-flooded conditions revealed that total As concentrations in rice grains were 10–15 times greater in rice grown under flooded conditions than in rice grown under non-flooded conditions (Xu et al., 2008). This is consistent with the study by Spanu et al. (2012) who showed that intermittent irrigation has produced rice grains with 2.8 μg kg^{-1} of total As whereas the corresponding figure for the continuous flooding irrigation was 163 μg kg^{-1}. Correlation analysis revealed that a significant positive correlation existed in total As concentrations between soil pore water and rice grains. Similarly, studies have indicated that an increased level of soil As lead to a high level of total As in rice straw and grains (Dittmar et al., 2010; Hossain et al., 2008). However, contradicting results have also been reported and these could be due to various factors including irrigation intensity, underlying geology, weather conditions, fertilizer applications, and diseases.

4. Arsenic risk to rice plant and humans

4.1. Impact on yield

Straighthead disease, in which the panicles remain upright due to lack of grain filling and sterility, is most common in rice due to a high level of As exposure (Li et al., 2016). Both chlorophyll *a* and b levels in rice leaves tend to decrease under As exposure and this may adversely affect the photosynthesis (Rahman et al., 2007a). Increased levels of As(V) in paddy soil pore water negatively affects plant growth parameters including plant height, grain yield, number of filled grains, and root biomass. Further, accumulation of As(V) and As(III) enhances the production of reactive oxygen species (ROS) which may damage the macromolecules and cell membranes (Finnegan and Chen, 2012). A recent study by J. Xu et al. (2017) demonstrated that As(III) binds with reduced cysteine residues in proteins, and hence, influences the catalytic functions. Moreover, the preferential translocation of DMA(V) into filial tissues such as embryo, endosperm, and aleurone causes a marked reduction in seed setting rate (i.e. spikelet sterility) and loss of yield (Wang et al., 2015). Rice cultivation in an environment with minimal As levels or As-tolerance rice cultivars is, therefore, an important aspect of achieving the goal of sustainable production of rice in terms of quality and quantity.

4.2. Contribution of rice to arsenic intake in humans

Rice and rice-based products with high As concentrations may increase the health risk for humans (Sandhi et al., 2017; Signes-Pastor et al., 2016). The levels of total As and As species in rice grains are dependent on the rice genotype, physico-chemical, and environmental interactions as summarized in Table 4. Arsenic-containing rice husks are used as cattle feed in several countries (i.e. Bangladesh and China) and may act as an indirect pathway of As exposure to humans through the food chain (Abedin et al., 2002). In addition, As in rice grains leads to loss of trace mineral nutrition such as Zn, Se, Ni and amino acid content (Dwivedi et al., 2010; Williams et al., 2009a). High levels of As in rice brans increases the health risk, particularly for children, and people on gluten-free products (Abedin et al., 2002; Althobiti et al., 2018). Removing the bran layer during rice processing is an effective way to eliminate inorganic As. However, from a nutritional point of view, removal of the bran results in a substantial loss of nutrients in rice grains.

There have been many studies focusing on the risk assessment of human exposure to As. For example, the lifetime cancer risk in West Bengal, India was found to be 7.62×10^{-4} which exceeds the United States Environmental Protection Agency (USEPA) threshold value range of 10^{-4}–10^{-6} (Mondal and Polya, 2008). Dittmar et al. (2010) predicted that under current irrigation practices in Bangladesh, total As concentrations in rice grains would increase from ~0.15 to 0.25–0.58 mg kg^{-1} by 2050. This figure subsequently increases the human As intake by 1.5–3.8 fold through rice consumption. Therefore,

Table 2
Arsenic concentration in irrigation water, soil and different plant tissues worldwide from field experiments.

Country	Rice variety	Total As in irrigation water (μg L^{-1})	Total As (mg kg^{-1})					References
			Soil	Root	Straw	Husk	Grain	
Bangladesh	BR29	–	~15	–	1.7 (0.3–8.5)	–	0.17 (0.08–0.43)	Dittmar et al. (2010)
	–	68–136	11.41–61.04	–	0.571–1.916	0.380–0.995	0.182–0.436	Hossain et al. (2008)
	Boro and Aman	–	–	–	2.64–12.52	1.20–2.48	0.22–0.81	Khan et al. (2010a)
	–	5.1–77.4	9.8–13	–	2.1	–	0.23	Rahman et al. (2010)
	BRRI dhan28	70	14.5	46.3	1.7	–	0.6	Rahman et al. (2007b)
	BRRI hybrid dhan 1	–	–	51.9	1.9	–	0.7	
Cambodia	–	–	0.8–18	–	–	–	0.2 (0.1–0.37)	Seyfferth et al. (2014)
China	Bollyou15	–	3 (0.1–45.9)	–	–	–	0.092 (0.005–0.309)	Fu et al. (2011)
	–	–	6–253	–	–	4.07	0.22	Williams et al. (2009b)
	–	–		–	–	–	0.62	Zhu et al. (2008)
	–	–	7.32 (2.5–19.2)	–	–	–	0.09 (0.02–0.18)	Jiang et al. (2014)
	–	110	17.9	98.9	3.9	1.2	0.7	Chou et al. (2014)
	F299	–	64.44 (52.49–83.86)	196.27	10.08	2.01	0.43	Lei et al. (2013)
India	Red Minikit	50–70	1.34–14.09	9.10–22.71	1.35–2.08	0.49–1.11	0.17–0.57	Bhattacharya et al. (2010)
	Megi	–	–	6.18–19.44	0.55–1.73	0.27–0.87	0.11–0.43	
	–	–	19.4–41.24	–	–	–	1.6	Shrivastava et al. (2017)
	CNI1643–3	–	12.43	155	–	–	–	Dwivedi et al. (2010)
	IR68144–127			–	–	–	1.7	
	–	5.33–17.27	1.09–2.48	–	0.004–0.015	–	0.001–0.006	Vicky-Singh et al. (2010)
France	–	–	5–10	–	10.2 (1.5–20.6)	–	0.32 (0.12–0.61)	Williams et al. (2007)
Japan	Kirara 397	–	3[a]	–	–	–	0.121	Kuramata et al. (2011)
	Koshihikari			–	–	–	0.166	
Span	–	–	4–11	–	3.3 (0.8–9.8)	–	0.16 (0.06–0.29)	Williams et al. (2007)
Taiwan	Taigon 2	25–67	11.8–112	20.3–188	0.70–5.79	1.05–4.31	0.29–0.66	Hsu et al. (2012)
USA	–	–	2-4	–	0.7 (0.4–1.3)	–	0.13 (0.08–0.18)	Williams et al. (2007)

The range of total As concentrations is shown in parenthesis.

[a] As concentration in soil solution.

Table 3
Arsenic speciation in different rice tissues from glasshouse experiments.

Rice genotype	Type of plant tissue	Total As (mg kg^{-1})	As species (mg kg^{-1})				Reference
			As(III)	As(V)	DMA(V)	MMA(V)	
Jiahua	Root	–	83.9	105.2	2.6	0.22	Jia et al. (2012)
	Shoot		4.39	1.29	0.42	0.008	
29 Japonica subspecies and 8 indica subspecies	Grain	0.163	–	–	–	–	Spanu et al. (2012)
–	Straw	0.7–13.4	–	–	–	–	Bogdan and Schenk (2008)
C039	Straw	25.77	–	–	–	–	Mei et al. (2009)
	Grain	2.72					
Guanglua	Straw	25.13	–	–	–	–	
	Grain	2.28	–	–	–	–	
Azucena	Root	–	–	13	4.6	7.1	Raab et al. (2007)
	Shoot	–	–	2.5	4.0	1.1	
Bala	Root	–	–	26	8.9	18	
	Shoot			0.2	1.0	1.1	
BR11	Root	107.5	–	–	–	–	Abedin et al. (2002)
	Straw	91.5	–	–	–	–	
T116	Root	–	4.25	0.55	0.16	1.95	Geng et al. (2017)
	Stem	–	0.70	0.56	1.08	1.30	
	Grain	–	0.83	–	0.98	0.42	
Y2	Root	–	1.34	0.09	0.16	1.96	
	Stem	–	0.75	0.23	0.85	0.30	
	Grain	–	0.83	2.18	–	–	
Quest	Root	–	57–78[a]	16–27[a]	–	–	Smith et al. (2008)
	Shoot	–	32–63[a]	14–28[a]	–	–	
	Grain	–	1–6[a]	–	85–94[a]	–	
6 different genotypes	Grain	–	19.8–54.4[a]	–	45.6–80.2[a]	–	Syu et al. (2015)
Shatabdi	Grain	–	33–77[a,b]		23–67[a]	–	Khan et al. (2010b)
M-206	Bran	–	–	69–88[a]	12–31[a]	–	Seyfferth et al. (2011)
	Germ	–	20[a]	80[a]	–	–	

[a] Values are as percentages.

[b] Sum of the As(III) and As(V).

Table 4
Arsenic speciation in market-based rice in different countries.

Country	Rice product	Number of samples	As species (μg kg^{-1})				Total As (μg kg^{-1})	Reference
			As(III)	As(V)	DMA(V)	MMA(V)		
Bangladesh	–	29	129 (3–402)	66 (7–570)	14 (0–53)	2 (0–3)	283 (28–961)	Halder et al. (2014)
China	–	43	107.1 (45–198)	4.7 (0–23.8)	9.6 (1.4–30.6)	0.3 (0–2.8)	129.4 (50–253)	Ma et al. (2016)
Italy	–	61	91	8.2	55	–	162	Tenni et al. (2017)
Taiwan	White rice	51	61.6	4.3	12.1	2.7	116.6	H.L. Chen et al. (2016)
Australia	Brown rice (whole, medium grain)	3	178^{a} (156–200)		68 (65–71)	<dl	287 (290–284)	Rahman et al. (2014)
USA	White long grain	7	98^{a} (62–134)		157 (85–229)	<dl	264 (85–344)	Heitkemper et al. (2009)
South Korea	Brown rice	30	80	5	30	2	–	Kim et al. (2013)
Nigeria	–	23	47^{a}		11.5	0.33	58.8	Adeyemi et al. (2017)
Thailand	White rice	79	81.44 (14–154)	3.74 (<2–8)	29 (2–86)	<2 (<2–6)	139.48 (23–304)	Nookabkaew et al. (2013)
India	Red rice	–	50^{a}		10	<dl	80	Williams et al. (2005)
Japan	Rice noodles	6	120^{a}		20^{b}		190	G.X. Sun et al. (2009)
Japan	Rice crackers	11	210^{a}		70^{b}		390	G.X. Sun et al. (2009)
UK	Rice cereals	30	75^{a} (8–188)		45 (13–82)	1 (1–4)	–	Signes-Pastor et al. (2016)
UK	Baby rice	–	60^{a}		30	–	120	Meharg et al. (2008)
Spain	Gluten-free infant rice	13	29–121^{a}		10–198	–	46–315	Carbonell-Barrachina et al. (2012)
USA	Rice cereals	–	66	72	100	–	230	Juskelis et al. (2013)

dl - below the detection limit.
The range of concentration for respective As species is shown in parenthesis.
[a] Sum of As(III) and As(V).
[b] Sum of DMA(V) and MMA(V).

precautionary measures must be undertaken to control the intake of As through rice consumption considering special attention to regions prone to As contamination.

5. Concluding remarks

Arsenic dynamics in paddy soil-water systems and rice grains are a function of geochemical, microbial, and plant genetic factors. The knowledge gained with respect to speciation, mobility, and bioavailability of As in paddy soil-water systems, and subsequent uptake, efflux, translocation, and sequestration of As in rice plants is an important aspect to develop mitigation measures against the As issue. Rice production must increase to meet the demand for ever-growing populations. Therefore, there is an urgent need to adapt As mitigation measures in As-prone areas around the world. Firstly, the mobility and bioavailability of As in paddy-soil water systems could be decreased by using agronomical, physico-chemical, and biological approaches. Alternative water management practices may play an important role in reducing As mobility and bioavailability in the paddy environment. Supplementation of fertilizer and soil amendments can lead to reducing the bioavailability of As in the soil solution. The optimum supplementation rates for fertilizer and soil amendments are required to be examined before being introduced into As-contaminated paddy lands. Microorganisms are influencing the mobility and bioavailability of As in paddy soil-water systems through direct and indirect pathways. The kinetics of microbe-driven As biotransformations and related rate limiting factors need to be examined with respect to different As-contaminated localities. Secondly, uptake and translocation of As species in rice plants could be decreased by altering the expressions of transporter genes associated with As uptake, efflux, translocation, and sequestration. It is important to study whether changes in expression of As transporters may influence the uptake and translocation of other essential elements and compounds in rice plants.

The key knowledge gaps regarding the uptake and translocation of As species in rice plants remain unanswered and must be the aim of future studies. These include the following: (1) the extent of active and passive As uptake and translocation in rice, (2) a detailed survey of possible As demethylation and volatilization mechanisms and identification of related genes involving in As demethylation and volatilization in rice tissues, (3) the spatial distribution of As species and temporal variations of their concentrations in rice grains at different development stages in various rice genotypes, under different water management regimes, and soil amendments, and (4) qualitative and quantitative determination of As-thiol complexes in different rice cultivars at different growth stages.

As-contaminated rice grains are a major source of As in humans, particularly for those consuming a large amount of rice and rice-based products. The development of advanced techniques regarding the in-situ measurement of As species, associated elements and genes at cellular and sub-cellular levels in rice plants is of significant importance in order to produce rice safe from As. Therefore, scientific-based studies in As dynamics would safeguard both paddy rice ecosystems and humans from the As burden.

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2.2.3 Concluding remarks

The As(III) is mainly acquired by nodulin 26-like intrinsic proteins, collectively called aquaglyceroporins. Phosphate transporters were found to have a high affinity for As(V) uptake in rice plants. Since OsNIP2;1 (Lsi1) and PO_4^{3-} transporters share the uptake pathway for $Si(OH)_4$ and PO_4^{3-} with As(III) and As(V), respectively, high concentrations of $Si(OH)_4$ and PO_4^{3-} in paddy pore water may reduce the uptake of As(III) and As(V) in rice plants. The exact mechanism for the uptake of MMA(V) and DMA(V) remains largely unknown. It is suggested that Lsi1 mediates the uptake of both MMA(V) and DMA(V) in rice plants. Various metabolic pathways including As(III) efflux back into the external environment, As(III)-thiol complexation and consequent sequestration, and As(V) reduction to As(III) play a key role in governing the translocation and accumulation of inorganic As species in rice plants. The translocation efficiency (caryopsis-to-root) is higher for organic As species than for the inorganic As species. The maturity stage and the genetic makeup of rice plants largely affect the loading pattern of As species in rice grains. Arsenic stress in paddy rice soils leads to straighthead disease and adversely affects plant growth parameters (i.e. plant height, root biomass, grain yield, and number of filled grains). Therefore, supplementation of scientific-based amendment into the As contaminated paddy rice soils may reduce the uptake of As species, due to the release of $Si(OH)_4$ and PO_4^{3-} into the paddy pore water, and may increase the quality and quantity of rice yield by releasing important nutrients into the paddy soil solution.

2.3 Water management, biochar/biochar composites, and other physico-chemical and biological methods to reduce arsenic accumulation in rice plants

Article III

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2.3.1 Introduction

Development and implementation of mitigation measures are urgently needed in order to produce rice grains at non-toxic As levels. Over the last few decades, studies have focused on the efficiency, practical applicability, and technical feasibility of various methods to decrease the As accumulation in rice grains. This review critically evaluates intermittent and aerobic irrigation practices in comparison to conventional, flooded irrigation practices to produce rice grains with reduced As. The effects of supplementation of nutrients (i.e. P, Si, S, and N) and incorporation of amendments (i.e. Fe, Mn, and pristine and modified BC) to reduce As content and to improve the rice yield have been discussed in detail in this review. Biological methods (i.e. inoculation and stimulation of microorganisms and use of hyperaccumulating plants) and genetic engineering approaches (i.e. screening low As accumulation rice cultivars and producing transgenic rice cultivars) have also been evaluated to produce rice grains with decreased As content. In addition, this review concerns the applicability and limitations of water management, physico-chemical, and biological methods, considering the environmental and socio-economic points of view.

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Mitigation of arsenic accumulation in rice: An agronomical, physico-chemical, and biological approach – A critical review

Prasanna Kumarathilaka[a], Saman Seneweera[b,c], Yong Sik Ok[d], Andrew A. Meharg[e], and Jochen Bundschuh[a,f]

[a]School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, Toowoomba, Queensland, Australia; [b]Center for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, Toowoomba, Queensland, Australia; [c]National Institute of Fundamental Studies, Kandy, Sri Lanka; [d]Korea Biochar Research Center & Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Korea; [e]Institute for Global Food Security, Queen's University Belfast, Belfast, UK; [f]UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, Toowoomba, Queensland, Australia

ABSTRACT

Human exposure to As through rice consumption is a worldwide health concern. There is an urgent need to either remediate As contaminated paddy soils, or to screen for low As accumulating rice varieties, thereby limiting the build up of As in their grains. This review presents a number of agronomic, physico-chemical, and biological approaches that may reduce the As content in paddy agroecosystems. Studies have shown that alternative water management practices significantly reduce As accumulation in rice grains. The application of Si sources into As contaminated paddy soils may limit As(III) uptake. The supplementation of redox-sensitive elements (i.e. Fe and Mn) and the incorporation of biochar (BC) may also immobilize As in the paddy environment. Inoculation of microorganisms is another in-situ method to reduce As in rice grains. Accumulation of As in rice grains can also be largely reduced through altering the expression of genes in rice plants. However, applicability of potential As mitigation approaches is dependent on the biogeochemical properties of the paddy agroecosystems, water management practices, availability of sources, and cost. This article expands on research gaps and provides future research directions to enable the production of safer rice grains with reduced As accumulation.

KEYWORDS
Arsenic methylation; biochar; microorganisms; rice; soil amendments; water management

CONTACT Jochen Bundschuh ✉ jochen.bundschuh@usq.edu.au School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia.

1. Introduction

Arsenic is a class (I) carcinogen (IARC, 2004). Over the last few decades, millions of people around the world have suffered from numerous chronic diseases and deaths, related to the consumption of As contaminated drinking water (Bundschuh et al., 2012; McClintock et al., 2012). Rice consumption is another important agency of dietary exposure to inorganic As in humans (Chatterjee et al., 2010; Kumarathilaka, Seneweera, Ok, Meharg, & Bundschuh, 2019). Recent risk assessment studies have also revealed that the consumption of As contaminated rice and rice-based food products leads to increased health risks in humans (Signes-Pastor, Carey, & Meharg, 2016; Signes-Pastor et al., 2017).

Different As sources (i.e. geogenic and anthropogenic) have led to substantial As distribution in paddy agroecosystems (Sahoo & Kim, 2013). Traditional paddy rice cultivation practices in an As contaminated environment (i.e. flooding soils from the time of crop establishment to almost harvest time) may involve a greater accumulation of inorganic As in rice grains than other cereal crops (Kögel-Knabner et al., 2010; Williams et al., 2007). One reason is that submerged soil conditions change the redox chemistry in the paddy environment, increasing the bioavailability of inorganic As in the rice rhizosphere for uptake by rice plants (Awad et al., 2018; Sahrawat, 2015). Factors including dissolved organic matter, redox-sensitive elements (i.e. Fe, Mn, S, and N), formation of root plaque, competitive ions/compounds (i.e. phosphate (PO_4^{3-}) and silicic acid ($Si(OH)_4$)), and the activity of microorganisms also influence the mobility, bioavailability and speciation of As in paddy agroecoystems (Bhattacharya et al., 2007; Kumarathilaka, Seneweera, Meharg, & Bundschuh, 2018a; Xu, Chen, Wang, Kretzschmar, & Zhao, 2017). Arsenite (As(III)) and arsenate (As(V)), and monomethylarsonoic acid (MMA(V)) and dimethylarsinic acid (DMA(V)) are the most commonly found inorganic and organic As species in paddy agroecosystems, respectively (Kumarathilaka, Seneweera, Meharg, & Bundschuh, 2018b; Williams et al., 2007). A number of transporters are involved in the uptake, translocation and grain filling of different As species in rice plants (Ma et al., 2008; Tang, Chen, Chen, Ji, & Zhao, 2017; Tiwari et al., 2014).

Therefore, in order to produce safer rice grains with reduced As accumulation, implementation of mitigation measures is essential in As contaminated paddy agroecosystems. Over the last few decades, many researchers have investigated the efficiency, practical applicability and technical feasibility of different mitigation methods to reduce the As content of rice grains. In this review, we critically evaluate potential agronomical, physico-chemical and biological techniques which can be used to reduce both the bioavailability of As in the paddy soil solution and substantial uptake and accumulation in rice grains. This review is mainly concerned with the applicability and limitations of potential agronomical, physico-chemical, and biological techniques with respect to the mitigation of As in paddy agroecosystems, considering the environmental and socio-economic points of view. Research gaps and future research orientations are also highlighted to produce rice grains with reduced As accumulation.

2. Alternative water regime management to reduce As accumulation in rice plants

Paddy soils under conventional rice cultivation practices undergo flooded (reductive) followed by non-flooded (oxidative) conditions (Kögel-Knabner et al., 2010). The development of reductive conditions in paddy soils prevents aerobic microbe-mediated disease damage (Minamikawa, Takahashi, Makino, Tago, & Hayatsu, 2015). Alternative water management practices have been proved to ensure the least total As levels in the paddy soil solution and substantially less in rice tissues (i.e. root, straw, husk, and grain). Intermittent and aerobic water management practices, as alternative water management strategies, have recently been examined to mitigate As accumulation in rice grains (Hu, Huang, et al., 2013; Huq, Shila, & Joardar, 2006; Liao et al., 2016). During intermittent ponding, paddy soil is flooded with irrigation water to a height of about 3–5 cm. The water level under the intermittent irrigation practice gradually decreases via evaporation and seepage. When the soil becomes dry, it is flooded again allowing for wet and dry cycles throughout the rice growing period. In contrast to intermittent irrigation practices, the water is discharged from the field for maintaining the aerobic water management. Soil is irrigated to approximately 1 cm on alternate days to ensure wet cultivation of rice even during the aerobic water management practice (Basu et al., 2015; Hu, Ouyang, et al., 2015).

Water management practices mainly alter physico-chemical and biological properties of the paddy soil-water system. For example, under continuous flooded irrigation practice, As retained in the soil matrix is solubilized from As(V) to As(III), which is more mobile than As(V) (Honma, Ohba, Kaneko, et al., 2016; Sahrawat, 2015). The reverse is true under intermittent and aerobic water management practices where the As(V)/As(III) ratio is high in the paddy soil solution (Dittmar et al., 2007).

The concentrations of both inorganic and methylated As species under conventional and alternative water management practices are summarized in Table 1. Xu, McGrath, Meharg, and Zhao (2008) demonstrated that an increased DMA(V) proportion under continuous flooded irrigation practice led to an increase in total As levels in rice grains. The proportion of methylated As levels in rice grains tends to decrease under intermittent and aerobic water management practices, whereas the reverse is true for inorganic As

Table 1. Total As and As speciation concentrations and grain yield under different types of water management regimes.

Rice variety	Water management strategy	As concentrations in rice grains ($\mu g\ kg^{-1}$)					Grain yield (kg ha^{-1})	Reference
		Total As	As(III)	As(V)	DMA(V)	MMA(V)		
Gladio	Continuous flooding	547	138[a]		353[b]		3250	Moreno-Jiménez et al. (2014)
	Intermittent (natural rainfall supplemented by sprinkler irrigation) (1 year)	200	70[a]		88[b]		2519	
	Intermittent (natural rainfall supplemented by sprinkler irrigation) (7 year)	89	25[a]		31[b]		3301	
Koshihikari	Continuous flooding	950	450[a]		480	—	—	Arao et al. (2009)
	Flooding from transplanting for 2 weeks	100	120[a]		10	—	—	
—	Continuous flooding (5 cm of water applied at every 3-day interval)	627	—	—	6790	—	6790	Basu et al. (2015)
	Intermittent (5 cm of water applied if cracks were found)	495	—	—	5930	—	5930	
	Aerobic (1 cm of water applied on alternative days to aeration)	549	—	—	6450	—	6450	
Koshihikari	Continuous flooding	390	290	20	—	—	—	Honma, Ohba, Kaneko, et al. (2016)
	Rainfed	110	70	4	—	—	—	
IAPAQ-9	Continuous flooding	210	—	—	—	—	—	Hu, Ouyang, et al. (2015)
	Aerobic (water was discharged to leave a water layer ~5 cm deep)	140	—	—	—	—	—	
	Intermittent (3 cm of water applied if was dried)	140	—	—	—	—	—	
	Conventional (flooded until full tillering stage followed by intermittent irrigation)	150	—	—	—	—	—	
37 genotypes	Continuous flooding	95–235	—	—	—	—	—	Spanu, Daga, Orlandoni, and Sanna (2012)
	Sprinkler irrigation	1.3–5.1	—	—	—	—	—	

[a] Sum of As(III) and As(V) as inorganic As species.

[b] Sum of DMA(V) and MMA(V) as organic As species.

species. However, inorganic As levels in rice grains under intermittent and aerobic water management practices are far less than that of rice grains under continuous flooded irrigation practice (Newbigging, Paliwoda, & Le, 2015). Microbial methylation of inorganic As in the rice rhizosphere may enhance the level of methylated As species in the paddy soil solution, and subsequent accumulation in rice tissues, under continuous flooded irrigation practice (Jia, Huang, Sun, Zhao, & Zhu, 2012). The reported variabilities in As species concentrations among different localities and rice genotypes may be due to the site-specific physico-chemical, biological, and environmental factors along with water management practices (Table 1). The influence of different physico-chemical and biological factors on As dynamics in paddy environments is discussed in detail in the following sections, starting from section 3. Overall, the least concentrations of total As, inorganic and methylated As species in rice grains are found under the aerobic water management practice followed by the intermittent water management practice and continuous flooded irrigation practice (Arao, Kawasaki, Baba, Mori, & Matsumoto, 2009; Moreno-Jiménez et al., 2014).

Alternative water management practices, on the other hand, ensure efficient water usage per hectare and thereby decrease the cost of water consumption. Aerobic and intermittent water management practices significantly reduce water consumption throughout the rice growing season by approximately one-third of that used for flooded systems (Moreno-Jiménez et al., 2014). However, reduction of irrigation water volume can lead to reduction in grain yield under aerobic and intermittent water management practices (Basu et al., 2015; Devkota et al., 2013). There is a sizable literature to support the reduction in grain yield by changing the water management practices (Table 1). Soil drying also limits the root growth and thus reduces the water uptake in rice plants (Bengough, McKenzie, Hallett, & Valentine, 2011). Yield loss is probably due to a lower number of spikelets per panicle, a smaller portion of filled grains, lower 1000-grain weight and thus lower grain yield per unit area (Chou et al., 2016). For example, Moreno-Jiménez et al. (2014) demonstrated that grain yield under intermittent water management was lowered by 25% compared to crops under the continuous flooded water management on a short-term basis (1 year). A lower As level in raw rice grains is extremely beneficial from a health perspective; however, lower production might not be acceptable to the farmers. Therefore, long-term investigations are required to provide a better understanding of how water management regimes not only mitigate As accumulation in rice grains but also impact on rice grain quality and production. Moreno-Jiménez et al. (2014) found that grain yield under intermittent water management becomes more or less similar to flooded irrigation on a long-term basis (7 years). These results are consistent with those obtained by Norton et al. (2017) who demonstrated that intermittent water management increased grain mass. The availability of water and the presence of roots along the paddy soil profile (25–35 cm) even during the drying periods in alternative water management practices ensured that the rice plants did not suffer from drought conditions and thus yields were maintained as in the flooded water management (Carrijo et al., 2018). Therefore, it is important to investigate the surface and subsurface hydrology along the paddy soil profile to understand the potential of alternative water management practices on rice yields.

Natural rainfall can also affect the As dynamics in the paddy agroecosystem governed by different water management approaches. More precisely, As content in the top soil in seasonally flooded rice fields decreases during the wet season following dry season irrigated cultivation, suggesting that natural rainfall attenuates As levels in paddy soils. Roberts et al. (2010) assessed that 51–250 mg m^{-2} of total As in top paddy soils were released into the flood water during the wet season, which corresponded to a loss of 13–62% of the total As added to rice fields through groundwater irrigation. Therefore, the effect of natural rainfall on As dynamics in the paddy environment needs to be further investigated.

There is a tradeoff relationship between As and Cd bioavailability in the paddy soil solution and their concentrations in rice tissues under different water management regimes (Honma, Ohba, Kaneko, et al., 2016; Hu, Li, et al., 2013; Liao et al., 2016) because Cd solubility and substantial bioavailability in the paddy soil solution also depend on the changes in redox chemistry. For example, under flooded conditions, Cd(II) reacts with sulfide (S^{2-}) to form a less soluble CdS, thereby suppressing the bioavailability of Cd(II) (Hu, Ouyang, et al., 2015; Rizwan et al., 2017). Apart from these considerations, under flooded conditions, Cd can be sorbed onto the Fe and/or Mn hydro(oxides) or precipitated as $CdCO_3$ (Arao et al., 2009; Saraswat & Rai, 2011). Phosphorous in the soil matrix may also precipitate Cd as insoluble phosphate complexes. However, oxidation of CdS to Cd(II) and sulfate (SO_4^{2-}) enhances Cd(II) bioavailability in the paddy soil solution and increases Cd accumulation in rice grains during the aerobic water management practice (Honma, Ohba, Kaneko-Kadokura, et al., 2016). The solubility of Cd in the paddy environment is affected by the soil pH which influences the surface charge of the sorption sites and, thereby affects the affinity of Cd for sorption sites. For example, increased pH from 6.1 to 6.9 in paddy soils, due to the supplementation of MgO, has led to decreased Cd levels in rice grains in both flooded and upland rice cultivars (Kikuchi et al., 2008). $Cd(OH)_2$ may have precipitated on the surface of MgO amendments (Kikuchi et al., 2008). Taking these possible impacts into account, intermittent and aerobic water management practices for As mitigation should not be used for paddy soils until a solution for the optimization of the tradeoff relationship between As and Cd is found. Moreover, physico-chemical properties of As-Cd contaminated soils need to be evaluated in detail to reduce the accumulation of both As and Cd in rice grains. Overall, alternative water management practices seem to be the most cost-effective approach to mitigate As accumulation in rice tissues in environments that have no Cd issues.

3. Soil amendments to reduce As uptake and accumulation in rice plants

3.1. Nutrients supplementation

3.1.1. Phosphorus

Phosphorus, in the form of PO_4^{3-} fertilizers, plays a key role in plant metabolism. Phosphate fertilization can promote plant growth characteristics (i.e. total chlorophyll, chlorophyll-a, and chlorophyll-b) and increase the production of antioxidant scavenging enzymes to promote better growth of rice seedlings (Choudhury, Chowdhury, & Biswas, 2011). Phosphate application into As contaminated paddy soils mainly reduces the uptake and accumulation of As(V) in rice plants (Figure 1). The reason is that As(V) is a chemical analog of PO_4^{3-} and shares the same uptake pathway from the paddy soil solution to the rice roots. The PO_4^{3-} transporter, OsPHT1;8 (OsPT8), mediates both PO_4^{3-} and As(V) uptake by rice roots (Wang, Zhang, Mao, Xu, & Zhao, 2016). A higher PO_4^{3-}/As(V) ratio in the rhizosphere may thus decrease As(V) uptake by the rice plant, making this ratio an important parameter. However, a number of factors (i.e. soil properties and application rate of PO_4^{3-}) affect the roles of PO_4^{3-} in As(V) uptake in rice plants.

Phosphate supplementation has also been found to increase As(V) concentration in the soil solution. This could be attributed to competitive adsorption between PO_4^{3-} and As(V) on the soil matrix and root plaque. Lee et al. (2016) demonstrated that application of PO_4^{3-} fertilizer (6–8 mg kg^{-1} per a growing season) into As contaminated paddy soils (9–102 mg kg^{-1}) did not inhibit the As uptake and accumulation in rice plants. Hossain et al. (2009) also found that the addition of PO_4^{3-} fertilizer increased the total As concentration in both rice straw and grain. Moreover, Geng, Zhu, Liu, and Smith (2005) revealed that the addition of PO_4^{3-} decreased the percentage of total As retention in Fe plaque from 70 to 10% and consequently increased the percentage of total As concentration in rice roots and shoots by 20–60%. Therefore, optimum supplementation of PO_4^{3-} fertilizers across As contaminated paddy environments needs to be accurately measured to decrease As accumulation in rice grains.

Phosphate fertilization in As contaminated paddy soils is not feasible for a number of reasons. The global PO_4^{3-} fertilizer production depends on PO_4^{3-} rock resources. The scarcity of PO_4^{3-} rock mines and the impact of rising market prices for PO_4^{3-} fertilizers limit PO_4^{3-} supply to As contaminated

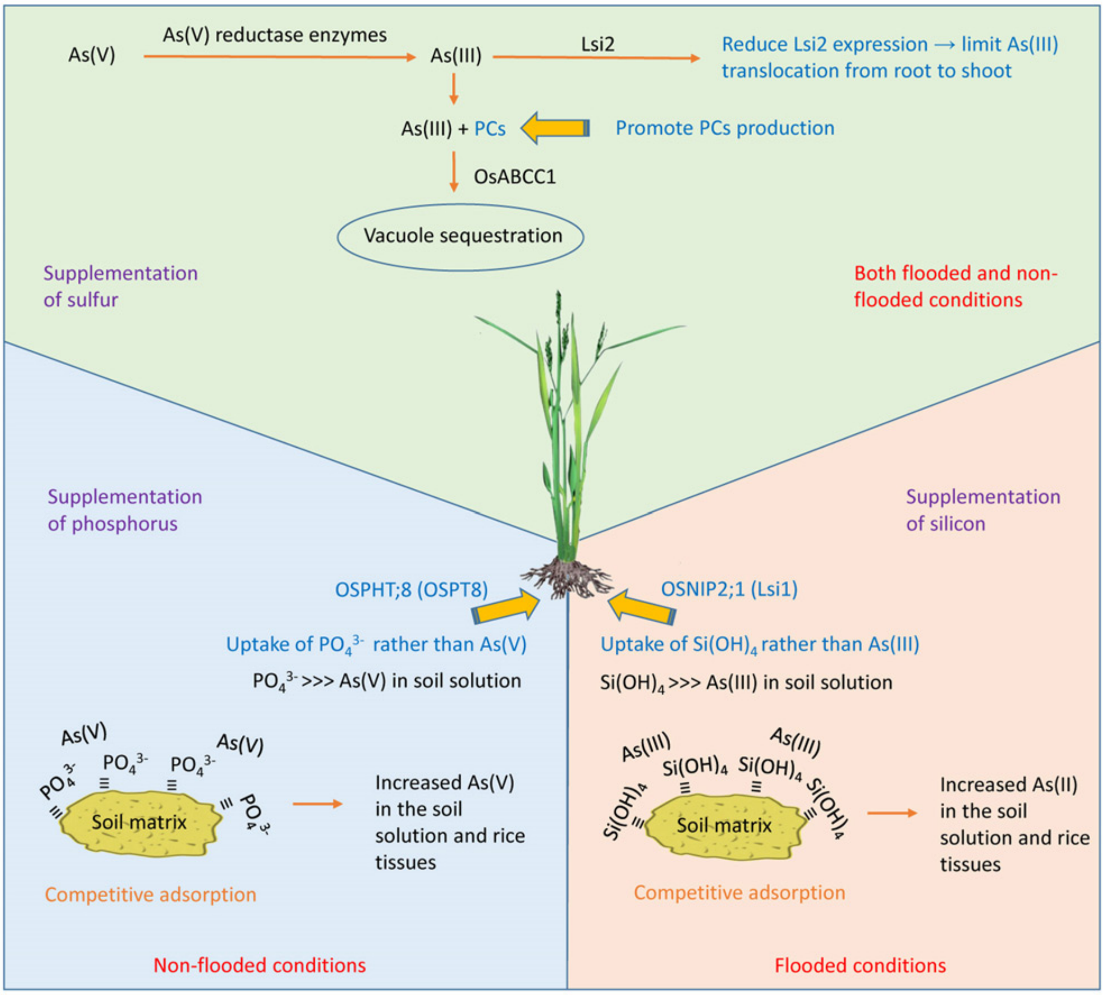


Figure 1. Effects of nutrient supplementation on As bioavailability in paddy soils and As uptake and translocation in rice plants.

paddy environments (Mew, 2016; Neset & Cordell, 2012). The increased input of toxic elements such as As and Cd to the paddy soils is another issue with PO_4^{3-}-based fertilizer supplementation on a long-term basis (Charter, Tabatabai, & Schafer, 1995; Fayiga & Saha, 2016; Molina, Aburto, Calderón, Cazanga, & Escudey, 2009). Charter et al. (1995) found that the level of As in commonly used PO_4^{3-} fertilizers (i.e. triple super phosphate, monoammonium phosphate, diammonium phosphate, and rock phosphate) ranged between 2.4 to 32.1 mg kg^{-1}. Phosphate losses due to surface runoff and vertical leaching may reinforce eutrophication in streams, lakes and reservoirs. Most importantly, PO_4^{3-} fertilizers are not as effective as other potential mitigation measures to reduce As accumulation in rice grains.

3.1.2. Silicon

Silicon is the second most abundant element in the earth's crust (Marschner & Tilley, 2017). It can increase plant resistance to both biotic (i.e. fungal and insect pests) and abiotic stresses (i.e. strong rain, wind, and salinity) (Adrees et al., 2015). Rice shoots contain approximately 10% of the total Si taken up by rice roots (Meharg & Meharg, 2015). Plant-available $Si(OH)_4$ in paddy soils originates from irrigation water, weathering and desorption of Si-bearing minerals in the soil matrix and deposition of crop residues (i.e. remaining straws after harvest) (Babu, Tubana, Datnoff, Yzenas, & Maiti, 2016; Meharg & Meharg, 2015; Song et al., 2017).

Silicon application into the As contaminated paddy environment leads to reduction of As(III) accumulation in rice grains. Both silicic acid and As(III) share the same uptake pathway to enter the rice root cells (Figure 1). The aquaporin channel, OsNIP2;1 (Lsi1) mediates both $Si(OH)_4$ and As(III) uptake in rice plants (Ma et al., 2008). This may be due to the similar sizes and similar dissociation constants (pK_a) for both compounds (Fleck, Mattusch, & Schenk, 2013; Ma et al., 2008). Therefore, an increase in the Si/As(III) ratio in the rhizosphere is a critical factor for decreasing As(III) uptake in rice plants (Figure 1). Studies have clearly demonstrated that As(III)/total As ratio in rice tissues decreases markedly with increasing Si supplementation into As contaminated paddy soils (Fleck et al., 2013; Wu et al., 2015). This could correspond to the lack of As(III) uptake and increased $Si(OH)_4$ uptake by the $Si(OH)_4$ uptake transporters (Figure 1).

Silicon supplementation rates and the mineralogy of Si sources were found to have different effects on As(III) uptake in rice plants. For example, a study by Lee, Huang, Syu, Lin, and Lee (2014) demonstrated that Si application rates ($0.375\,g\,kg^{-1}$) did not decrease As accumulation in rice plants. A relatively lower concentration of Si application may increase As(III) level in the paddy soil solution due to competitive adsorption between $Si(OH)_4$ and As(III) on soil particles. In this sense, rice plants acquire and accumulate high As(III) levels in vegetative and reproductive parts. Seyfferth and Fendorf (2012) revealed that two silicate minerals (diatomaceous and SiO_2 gel), with different solubility constants, had differing effects on As accumulation in rice grains. Addition of SiO_2 gel significantly reduced the total As level in rice grains, whereas diatomaceous application did not decrease the total As level in rice grains. Therefore, optimization of Si supplementation rates into As contaminated paddy environments and understanding of the mineralogy of Si sources are important aspects to ameliorate As stress in rice plants.

Silicon fertilization in As contaminated paddy soils is limited due to the scarcity of the resource and its high cost. Desplanques et al. (2006) estimated that annual off-take of Si by rice crops was $270\,kg\,ha^{-1}$. Therefore, the reincorporation of Si-rich rice straw after composting may introduce Si fertilizers back into the paddy fields. In addition, BC production using Si-rich feedstocks and subsequent incorporation into As contaminated paddy soils may be another cost-effective method of releasing Si slowly into the paddy soil-water system (Seyfferth et al., 2016).

3.1.3. Sulfur

Sulfur is an essential macronutrient for plant growth (Boldrin et al., 2016) and its addition leads to the mitigation of As accumulation in rice tissues by changing the mineralogy of the rhizosphere. During flooded conditions, SO_4^{2-}in the soil-water system reduces into S^{2-} (Sahrawat, 2015). As(III) in the paddy soil solution can react with S^{2-} and precipitate as an As_2S_3-like complex (Burton, Johnston, & Kocar, 2014). Therefore, bioavailability of As(III) for the uptake by rice plants is decreased.

Moreover, S addition causes the mitigation of As accumulation in rice grains by changing the metabolism of the rice plant. For example, Dixit et al. (2015) demonstrated that the addition of S (5.0 mM) resulted in a reduced transcript level of Lsi2 which mediates As(III) efflux in the direction of the xylem. Sulfur can also enhance the formation of low molecular weight thiol-rich peptides (i.e. phytochelatins (PCs) and glutathione (GSH)) in rice roots (Zhang, Zhao, Duan, & Huang, 2011). These thiols possess a high affinity for As(III) (Figure 1). As(III)-thiol complexes are transported for vacuole sequestration through a C-type ATP-binding cassette transporter (OsABCC1) in rice roots (Zhao, Ma, Meharg, & McGrath, 2009). The OsABCC1 present in the tonoplast of phloem in nodes also mediates transporting of As(III)-thiol complexes for vacuole sequestration (Song et al., 2014). As(V), the major As species under non-flooded conditions, is readily reduced into the As(III) by As(V) reductase enzymes in rice roots (Shi et al., 2016; Xu, Shi, et al., 2017). As(V) reduction and its consequent As(III)-thiol complexation and sequestration in the vacuoles reduces As(V) translocation in the rice plants.

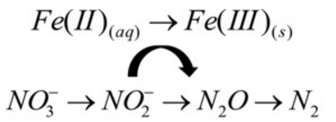
Even though a number of studies have shown that rice varieties with a low level of As in their grains had a significantly high PC level in their roots some recent studies have reported contrasting results. For example, Batista et al. (2014) demonstrated that the production of PCs at a high concentration in rice varieties have not necessarily decreased the total As level in rice grains. Even under decreased S availability, some rice varieties have been found to decrease As accumulation in their grains. Srivastava, Akkarakaran, Sounderajan, Shrivastava, and Suprasanna (2016) demonstrated that rice variety IR64 can decrease As accumulation in its grains even at zero S supplementation. Therefore, quantification of As-thiol complexation in rice varieties across different field sites may provide better understanding of the effects of S in As contaminated paddy environments.

Recent findings have also revealed that S fertilization leads to the formation of thioarsenates ($HAsS_nO_{4\text{-}n}{}^{2-}$, $n = 1–4$) in As contaminated rice agroecosystems (Kerl, Rafferty, Clemens, & Planer-Friedrich, 2018). More precisely, thioarsenates are formed spontaneously under sulfate reducing conditions from As(III) through the exchange of OH^-/SH^--ligands and the oxidative addition of S (Planer-Friedrich et al., 2015; Planer-Friedrich, Suess, Scheinost, & Wallschläger, 2010). However, different factors such as S(-II)/As(III) and S(0)/As(III) ratios, pH, and availability of microorganisms also affect the formation of individual thioarsenates (Edwardson, Planer-Friedrich, & Hollibaugh, 2014; Planer-Friedrich et al., 2015). For example, monothioarsenate, which has more or less similar toxicity to As(V), can occur over a wide pH range found in paddy environments (pH 2.5–8.0) (Planer-Friedrich et al., 2017; Zeng et al., 2011). Unlike As(III) and As(V), thioarsenates have less complexation capacity with Fe(III) hydro(oxides) (FeOOH), leading to an increased thioarsenate level in the paddy soil solution and consequently in the rice tissues (Couture et al., 2013). Therefore, it is essential to study the behavior and toxicity of thioarsenates in As contaminated paddy environments before promoting S amendments.

Sulfur supplementation in As contaminated paddy soils is limited, mainly due to the sharp increase in the price of S in the global market. For instance, Fixen and Johnston (2012) reported that in 2008 there was a price rise for S in the USA market from less than \$100 to \$800 per metric ton. In summary, there is no conclusive data regarding S fertilization in As contaminated paddy agroecosystems. Most of the studies reported in the literature were performed only as short-term hydroponic experiments. Longer term field experiments may thus give a better overview of S vs total As in rice tissues.

3.1.4. Nitrogen

Nitrogen fertilizer is supplied as urea and ammonium sulfate in order to increase rice yield. The coupling of N and Fe cycles has the potential to influence As dynamics in paddy soil-water systems and subsequent As uptake by rice roots (Burgin, Yang, Hamilton, & Silver, 2011). Under flooded conditions, denitrification occurs when Eh decreases (Sahrawat, 2015). The supplementation of N in the form of NO_3^- in paddy soils could increase microbially catalyzed Fe(II) oxidation (Eq. 1). Studies have revealed that NO_3^- dependent Fe(II) oxidizing microorganisms are widespread in paddy soils (Klüber & Conrad, 1998; Li, Yu, Strong, & Wang, 2012). The formation of FeOOH assists in retaining both As(V) and As(III) in the paddy soil matrix (discussed in detail in section 3.2.1). Chen, Zhu, Hong, Kappler, and Xu (2008) demonstrated that application of KNO_3 (1 mM kg^{-1}) to As contaminated paddy soil (84.92 mg kg^{-1}) significantly reduced the total As concentration in the rice roots and shoots by approximately 40%. Under flooded conditions, anammox-bacteria has been found to promote NH_3 oxidation which is linked to the reduction of Fe(III) (Shrestha, Rich, Ehrenfeld, & Jaffe, 2009). However, effects of microbes-driven NH_3 oxidation on Fe(III) reduction in the paddy environment need to be studied.



NO_3^- - dependent Fe(II) oxidizing microorganisms (1)

Detailed investigations on the influence of N supply on As mobility, bioavailability, and accumulation in rice tissues are lacking. Since addition of N fertilizer indirectly affects the total As concentration in rice tissues, the abundance of microorganisms and indigenous Fe content in paddy soils may influence the impact of N supply on As in rice tissues (Ding, Su, Xu, Jia, & Zhu, 2015). Furthermore, excess N supplementation may cause secondary effects such as reservoir eutrophication. Therefore, it is essential that the impacts of N fertilizer incorporation into As contaminated paddy soils are further examined.

3.2. Incorporation of stabilization agents

3.2.1. Immobilization of As by the sorption onto Fe(III)

Iron is an essential element for plant growth and has a strong affinity with As in As contaminated soils (Chen et al., 2014; Kim et al., 2017). Paddy soils may comprise indigenous FeOOH including ferrihydrite ($Fe_5HO_8 \cdot 4H_2O$), lepidocrocite (γ-FeOOH), hematite (α-F_2O_3), and goethite (α-FeOOH) (Zhuang, Xu, Tang, & Zhou, 2015). The phase conversion from poorly crystalline ferrihydrite to other crystalline Fe oxides (i.e. goethite and hematite) may affect As adsorption since the number of adsorption sites diminishes with increasing crystallinity (Komárek, Vaněk, & Ettler, 2013).

External Fe supplementation (i.e. Fe oxides, Fe-containing industrial byproducts and mixed Fe sources) may enhance As sorption capacity in paddy soils and hence reduce As accumulation in rice grains (Table 2) for a number of reasons. Firstly, Fe amendments can directly affect the portion of Fe fractions in the paddy environment. When Fe(0) and Fe(II) compounds are applied to paddy soils, they are oxidized, forming poorly crystalline Fe oxides as shown in Eq. 2, 3, and 4 (Miretzky & Cirelli, 2010).

$$Fe(0)_{(s)} + 2H_2O_{(l)} + O_{2(g)} \rightarrow Fe(II)_{(s)} + 4OH^-_{(aq)} \tag{2}$$

$$Fe(II)_{(s)} + 2H_2O_{(l)} + O_{2(g)} \rightarrow Fe(III)_{(s)} + 4OH^-_{(aq)} \tag{3}$$

$$Fe(III)_{(s)} + 2H_2O_{(l)} + O_{2(g)} \rightarrow FeOOH_{(s)} + 3H^+_{(aq)} \tag{4}$$

The replacement of OH_2 and OH^- for the anionic As in the coordinate spheres of FeOOH leads to the formation of monodentate, bidentate, or binuclear bridging complexes (Fendorf, Eick, Grossl, & Sparks, 1997; Luong et al., 2018). Due to this, As is readily adsorbed onto FeOOH.

Secondly, Fe supply stimulates Fe(III) plaque formation on rice roots and consequently sequesters both As(V) and As(III) on the root surface (Liu, Zhu, Smith, & Smith, 2004). However, the process of As adsorption onto the Fe(III) plaque could be reversible due to the changes in redox chemistry in the paddy environment. During flooded conditions, FeOOH in root plaque and bulk paddy soil reduces into Fe(II) as the redox potential decreases (Sahrawat, 2015). As a result, sorbed As species on FeOOH are released into the soil solution as As(III). Radial oxygen loss (ROL), the process of releasing O_2 in rice plants to the rhizosphere, can increase the redox potential and promote Fe(III) plaque formation (Mei, Ye, & Wong, 2009). Therefore, the formation of root Fe(III) plaques under flooded conditions plays a vital role in sequestrating As into the rhizosphere, and hence may limit the uptake and consequent accumulation of As in rice grains (Table 2). However, further

Table 2. Effects of different Fe amendments to alleviate As in the paddy soil solution and rice grains.

Rice genotype/s	Background Fe concentration (g kg^{-1})	Fe form and application rate	Decrement of total As compared to control (%)		Reference
			Soil solution	Rice grain	
Jiahua-1	18.8[a]	Ferrihydrite; 1.5 % (wt/wt)	—	36 (shoot)	Chen et al. (2014)
Zhe733 and Cocodrie	7–10	Fe oxide (Fe_3O_4: 80% and Fe_2O_3: 20%); 0.5–2.0 (wt/wt)	—	~50	Farrow et al. (2015)
—	—	Fe oxide; 5 g kg^{-1}	—	~50	Yu, Wang, et al. (2017)
BR28	33.6	Amorphous Fe hydroxide; 0.1 w/w	—	~85 (shoot)	Ultra et al. (2009)
Koshihikari	20.8[a]	Water treatment residue containing polysilicate (Fe (401 g kg^{-1})); 0–20 t ha^{-1}	15.0–43.1	19.8–31.7 (husk) 18.6–21.0	Suda, Baba, Akahane, and Makino (2016)
—	—	Steel slag; 5% (wt/wt)	32	—	Yun et al. (2016)
Milyang 23	—	Steel slag; 3% (wt/wt)	48.62[b] (soil)	—	Kim et al. (2017)
—	9–33	Steel slag: 2 kg m^{-2}	20.5	32.6	Makino et al. (2016)
		Non-crystalline Fe hydroxide: 1 kg m^{-2}	53.3	31.1	
		Zero-valent iron: 1 kg m^{-2}	81.9	53.6	
Koshihikari	—	Steel slag: 1 kg m^{-2}	44.8	17.1	Matsumoto, Kasuga, Makino, and Arao (2016)
		Non-crystalline Fe hydroxide: 1 kg m^{-2}	71.8	47.3	
		Zero-valent iron: 1 kg m^{-2}	89.2	44.7	
Koshihikari	—	Steel slag: 0.5 kg m^{-2}	—	21.6	Matsumoto et al. (2015)
		Non-crystalline Fe hydroxide: 0.5 kg m^{-2}		46.7	
		Zero-valent iron: 0.5 kg m^{-2}		50.6	

studies are required to establish conclusively that Fe plaque in rice rhizosphere limits As accumulation in rice grains, since Fe plaque may also serve as a sink for As in the paddy environment.

The effect of Fe amendment on As accumulation in rice grains also depends on the growth stages of the plant. Studies related to Fe amendment have not focused on As accumulation in rice grains for a fully rice growing cycle and were mainly performed in hydroponic cultures. In a recent study, Yu, Wang, et al. (2017) demonstrated that Fe supplementation significantly decreases As accumulation in rice plants at the grain filling stage. Changes in the physico-chemical properties also affect As bioavailability in the rhizosphere under Fe amendment. For example, natural organic matter derived from the decomposition of plants and animals can be adsorbed onto the FeOOH and thus limits both As(III) and As(V) adsorption onto FeOOH (Mladenov et al., 2015). Similarly, PO_4^{3-} has a strong affinity with FeOOH and inhibits As(V) adsorption onto FeOOH (Zeng, Fisher, & Giammar, 2008). Therefore, the growth stage at which least As accumulation occurs can vary among rice cultivars and different localities.

Iron supplementation to reduce As accumulation in rice grains should also be considered for the following aspects. Adsorptive properties such as specific surface area and solubility of Fe sources need to be assessed before applying them in paddy soils as these properties greatly determine the adsorption capacity for As (Matsumoto, Kasuga, Taiki, Makino, & Arao, 2015). The optimum supplementation rates of Fe also need to be carefully determined. Higher application rates may cause thick layers of Fe plaque on the root surfaces and this might hinder nutrient uptake and O_2 diffusion through roots (Ultra et al., 2009). Iron-bearing industrial byproducts are economically feasible options, however, caution is necessary as there is the potential for the release of other contaminants contained in the Fe surface into the paddy environment. Acidification may be caused due to Fe amendments, which can further mobilize other trace elements in the soil matrix. Iron supplementation, together with basic materials such as lime, may be a possible option, and is worthy of future studies.

3.2.2. As(III) oxidation and As(V) adsorption by manganese oxides

Manganese oxides are generally present in soils as fine-grained coatings of soil particles or as nodules (Essington, 2015). However, there is a lack of detailed investigations into the influence of indigenous or exogenous supplementation of manganese oxides on As content in rice tissues. A recent study by Xu, Chen, et al. (2017) demonstrated that the application of synthetic manganese oxides (mainly as hausmannite) at a rate of 1200 mg Mn kg^{-1} to As contaminated paddy soils reduced total As concentration in rice straw and grains by 30–40%. This is possible because manganese oxides supplementation may slow down the decrease of Eh in flooded paddy soils

(Ehlert, Mikutta, & Kretzschmar, 2014). In general, Mn(IV) reduction takes place at relatively higher Eh than that of Fe(III) reduction. Therefore, manganese oxides in As contaminated paddy soils might retard the release of highly mobile As(III) through reductive dissolution of FeOOH (Ehlert et al., 2014; Lafferty, Ginder-Vogel, & Sparks, 2010). As(III) oxidation and substantial As(V) complexation/co-precipitation in paddy soil-water may also be involved in reducing the inorganic As concentration in rice grains (Eq. 5, Eq. 6, Eq. 7, and Eq. 8) (Komárek et al., 2013; Manning, Hunt, Amrhein, & Yarmoff, 2002; Tournassat, Charlet, Bosbach, & Manceau, 2002). Moreover, Mn plaque formation on rice roots may promote As(III) oxidation in the rice rhizosphere (Liu, Zhu, & Smith, 2005). However, more investigations are required to confirm whether Mn plaque plays a vital role in As oxidation in the rhizosphere of rice.

$$MnO_{2(s)} + H_3AsO_{3(aq)} + 2H^+_{(aq)} \rightarrow Mn^{2+}_{(aq)} + H_3AsO_{4(aq)} + H_2O_{(l)} \tag{5}$$

$$2Mn{-}OH_{(s)} + H_3AsO_{4(aq)} \rightarrow (MnO)_2AsOOH_{(s)} + 2H_2O_{(l)} \tag{6}$$

$$Mn^{2+}_{(aq)} + H_2AsO^-_{4(aq)} + H_2O_{(l)} \rightarrow MnHAsO_4.H_2O_{(s)} + H^+_{(aq)} \tag{7}$$

$$3MnOOH_{(s)} + 2HAsO^{2-}_{4(aq)} + 7H^+_{(aq)} \rightarrow Mn_3(AsO_4)_{2(s)} + 6H_2O_{(l)} \tag{8}$$

Contradictory results indicating that manganese oxides supplementation does not support As immobilization in paddy soils due to their lower surface charge (pH_{zpc}) (1.8–4.5) have also been reported (Komárek et al., 2013). Xu, Chen, et al. (2017) observed that the impact of manganese oxides on As immobilization ceased in the final growth stages of the rice plant. This could be due to the fact that the surfaces of manganese oxides could be readily passivated by the buildup of Mn(II) and Fe(II) (Ehlert et al., 2014). Furthermore, potential secondary effects of exogenous manganese oxides supplementation are yet be examined. For instance, contamination of drinking water sources with Mn (400 $\mu g\ L^{-1}$) may increase the health risks for humans (WHO, 2004). The dissolution of an excessive amount of manganese oxides in the paddy soil solution may cause toxicity in the rice tissues as well. Therefore, detailed studies are required to assess the effect of manganese oxides supplementation to reduce As accumulation in rice grains and its secondary effects in rice growing environments.

3.3. Immobilization and phytotoxicity reduction of As through biochar

The application of BC in agricultural soils has recently gained significant attention because of its potential agronomic, environmental, and economic benefits (Jayawardhana et al., 2018; Kumarathilaka, Mayakaduwa, Herath, & Vithanage, 2015; Lee et al., 2018). Biochar is produced through the thermal decomposition of organic biomass under low levels of O_2 (pyrolysis) (Lehmann & Joseph, 2015). The type of feedstock, pyrolysis temperature, heating rate and residence time determine the physico-chemical properties such as pH, surface properties (i.e. pore volume, pore size, functional groups, pH_{zpc}, cation exchange capacity (CEC)), and nutrient content in BC (He et al., 2018; Jayawardhana, Mayakaduwa, Kumarathilaka, Gamage, & Vithanage, 2017; Vithanage et al., 2017). In contaminated soils, a remarkable reduction in metal mobility and bioavailability has been observed with BC supplementation (Bandara et al., 2017; Herath, Kumarathilaka, Navaratne, Rajakaruna, & Vithanage, 2015; Kumarathilaka, Ahmad, et al., 2018; Kumarathilaka & Vithanage, 2017). However, very limited studies have focused on the role of BC (pristine and modified) in As contaminated paddy environments (Table 3).

Supplementation of pristine BC decreases uptake of As in rice plants for a number of reasons (Figure 2). Biochar has a well-developed pore structure (i.e. micropores, mesopores, and macropores) and facilitates the diffusion of As into the pores through physical adsorption (Khan, Reid, Li, & Zhu, 2014). Biochar contains oxygenated functional groups (i.e. alcoholic, phenolic, and carboxylic) which may control As sorption through surface complexation (Beiyuan et al., 2017; Mohan, Sarswat, Ok, & Pittman, 2014). Several studies have indicated that Fourier Transform Infrared Spectroscopy (FTIR) bands of oxygen-containing functional groups in As adsorbed BCs have shifted, suggesting that As complexation occurs with oxygen-containing functional groups (Hu, Ding, Zimmerman, Wang, & Gao, 2015; Samsuri, Sadegh-Zadeh, & Seh-Bardan, 2013). Electrostatic interactions are also an important mechanism of As adsorption onto BC. However, pH-Eh of the medium and pH_{zpc} might alter the adsorption process. Biochar may add and/or increase competitive ions such as PO_4^{3-} and $Si(OH)_4$ content in paddy soils. Khan et al. (2014) demonstrated that the application of sewage sludge BC at 5 and 10 w/w% into paddy soils increased their PO_4^{3-} level by 3.5 to 4.9 fold. Since both PO_4^{3-} and As(V) are acquired by the same transporter, a high concentration of PO_4^{3-} in the soil solution may lead to reduced uptake and accumulation of total As in rice grains. Seyfferth et al. (2016) found that the incorporation of Si-rich rice husk BC (1%) decreased inorganic As in the grain by 30%. This is because $Si(OH)_4$ addition may limit the As(III) uptake by rice roots. Moreover, BC may increase the dissolved organic carbon (DOC) concentration in the soil solution and consequently, the formation of As-DOC complexes may also immobilize As in paddy soils (Khan et al., 2014). The long-term use of BC can increase the Fe(III) concentration in the rhizosphere. As mentioned earlier, Fe(III) plaque formation in the rice rhizosphere leads to the immobilization of both As(III) and As(V).

The use of pristine BC has been shown conflicting behavior in rice ecosystems since some BC types increase As accumulation in rice tissues (Wang, Xue, Juhasz, Chang, & Li, 2017; Yin, Wang, Peng, Tan, & Ma, 2017). Biochar-induced

Table 3. Pristine and modified biochar types to mitigate As species accumulation in rice grains.

				Physico-chemical properties of BC					
Biomass type	Pyrolysis temperature (°C)	Agent/s used for modification	Application rate (%)	pH	pH_{zpc}	Specific surface area ($m^2\ g^{-1}$)	Elements concentration (mg kg^{-1})	Arsenic increment (↑) / decrement (↓) compared to the control in soil solution / rice tissues (%)	Reference
Rice straw	500	—	3	10.50	—	—	K – 23,684 Fe – 750 As – 1.4	↑ 20.2 – total As (soil solution)	Wang et al. (2017)
Rice straw	450	—	1–3	10.70	—	—	P – 46.9 Fe – 740 As – 10.6	↑ 8.1–39 total As (root)	Yin et al. (2017)
Rice straw	450	Fe	0.5–2	4.87	—	—	P – 55.1 Fe – 35,500 As – 9.3	↓ ~28 total As (root)	Yin et al. (2017)
Sewage sludge	—	—	5–10	7.18	—	5.57	—	↓ 60–68 Total As (grain) ↓ 67–72 As(III) (grain) ↓ 50–62 As(V) (grain) ↓ 39–74 DMA(V) (grain) ↓ 78–88 As(III) (leaves) ↓ 74–81 As(V) (leaves) ↓ 33–62 DMA(V) (leaves) ↓ 85–92 As(III) (stem) ↓ 52–63 As(V) (stem) ↓ 27–69 DMA(V) (stem)	Khan et al. (2014)
Rice straw, husk, and bran	500	—	5	7.20–8.2		—	—	↑ 327 total As (root)	Zheng et al. (2012)
Palm shell	500	Surface amination and nano zero-valent iron				244	—	↓ 47.9 total As (straw)	Liu et al. (2017)
Corn straw	600	MnO_2	0.5–2.0	10.8		3.18	Mn – 7.41[a]	↓ 13.9–19.8 total As (grain)	Yu, Qiu, et al. (2017)
Corn straw	600	— BC:$KMnO_4$:$Fe(NO_3)_3$; 25:4:1 BC:$KMnO_4$:$FeSO_4$; 18:3:1	0.5–2.0		8.93 9.60 3.17	60.97 208.0 7.53	K – 112.3 K – 261.4 K – 259.2	↓ 13.8–39.0 total As (root) ↓ 11.1–20.5 total As (stem) ↓ 21.6–30.4 total As (brown rice) ↓ 53.7–71.9 total As (root) ↓ 14.1–49.4 total As (stem) ↓ 29.7–54.7 total As (brown rice) ↓ 49.2–79.1 total As (root) ↓ 16.1–64.7 total As (stem) ↓ 43.9–75.7 total As (brown rice)	Lin et al. (2017)

[a]Percentage (%).

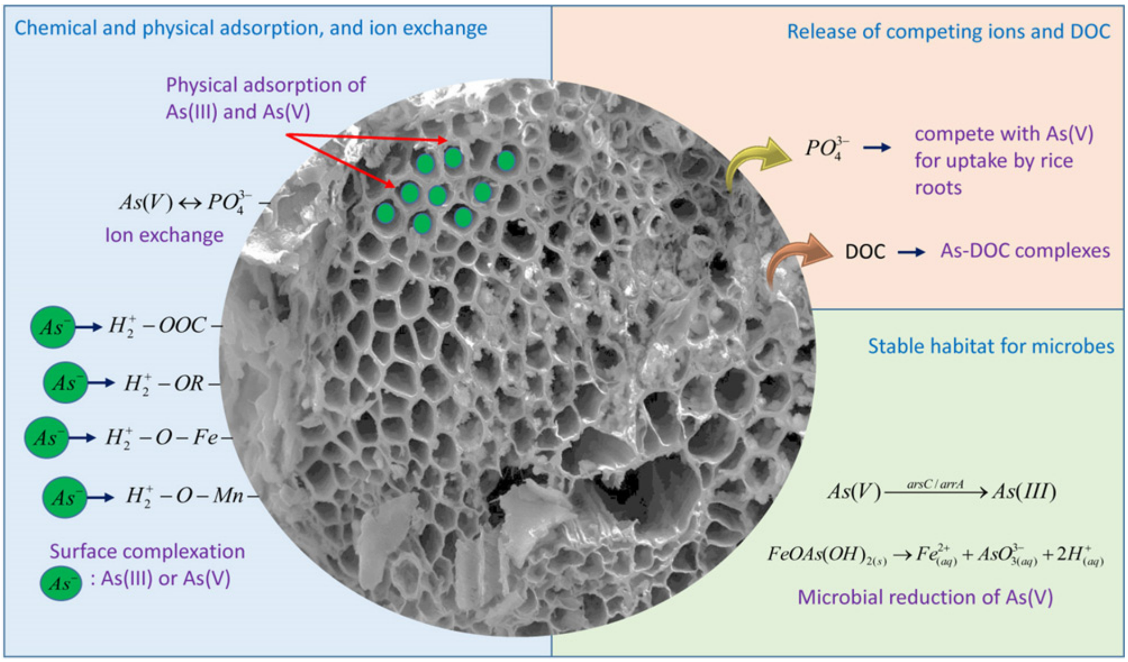


Figure 2. Possible interactions between BC (pristine and modified) and As in the paddy agroecosystem.

high As concentrations in the paddy soil solution and rice tissues could be mainly attributed to microbial growth. For example, BC supplementation has increased the abundance of genes (*arrA* and *arsC*) associated with As(V) reduction into As(III) (Wang et al., 2017). Recent studies revealed that BC application leads to an increase in the abundance of Fe(III) reducing bacteria. Wang et al. (2017) showed that the relative abundance of *Clostridium, Geobacter*, *Bacillus*, *Caloramator*, *Desulfitobacterium*, and *Desulfosporosinus*, which are closely involved in Fe(III) reduction, increased with rice straw BC application. The high concentration of salts in BC results in increased electrical conductivity in the soil solution and promotes electron transfer between Fe(III) reducing bacteria and Fe(III) minerals (Kappler et al., 2014). As a result, As(V) retained in FeOOH could be released into the soil solution in the form of As(III) (Figure 2) The increased abundance of microorganisms could correspond to the relatively high surface area and development of pore structure in BC, which provides a stable habitat for microbial growth. Another possible reason for increased microbial growth is the DOC released from BC. Furthermore, changes in the physicochemical properties in the paddy soil system with BC supplementation can increase As mobility. For instance, BC application increases soil pH. As a result, soluble minerals from BC are hydrolyzed, leading to elevated OH^- levels in the soil solution (Yin et al., 2017). For this reason, As retained in the soil matrix could be desorbed through ligand exchange.

The use of surface modified BC instead of pristine BC is, therefore, an alternative method to alleviate As uptake and accumulation in rice tissues (Table 3). Redox-sensitive elements such as Fe and Mn can be successfully used for surface modification of BC (Lin et al., 2017; Liu et al., 2017). Surface modification may enhance the physico-chemical properties of BC. For example, modification of BC with nano-zero valent Fe increases the surface area in modified BC which markedly increases the reaction sites in BC (Liu et al., 2017). Both Fe and Mn lead to complexation with As(V) and As(III), thereby decreasing As bioavailability in the rice rhizosphere (Figure 2). In addition, recent studies have revealed that Fe and/or Mn-modified BC increases the root plaque formation and consequently the As retention capacity in root plaques. Therefore, BC composites (i.e. impregnating both Fe or/and Mn) greatly reduce As accumulation in rice tissues (Yin et al., 2017; Yu, Qiu, et al., 2017). Application of Fe amendments through Fe-modified BC might be a cost-effective and time-saving option to reduce As bioavailability in the paddy soil solution. The addition of modified BC may simultaneously reduce As accumulation in rice tissues and enhance plant growth parameters since it contains important plant nutrients such as K (Awad et al., 2018; Lin et al., 2017).

The contrasting outcomes due to BC supplementation could be well related to the physico-chemical properties of BC under which it is produced, as well as the specifics of the soils. Some BC types have reduced both inorganic and methylated As species in rice tissues (Khan et al., 2014). This is because BC amendment could increase the *arsM* gene abundance in paddy soils which may promote the volatilization of As. However, the role of BC on microbial As methylation and volatilization is yet to be investigated in detail. Furthermore, precautions are required before applying BC into paddy soils since BC may also contain As itself (Wang et al., 2017; Yin et al., 2017). Last but not least, in-depth studies would provide a mechanistic understanding of the interaction between As species and pristine/modified-BC in paddy soil-water systems and the optimal BC application rate.

4. Arsenic mitigation strategy through biological methods

4.1. Bioremediation potential of rhizospheric microorganisms

Rhizosphere microbiome activities can influence As speciation in the rice rhizosphere (Figure 3). Arsenic-resistant microorganisms can grow in environments containing a high concentration of As that would be highly toxic to other organisms (Bachate, Cavalca, & Andreoni, 2009; Hayat, Menhas, Bundschuh, & Chaudhary, 2017). Singh, Srivastava, Rathaur, and Singh (2016) found that inoculation of As-tolerant bacterial strains (i.e. *Staphylococcus arlettae* (NBRIEAG-6), *Staphylococcus* sp. (NBRIEAG-6), and *Brevibacillus* sp. (NBRIEAG-6)) can decrease the total As accumulation in rice grains by 30–40% when rice plants are grown in As contaminated paddy soils (30 and 15 mg kg^{-1} of As(V) and As(III), respectively). Microbes-mediated As(III) oxidation into As(V) can be considered as a detoxification phenomenon in the rice agroecosystems (Zhang, Zhao, et al., 2015). Some of the As(III) oxidizing microorganisms are autotrophs which utilize As(III) as the electron donor for the respiration, while others are heterotrophs depending on organic C for growth (Sun et al., 2009). Some microorganisms (i.e. *Paracoccus* sp.) could oxidize As(III) by using either NO_3^- (flooded conditions) or O_2 (non-flooded conditions) as the electron acceptor (Zhang, Zhou, et al., 2015). Zhang et al. (2017) found that NO_3^- additions into flooded paddy soils have significantly increased the abundance of As(III) oxidizing microorganisms and subsequently decreased the bioavailable As levels in the paddy soil solution. The most likely reason is that the oxidation of As(III) to As(V) by As(III) oxidizing microorganisms may lead to sequestration of As(V) in the root plaque and the paddy soil matrix. Even though microbes-driven As(III) oxidation is a slow phenomenon, it becomes prominent in flooded paddy soils once the abiotic conversion of As(III) oxidation ceases (Dong, Yamaguchi, Makino, & Amachi, 2014).

Microbial driven As methylation and volatilization is another important aspect for decreasing As accumulation in rice grains (Ye, Rensing, Rosen, & Zhu, 2012). Both As(III) and As(V) are converted to methylated As species through a sequential conversion by microorganisms (Figure 3): MMA(V) → monomethylarsonous acid (MMA(III)) → DMA(V) → dimethylarsinous acid (DMA(III)) → arsines (monomethylarsine ($(CH_3)AsH_2$), dimethylarsine ($(CH_3)_2AsH$), trimethylarsine ($As(CH_3)_3$, and arsine (AsH_3)) (Jia et al., 2012; Qin et al., 2006). Indigenous microorganisms in paddy soil have a limited effect in converting inorganic As species into volatile As compounds, possibly due to

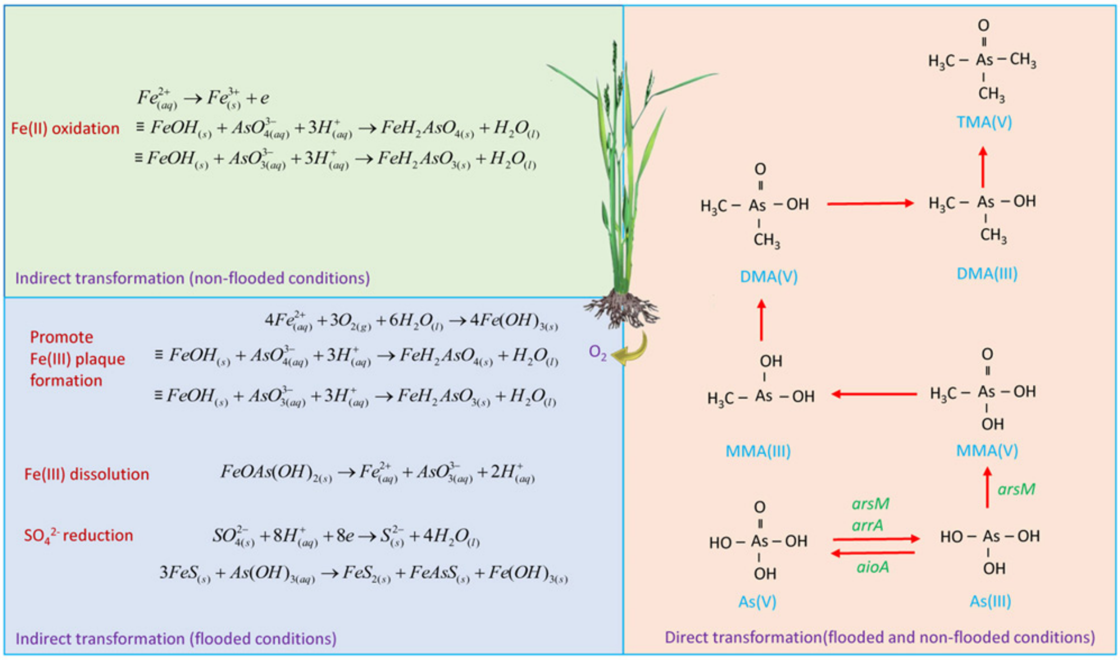


Figure 3. Direct and indirect As transformation pathways mediated by microbes in the rice rhizosphere.

the slower conversion rates (Majumder, Bhattacharyya, Kole, & Ghosh, 2013). However, the conversion rate of As methylation and volatilization can be increased through different approaches such as bioaugmentation (i.e. inoculation of microbes) and biostimulation (i.e. promoting microbial activities). Chen, Li, Wang, Zheng, and Sun (2017) showed that incorporation of rice straw (5%) and *pseudomonas putida* KT2440 removed 483.2 μg kg^{-1} of total As annually from arseniferous soils. Similarly, Ma et al. (2014) found that rice straw incorporation (6 t ha^{-1}) under field conditions increased the *arsM* gene abundance in the paddy soils. The decomposition of rice straw introduces DOC into the paddy soil solution and increases As(III) substrate availability for the stimulation of As volatilization. Since the half-life of arsines is approximately 8 h, emission of volatilized As compounds into the atmosphere would not be toxic to organisms (Hayat et al., 2017; Mestrot, Merle, Broglia, Feldmann, & Krupp, 2011). In addition, the possible dilution effect by air may decrease the concentration of volatilized As compounds. However, microbial-mediated As volatilization flux represents very small portion of the total As in paddy soils. Therefore, further studies are needed for enhancing the portion of microbial-driven As volatilization in the paddy environment.

Microbial driven indirect pathways also lead to a reduction in As accumulation in rice tissues (Figure 3). The presence of Fe(II) and Mn(II) oxidizing microbes may increase root plaque formation to sequestrate As (Dong et al., 2016; Somenahally, Hollister, Yan, Gentry, & Loeppert, 2011). Dong et al. (2016) observed that inoculation of Fe(II)/Mn(II) oxidizing bacterial strains (i.e. D54 and TWD-2) increased the Fe(III)/Mn(IV) plaque formation in rice roots and subsequently decreased the total As accumulation in rice tissues. Similar studies by Lakshmanan et al. (2015) also demonstrated that indigenous bacterium, EA106, promotes Fe plaque formation in rice roots. Microbial SO_4^{2-} reduction into S^{2-} also immobilizes As(III) due to the precipitation of As_2S_3-like complex and FeS minerals. For example, Jia, Bao, and Zhu (2015) found that the inoculation of SO_4^{2-} reducing bacteria and supplying SO_4^{2-} into paddy soils reduced the total As level in rice roots by approximately 23%. As mentioned earlier, water management practices remarkably affect the abundance of rhizosphere microbes. The abundance of As-, Fe-, and SO_4^{2-}-reducing bacteria increased in flooded paddy soils, whereas the abundance of As-, Fe-, and SO_4^{2-}-oxidizing bacteria increased in non-flooded paddy soils (Ghosh & Dam, 2009; Weber, Achenbach, & Coates, 2006). Das, Chou, Jean, Liu, and Yang (2016) observed that the As(V), Fe(III), and SO_4^{2-}-reducing genera (i.e. *Anaeromyxobactor*, *Desulfuromonas*, *Desulfocapsa*, *Desulfobulbus*, *Geobacter*, *Lacibactor*, and *Ohtaekwangia*) were present in higher abundance under flooded conditions, while the Fe(II) and S^{2-}-oxidizing genera (i.e. *Acinetobacter*, *Lysobacter*, *Ignavibaterium*, and *Thiobacillus*) were found in higher abundance under non-flooded conditions.

The main drawback of microbial inoculation is that inoculated microorganisms may not adapt to field conditions and may be out-competed by indigenous microorganisms. Therefore, identification and inoculation of microorganisms in multifarious paddy environments are still not practically employable. Inorganic and methylated As species uptake kinetics driven by microorganisms also need to be investigated in detail to identify rate-limiting factors.

4.2. Use of As hyperaccumulating plants

Phytoremediation is a commonly applied technology which aims to remove detrimental substances from contaminated soil and water; it has high public acceptance because it is a cost-effective option and is environmentally friendly (Kumarathilaka, Wijesekara, Bolan, Kunhikrishnan, & Vithanage, 2017; Usman et al., 2012). The prerequisite for efficient phytoremediation is the existence of metal hyperaccumulators (Mandal, Purakayastha, Patra, & Sanyal, 2012a; Rizwan et al., 2016; Shelmerdine, Black, McGrath, & Young, 2009; Srivastava, Ma, & Santos, 2006; Wang et al., 2002). Introduction of hyperaccumulating plant species into As contaminated rice fields may decrease total As level in rice plant tissues over time. The key mechanisms, including As(V) reduction and subsequent sequestration of As(III)-thiol complexes, may be involved in the successful accumulation of As in hyperaccumulating plant species (Wang et al., 2002; Zhang, Cai, Downum, & Ma, 2004). Ye, Khan, McGrath, and Zhao (2011) demonstrated that *Pteris vittata* grown (9 months) in As contaminated paddy soils (7.6–74.3 mg kg^{-1}) decreased the total As concentration and bioavailable As fractions (i.e. phosphate-extractable fraction) by 3.5–11.4% and 18–77%, respectively. In a recent study by Praveen, Mehrotra, and Singh (2017), rice plants were grown alongside other As accumulators (*Phragmites australis*, *Vetiveria zizanioides*, *P. vittata*) to minimize As accumulation in rice shoots and grains. Hyperaccumulating plants are capable of decreasing methylated As species in rice grains as well. *P. vittata*, for instance, decreased 100% of DMA(V) in rice grain samples (Ye et al., 2011). The influence of growing *P. vittata* on decreasing the level of methylated As species in rice grains could be possibly due to the least inorganic As concentrations in soil pore water, which might consequently decrease As methylation mediated by microorganisms (Ye et al., 2011).

The key factor behind an efficient phytoremediation process is the high concentration of bioavailable fractions of As in the rice rhizosphere (Antoniadis et al., 2017; Petruzzelli, Pedron, Rosellini, & Barbafieri, 2015). The supplementation of chelating agents and soil amendments has a great impact on As bioavailability in the soil solution. Mandal, Purakayastha, Patra, and Sanyal (2012b) reported that growing *P. vittata* with PO_4^{3-} fertilizers (i.e. $(NH_4)_2HPO_4$) reduced the total As concentration in rice grains by 52% compared with the controls after two growth cycles. As mentioned earlier, PO_4^{3-} competes with As(V) for adsorption sites in the soil matrix and enhances As(V) availability for *P. vittata* uptake. The presence of arbuscular mycorrhiza fungi (AMF) (i.e. *Gigaspora margarita* and *Glomus mossease*) is also found to increase the As translocation factor in *P. vittata* (Trotta et al., 2006).

Decreased plant growth and yield are common problems when rice plants are grown along with hyperaccumulators (Ye et al., 2011). Reduced growth and yield could be attributed to the competition for space, light, and nutrients between the rice crop and the hyperaccumulator, and/or growth inhibiting root exudates produced by hyperaccumulating plants (Ye et al., 2011). Proper pruning of hyperaccumulators and adequate fertilizer supply can be used to enhance growth and yield of rice. For instance, Mandal et al. (2012a) found that PO_4^{3-} supplementation enhanced grain yield by 14% after two rice growing cycles in succession when grown along with *P. vittata*.

Field investigations are further required to confirm whether the effect is long lasting due to the possibility of a re-introduction of As species to the bioavailable pool after phytoremediation. Field experiments are yet to be performed to examine whether typical rice growing conditions are suitable for As hyperaccumulating plant species on a long-term basis. For example, the hyperaccumulator *P. vittata* prefers to grow in alkaline soils and in the presence of PO_4^{3-} rock (Lessl, Luo, & Ma, 2014; Wei, Sun, Wang, & Wang, 2006). Such conditions may not be suitable for high rice yields. Therefore, phytoremediation method requires further development to be a feasible option for remediation of As contaminated paddy fields. Another limiting factor in the phytoremediation approach is that subsistence farmers are not going to take rice fields out of production.

Gentle soil remediation options (GROs) were introduced in Europe as a sustainable means of phyto-managing of heavy metal contaminated soils (Kumpiene et al., 2014; Quintela-Sabarís et al., 2017). GROs consist of in-situ stabilization (i.e. by incorporating soil amendments) and plant-based options (i.e. phytoexclusion) which have been designed to reduce total trace metal levels in the soil and the bioavailable pool in the soil solution (Kidd et al., 2015; Touceda-González et al., 2017). The introduction of the GRO based approach into As affected paddy areas may decrease the accumulation of As in rice grains and minimize risks of As transfer via the food chain.

5. Manipulation of rice grain As though screening and genetic engineering

5.1. Screening of low As accumulating rice cultivars

Selection and breeding of rice cultivars which accumulate the least levels of As are among the best options to mitigate As exposure. Quantitative trait loci

(QTL) mapping is a powerful genetic approach to identify the number, position and effects of genetic factors, which control As concentration in rice tissues (Syed et al., 2016; Zhang et al., 2008). Norton et al. (2012) showed that rice varieties with a longer vegetative stage accumulate the least As concentration in their grains in comparison to varieties which have a shorter vegetative stage. For example, among the world's rice collection (WRC) consisting of 69 accessions, Local Basmathi and Tima (*indica* type) contained the lowest total As and inorganic As concentrations (Kuramata et al., 2013). QTL mapping studies further demonstrated that there are significant genotype, environment, and genotype-environment effects on As concentrations in rice grains (Kuramata et al., 2013; Syed et al., 2016).

The anatomy of the rice plant plays a crucial role in rice plants with the least As accumulation. For example, rice cultivars with high rates of ROL and a high volume of root porosity diffuse more O_2 into the rhizosphere than rice cultivars with low rates of ROL and a low volume of root porosity. Mei et al. (2009) observed a significant negative relationship between total As concentration in rice tissues (i.e. straw and grain) and rates of ROL and root porosity. This is because a high amount of O_2 diffused into the rhizosphere may oxidize highly mobile As(III) to less mobile As(V) and also enhance Fe(III) plaque formation which could sequester both As(III) and As(V). Background Fe concentration is an important aspect of controlling ROL and subsequent As sequestration in root plaques. For example, Wu, Li, Ye, Wu, and Wong (2013) demonstrated that same cultivar in the presence of gradient As concentrations has shown a gradual decrease in the rate of ROL. This could be attributed to the increased formation of Fe(III) plaque which then might act as a barrier to prevent O_2 being released from the roots under high As stress (Wu et al., 2013). Therefore, it is suggested that field geochemical parameters need to be investigated before introducing the less As accumulating rice cultivars since they may behave differently in different local geological settings.

5.2. Transgenic rice with low As

Arsenic detoxification mechanisms in rice plants are important aspects of selecting cultivars for breeding purposes and producing transgenic rice plants (Awasthi, Chauhan, Srivastava, & Tripathi, 2017; Chen, Han, et al., 2017). In rice roots, As(V) is reduced into the As(III) and As(V) reductase enzymes (OsHAC1;1 and OsHAC1;2, and OsHAC4) govern the reduction process (Shi et al., 2016; Xu, Shi, et al., 2017). The reduction of As(V) can be considered as a detoxification mechanism in rice plants for different reasons. Firstly, As(III) possesses a high affinity to GSH and PCs (Tripathi et al., 2013). Even in rice shoots and nodes, As(III) is complexed with thiol-rich peptides limiting As(III) loading into rice grains. Secondly, As(III) can be released back into the environment via efflux transporters such as Lsi1 (Zhao et al., 2010).

A number of studies have reported that introducing exogenous genes leads to minimized grain As accumulation in transgenic rice plants (Duan, Kamiya, Ishikawa, Arao, & Fujiwara, 2012; Shri et al., 2014). The introduction of Arsenic Compounds Resistance protein 3 (ACR3) in yeast was found to decrease total As accumulation in transgenic rice grains by approximately 20% (Duan et al., 2012) because ACR3 enhances As(III) release from the rice root into the soil solution. The introduction of As methylation and volatilization genes into rice plants may also reduce highly toxic inorganic As species levels in rice grains. For example, Meng et al. (2011) found that transformation of the *arsM* gene into rice has increased volatile As species in transgenic rice plants by 10-times compared to the control rice plants. Nevertheless, the percentage of the volatile As species was only 0.06% out of the total As in rice plants (Meng et al., 2011). Therefore, enhancement of As methylation and volatilization rates coded by *arsM* gene in transgenic rice plants needs to be further studied. Moreover, it is essential to examine in detail the phytotoxicity of the intermediate organic As species during the methylation process catalyzed by the *arsM* gene in transgenic rice plants.

Altering the expression of transporters associated with the As metabolism may decrease As accumulation in rice tissues. Overexpression of transporters responsible for the vacuolar sequestration of As(III) in rice roots may lead to decreased As levels in rice shoots. However, As(III)-PC complexation is the critical step for As(III) transport into the vacuoles. Therefore, simultaneous expression of OsABCC1 transporter and PC synthase in transgenic rice plants might maximize As sequestration in roots. In addition, overexpression of As(V) reductase enzymes (OsHAC1;1 and OsHAC1;2, and OsHAC4) in transgenic rice plants may increase As(III) efflux back into the environment, leading to less As level in rice grains.

Gene editing techniques, such as clustered regularly interspaced short palindromic repeats and associated protein 9 (CRISPR/Cas9), are useful tools for gene function characterization in rice plants (Zhou et al., 2017). Therefore, the CRISPR/Cas system can be used to target critical genes (i.e. OsPHT1;8, Lsi1, and Lsi2) responsible for As uptake and translocation in rice plants. The mutations in OsPHT1;8, Lsi1, and Lsi2 through the CRISPR/Cas may lead to decreased As levels in rice grains. However, altering As(III) and As(V) transporters might reduce nutrient (PO_4^{3-} and $Si(OH)_4$) uptake in rice plants resulting in nutrient deficiency syndromes. Therefore, it is essential to investigate rice cultivars in which OsPHT1;8, Lsi1, and Lsi2 efficiently take up and transport PO_4^{3-} and $Si(OH)_4$ over inorganic and organic As species.

Overall, endogenous genes and an introduction of exogenous genes in transgenic rice plants may decrease the As burden in rice plants. However, growth parameters such as grain yield and grain quality in transgenic rice plants must be closely monitored to ensure the sustainability of rice production. Knowledge and technical skills are of paramount importance for these methods to succeed.

6. Concluding remarks

A multifaceted and interdisciplinary understanding of As biogeochemistry in paddy agroecosystems and the mechanisms in As metabolism in rice plants is important to ensure low As levels in the paddy soil solution and rice tissues. Water management, physico-chemical, and biological methods or combinations of these methods can be successfully adopted to decrease inorganic and methylated As species content in paddy agroecosystems. Even though each technique has its limitations, the advantages far outweigh the disadvantages.

Further studies should consider whether water management practices with different intervals are widely applicable across different geographical settings, weather conditions, and As-Cd levels in paddy environments. Even though different soil amendments such as Fe and Mn can decrease As uptake in rice environments, those may be toxic to microorganisms that promote rice plant growth, at high supplementation rates. Thus, estimation of the abundance of microorganisms under different soil amendments at the field scale must be conducted to ensure efficacy and safety for the practical application of amendments in As contaminated paddy environments. Recent studies have found that supplementation of S in paddy environments leads to the formation of thioarsenates which are more or less toxic, like As(V). Therefore, uptake, translocation and grain filling mechanisms of thioarsenates in rice plants and potential toxicity and detoxifying mechanisms developed in rice plants need to be assessed in detail before promoting S supplementation in As contaminated paddy environments. Sample preparation, preservation, and analytical methods need to be developed for the accurate and precise quantification of different thioarsenates. Another important question is whether Fe plaque can reduce As accumulation in rice grains. Evidently, Fe plaque sequesters both inorganic As species; however, whether Fe plaque restricts As uptake remains unclear. Therefore, a better understanding of As speciation dynamics near rice roots and consequent As uptake by rice roots is required. Since balanced nutrient capital of BC for As contaminated paddy environments is uncertain, the supplementation rate and complementarity of BC, together with fertilizer inputs, may also need to be assessed.

In terms of biological approaches, the rate of microbial-driven As(III) oxidation and As volatilization in As-contaminated paddy environments needs to be increased to utilize the eco-friendly approach in a sustainable manner. It is important to identify rate limiting factors in microbe-mediated As(III) oxidation and As volatilization processes in order to take measures to stimulate the reactions. Increased root porosity and high rate of ROL in rice roots may promote aerobic conditions in the rice rhizosphere and subsequently reduce As bioavailability. Studies related to the rice root anatomy would produce rice plants with increased root porosity and a high rate of ROL. It is also necessary to investigate how rice and other plant species metabolize As and thus new endogenous and exogenous genes become available for mitigation of As accumulation in rice grains.

Further studies are needed to confirm integrated approaches employing water management, soil amendments, and biological methods to select the best combination/s of countermeasures for the decreased As accumulation in rice grains. Cost-effectiveness for each agronomical, physico-chemical, and biological approach with respect to various As affected localities and among different rice cultivars also needs to be analyzed to select the most practical and economically feasible approach(es).

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2.3.3 Concluding remarks

Alternative water management practices such as intermittent irrigation and aerobic irrigation may reduce the accumulation of As in rice grains more than those of conventional flooded irrigation practices. However, studies are required to investigate the effects of alternative water management practices on rice yield. The incorporation of Si sources into As-contaminated paddy soils may lead to a reduced As(III) uptake in rice plants. The application of redox sensitive elements such as Fe and Mn into As-contaminated paddy rice soils may enhance the immobilization of As in the rice rhizosphere. The production of BC from Si-rich organic feedstocks would be a sustainable and cost effective way to tackle As accumulation in rice tissues since Si fertilization is limited in As-contaminated paddy rice soils, due to the scarcity and high cost of Si sources. Modification of BC with Fe and Mn may also stimulate the formation of Fe and Mn plaque in the rice rhizosphere. Biochar in paddy rice soils may also promote biostimulation and consequently, microbes may decrease bioavailability of As in the rice rhizosphere through methylation and volatilization of As. Since BC contains and slowly releases important plant nutrients into the soil solution, incorporation of pristine and modified BC into As-contaminated paddy rice soils may simultaneously reduce the accumulation of As and promote the quality and quantity of rice grains. There is no report of the effect of pristine/modified BC under different water management practices to reduce the accumulation of As in rice tissues and therefore, it is a worthwhile subject for study.

CHAPTER 3

An integrated approach of rice hull biochar-alternative water management as a promising tool to decrease inorganic arsenic levels and to sustain essential element contents in rice

Article IV

Kumarathilaka, P., Bundschuh, J., Seneweera, S., Ok, Y.S., (2021). An integrated approach of rice hull biochar-alternative water management as a promising tool to decrease inorganic arsenic levels and to sustain essential element contents in rice. Journal of Hazardous Materials, 405, 124188. (Published).

3.1 Introduction

The health risks associated with the ingestion of inorganic As through rice consumption are a global concern. Conventional, long-term flooded water management practices have increased bioavailable inorganic As concentrations in the paddy agro eco-system. Alternative water management practices such as intermittent irrigation have been found to decrease As concentration in rice tissues; however, loss of rice yield is associated with intermittent irrigation practices. Supplementation of BC to As-contaminated paddy rice soils would be a sustainable option in decreasing As bioavailability and increasing the rice yield. So far, no studies have examined the integrated effect of BC supplementation with alternative water management practices in As-contaminated paddy rice soils. Moreover, none of the studies have investigated essential elemental concentrations in rice grains, following the addition of BC into As-contaminated paddy rice soils. Taking these facts into account, this study investigated, for the first time, the integrated effect of rice hull BC-water management practices in As-contaminated paddy rice soils on As speciation in rice roots, shoots, husks, and unpolished rice grains, microbial diversity in the rice rhizosphere, and essential elemental concentration in rice grains.

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An integrated approach of rice hull biochar-alternative water management as a promising tool to decrease inorganic arsenic levels and to sustain essential element contents in rice

Prasanna Kumarathilaka[a], Jochen Bundschuh[a,b,*], Saman Seneweera[c,d], Yong Sik Ok[e]

[a] School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia
[b] UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia
[c] Centre for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia
[d] National Institute of Fundamental Studies, Hantana Road, Kandy 20000, Sri Lanka
[e] Korea Biochar Research Center & Division of Environmental Science and Ecological Engineering, Korea University, Seoul, South Korea

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Essential elements

ABSTRACT

Arsenic (As) in rice agroecosystems causes a loss of both rice yield and quality of rice grains. In this study, an integrated approach of biochar (BC) and alternative water management is proposed to reduce As content while sustaining essential elemental concentrations in rice. The rice cultivar, *Jayanthi*, was grown, irrigated with 1 mg L^{-1} of As-containing water, under rice hull BC (RBC)-flooded, RBC-intermittent, conventional flooded, and intermittent treatments. The RBC has increased rice yield by 11%−19% in RBC-intermittent and -flooded treatments compared to the flooded treatment. Inorganic As content in rice tissues and abundance of Fe(III) reducing bacteria in the rhizosphere were lowered by 10%−83% and 40–70%, respectively, in RBC-flooded, -intermittent, and intermittent treatments over flooded treatment. Essential elemental concentrations (Fe, Mn, Zn, Mg, and Ca) in unpolished rice grains increased by 45%−329% in RBC-flooded and -intermittent treatments compared to flooded treatment. Overall, the integrated approach of RBC-intermittent practices has lowered inorganic As concentration in unpolished rice grains, while sustaining the levels of essential elements in rice grains, compared to other treatments. An integrated approach of RBC-intermittent practices is suggested for rice grown with As-contaminated water to improve the quality of rice, as well as tackling food-related malnutrition in people.

1. Introduction

The health risks related to ingestion of inorganic arsenic (As) through rice consumption is a worldwide concern. Both natural processes (i.e. weathering of As-bearing rocks and alluvial deposits) and anthropogenic activities (i.e. mining operations, wastewater discharge, and use of arsenical pesticides) lead to the distribution of As in rice agroecosystems (Kumarathilaka et al., 2018a; Wu et al., 2019). Use of groundwater, with high concentrations of mostly geogenic As, for irrigating rice is the major cause of accumulating As in rice tissues. The Food and Agricultural Organization's (FAO) recommended level for total As in irrigation water is <100 μg L^{-1}. However, irrigation water exceeding the FAO's recommended level has been extensively used in major rice producing countries. For instance, Huang et al. (2016) demonstrated that groundwater containing 60–920 μg L^{-1} of As has been used in Vietnam for irrigating rice fields. In Bangladesh, India, and Nepal, As-contaminated groundwater (400–1010 μg L^{-1}) has also been extensively used for rice cultivation (Biswas et al., 2014; Dahal et al., 2008; Dittmar et al., 2007).

Rice accumulates higher levels of inorganic As than other cereal crops. Conventional rice cultivation practices (long-term flooded conditions and short-term non-flooded conditions) cause higher levels of bioavailable inorganic As in paddy soil-water systems and subsequently in rice tissues than other cereal crops (Bakhat et al., 2017). Physico-

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[*] Corresponding author at: School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia.
E-mail address: jochen.bundschuh@usq.edu.au (J. Bundschuh).

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chemical properties such as pH, redox potential (Eh), organic matter, redox sensitive ions (i.e. Fe, Mn, and S), and Si and PO_4^{3-} contents, and microbial diversity influence the mobility and bioavailability of As and As speciation (i.e. arsenite (As(III)), arsenate (As(V)), dimethylarsinic acid (DMA(V)), monomethylarsonic acid (MMA(V))) in paddy agroecosystems (Kumarathilaka et al., 2018b; Li et al., 2019a; Sun et al., 2019).

Physico-chemical and biological methods have been tested to minimize the accumulation of As in rice tissues. However, there are limitations to the practical employability of the above mentioned methods (Kumarathilaka et al., 2020; Li et al., 2019b). For example, supplementation of nutrients such as P, Si, and S to As-contaminated paddy environments is limited, due to the high cost and source scarcity (Desplanques et al., 2006; Mew, 2016). The incorporation of Fe and Mn sources into As-contaminated paddy soils needs to be considered, because of their cost and the potential to the secondary contamination. The main drawback of microbial inoculation to As-contaminated paddy agroecosystems is that inoculated microbes may not adapt to field conditions. Long-term field experiments need to be performed to investigate whether typical rice cultivation conditions are suitable for As hyperaccumulating plant species (Kumarathilaka et al., 2020). Alternative water management practices such as intermittent irrigation have proven to lessen As levels in paddy ecosystems more than conventional flooded water management. However, even though intermittent irrigation practices lead to lessen As accumulation in rice tissues, loss of grain yield has been reported (Chou et al., 2016; Moreno-Jiménez et al., 2014).

The application of biochar (BC) to As-contaminated paddy soils might be a viable option in terms of economic feasibility and practical employability. Biochar is produced through pyrolysis of different biomass types including those from forestry and agricultural crop residues, the organic portion of municipal solid wastes, invasive plant species, animal manures, and wood waste (Ghani et al., 2013; Jayawardhana et al., 2018; Tomczyk et al., 2020). Biochar in soil systems has a wide assortment of benefits such as reducing mobility and bioavailability of heavy metal(loids), improving soil nutrient content, increasing water holding capacity, providing habitat for microbial community, and minimizing greenhouse gas emissions through carbon sequestration (Kumarathilaka et al., 2018c; Xu et al., 2012).

Even though BC has been amended to As contaminated soils, very limited studies have focused on the role and potential of BC in As-contaminated paddy ecosystems. Yin et al. (2017) have applied rice straw BC produced at 450 °C to As-contaminated paddy soils. Khan et al. (2014) have produced sewage sludge biochar to be amended with As-contaminated paddy soils. The typical rice cultivation practices change the redox chemistry of the paddy soil-water system, therefore, in-depth study is needed to understand the interaction between BC and As species in paddy soil-water systems. Moreover, an integrated approach of BC supplementation and intermittent irrigation might sustain the rice yield and improve the rice quality for safer human consumption. There are no reports on the integrated effects of BC and water management practices on As dynamics in paddy agroecosystems. In addition, no study has reported essential elemental levels in rice grains under the integrated approach of BC-water management practices in As-contaminated paddy ecosystems. Taking these facts into account, this study, for the first time, investigated the effect of BC amendments to As contaminated paddy soils under different water management practices on plant growth parameters, As speciation in rice tissues, microbial diversity in the rice rhizosphere, and essential elemental concentration in rice grains. Rice hull was used as the feedstock for producing BC since rice hull could contain high level of Si. Since the supplementation of Si to As-contaminated paddy soils is limited, due to the scarcity of resource and its high cost, the application of Si-rich rice hull BC (RBC) may also be a cost-effective and practically employable option to decrease the accumulation of As in rice tissues. Both flooded and intermittent water managements were practiced in this study.

2. Materials and methods

2.1. Paddy soil collection and characterization

Paddy soils were collected from the ploughing layer (0–20 cm) in New South Wales, Australia (34° 35′ 53.5″ S, 146° 21′ 38.1″ E). A composite sample was prepared by mixing each subsample. After being air-dried, the soil samples for pot experiments and chemical analysis were passed through 5 and 2 mm sieves, respectively. The pH and electrical conductivity (EC) of the soil were measured in 1:10 suspensions of soil-to-distilled water using a digital pH and conductivity meter (PC2700, EUTECH Instruments). A moisture meter (ISSCO, MB 120, OHAUS Corporation) was used to measure the moisture content of soils. Total organic carbon content of the soil was determined through a loss-on-ignition method (Rayment and Lyons, 2014). Cation exchange capacity of the soil was determined using the 1 M/ammonium extraction (pH adjust to 7.0) method (Rayment and Lyons, 2014). Soil samples were digested in a microwave digestion system (Multiwave 3000, Anton Paar) before being analysed for total concentration of metal(loids). The EDTA (Ethylenediaminetetraacetic acid) extraction solution was used to determine bioavailable concentrations of metal(loids). In brief, 20 mL of 0.05 M / EDTA was added to 1 g of soil. The mixture was then stirred for 3 h, and centrifuged, and the supernatant was filtered through the membrane filter technique. Arsenic concentration in paddy soils was measured using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer NexION™ 300X). The concentration of other elements was determined using an atomic absorption spectrophotometer (AAS) (AAS-7000, Shimadzu).

2.2. Biochar production and characterization

Rice hulls were obtained from Grain and Grape, Victoria, Australia to produce RBC. Rice hulls were subjected to a slow pyrolysis process under continuous N_2 flow in a muffle furnace (CS2, RIO GRANDE) at 600 °C for 2 h to produce RBC. The heating rate of the pyrolysis process was set as 7 °C min^{-1}. The produced RBC was ground and sieved through a 2 mm aperture to gain homogeneous particle size.

The pH and EC of RBC were measured in 1:5 suspensions of RBC-to-distilled water using the digital pH and conductivity meter. The proximate analysis for RBC (moisture content, volatile matter, ash content, and resident matter) were performed according to the modified thermal analysis methods described elsewhere (Ahmad et al., 2012). In brief, moisture content was determined by calculating weight loss when RBC was heated at 105 °C for 24 h. Volatile matter content was measured as the weight loss when RBC was heated at 450 °C for 30 min in a covered porcelain crucible. Ash content was also determined as the weight loss when RBC was heated at 700 °C for 30 min in an open-top porcelain crucible. Resident matter content was calculated using the difference between the portion of RBC which was not ash and moisture, volatile matter, and ash contents. Specific surface area of the RBC was determined through adsorption isotherms using the Brunauer–Emmett–Teller (BET) equation. Total pore volume and average pore size of the RBC were measured from the N_2 adsorption data using the Barret–Joyner–Halender (BJH) method. The morphology of the RBC was characterized by Scanning Electron Microscopy (SEM) (JCM-6000, JEOL).

2.3. Pot experiment

A pot experiment was conducted to examine the influence of water management and RBC amendment on the mobility and phytoavailability of As species in the soil solution and substantial uptake and accumulation in rice tissues. The rice cultivar, *Jayanthi*, was used for this study. Rice seeds were surface sterilized with 10% H_2O_2 for 5 min and germinated in moist compost. After three weeks, plants of uniform size (three seedlings per pot) were transferred into polyvinylchloride pots containing paddy soils (2.5 kg). Before transplanting, each pot was flooded with As-containing water (1 mg L^{-1}) and amended with RBC (1% w/w). There were four different treatments: (1) continuous flooding with RBC (1% w/w); RBC-flooded, (2) intermittent irrigation with RBC (1% w/w); RBC-intermittent, (3) conventional flooding without RBC; Flooded, and (4) intermittent irrigation without RBC; Intermittent. To ensure the wet cultivation of rice, in continuous flooding treatments, pots were flooded (~5 cm) during the entire growing season, whereas in the intermittent irrigation treatments, pots were only flooded (~5 cm) when the soil was dry and cracks were formed. This depth was maintained by adding As-containing water to the appropriate pots, having a gauge marked on the side. The total volume of water consumed during the whole growing season was calculated. All the treatments were in triplicate and regularly randomized to ensure uniform light and temperature.

2.4. Soil solution analysis

Soil solution samples were collected at 30-day intervals until harvest. The pH and EC of soil solutions were measured. The redox potential (Eh) was recorded using an oxidation-reduction potential (ORP) meter (YK-23RP, Mother Tool) by inserting the platinum electrode into the paddy soil surface. The Fe, Mn, and Si concentrations in the paddy water were determined using AAS. Ions such as PO_4^{3-}, NO_3^-, NO_2^- and SO_4^{2-} concentrations in the paddy water were measured by an ion chromatography system (ICS-2000, Dionex).

2.5. Plant growth parameters

Plant height, root length, shoot biomass, and grain production per pot were measured to evaluate the plant growth and yield under different combinations of RBC and water management regimes. Plant height was recorded at 30 day intervals by using a meter scale. The root length and grain production per pot were measured after harvesting the plants. Shoot biomass was determined after drying the harvested rice shoots in an oven (STERIDIUM) at 60 °C for 72 h.

2.6. Arsenic speciation analysis

Rice tissue samples (roots, shoots, husks, and unpolished grains) were digested in the microwave digestion system for As speciation analysis. Acid cleaned digestion vessels were used and 0.5 g of rice tissues was added into the vessels with 7 mL of 0.2 M HNO_3. The digestion program consisted of a 20 min ramping time to 95 ºC with a 90 min holding time, and a 10 min holding time before cool down. All digested solutions were diluted to 20 mL by adding Milli-Q water. Each sample was filtered through a glass fiber filter prior to analysis. The concentration of As species in extracted solutions was determined using Ultra-High Performance Liquid Chromatography – Inductively Coupled Plasma Mass Spectrometry (UHPLC-ICP-MS) (Flexar, PerkinElmer – PerkinElmer NexION™ 300X). Arsenic species were separated using an anion exchange column (PRP-X100, 250 × 4.1 mm, 10 μm (Hamilton, USA)). A solution containing 8.5 mM of $NH_4H_2PO_4$ and 8.5 mM of NH_4NO_3 (1:1) at pH 6.0 was used as the mobile phase which was pumped through the column isocratically (Herath et al., 2020). Arsenic species in each sample were identified by comparing their retention times with those of the standards (As(III), DMA(V), MMA(V), and As (V)). A certified reference material (CRM), ERM-BC211, was used to verify digestion efficiency in rice tissue samples.

2.7. Determination of essential elements

Unpolished rice grain samples were digested in the microwave digestion system . A weight 0.5 g of rice tissues (oven-dried at 65 °C for 48 h) was added to the vessels with 6 mL of conc. HNO_3 and 1 mL of 30% H_2O_2. The digestion program comprised a 20 min ramping time to 180 °C with a 45 min holding time and a 10 min holding time before cool down. All digested solutions were diluted to 20 mL by adding Milli-Q water. Each sample was filtered through a glass fiber filter prior to analysis. A method blank and continuing calibration verification (CCV) were run for each analytical batch. In addition, a certified reference material (CRM), ERM-BC211 was used to verify digestion efficiency in rice tissue samples. The concentration of elements such as Fe, Mn, Mg, K, Ca, Zn, Ni, and Co, were determined using AAS.

Table 1
Selected physico-chemical properties and elemental composition in paddy soils.

Parameter	Value	
pH	6.59 ± 0.06	
EC (dS m^{-1})	0.04 ± 0.01	
Moisture content (%)	5.79 ± 0.21	
Organic matter (%)	7.22 ± 0.36	
Cation exchange capacity (cmol/kg)	14.37 ± 1.16	
Metal(loid) concentration (mg kg^{-1})	Total	EDTA-Extractable
As	3.10 ± 0.27	0.04 ± 0.01
Fe	12,720.26 ± 130	1147.74 ± 22.5
Mn	288.15 ± 9.56	197.82 ± 12.3
Si	2190.10 ± 24.3	153.43 ± 14.6
Zn	32.55 ± 2.58	2.23 ± 0.29
Mg	877.05 ± 26.8	37.78 ± 5.31
K	1284.50 ± 33.4	66.65 ± 4.68
Ni	23.99 ± 1.27	2.35 ± 0.62
Co	119.05 ± 7.92	42.5 ± 4.93
Ca	5531.58 ± 29.6	513.64 ± 15.6
Al	43,718.86 ± 147	563.88 ± 13.8

2.8. Microbial diversity analysis

DNA was extracted from the freshly collected rhizosphere soils at the flowering stage of the rice by using the DNeasy® PowerSoil® Pro kit (QIAGEN), according to the manufacturer's instructions. The fluorometry has been used for quality assurance of samples for Polymerase Chain Reaction (PCR) analysis. The V3-V4 region of the 16S rRNA gene was amplified using a set of primers (forward primer: 341F (CCTAYGGGRBGCASCAG) and reverse primer: 806R (GGACTACNNGGGTATCTAAT). The pooled DNA products were utilized to make an Illumina Pair-End library. Paired-ends reads were assembled by aligning the forward and reverse reads using PEAR (version 0.9.5) on the Miseq platform (San Diego, CA, USA). Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8.4) software.

2.9. Statistical analysis

Statistical analysis of the experimental data was performed with SAS 9.1 software. Statistical graphing was done using Origin 6.0 software package. The statistical significance of As concentration in rice tissues under different combinations of RBC amendments and water management practices were determined by using Duncan's multiple range test with $p < 0.05$.

3. Results and discussion

3.1. Paddy soil characterization

Paddy soils were slightly acidic with a moisture content of 5.79% (Table 1). Paddy soils were characterized by a high amount of organic matter (7.22%) and were in the range of cation exchange capacity in typical paddy soils (Dong et al., 2016). Total As concentration in paddy soils (3.10 mg kg^{-1}) was well below the European Union's

Table 2
Physicochemical properties and EDTA extractable elemental concentrations in RBC.

Parameter	Value
pH	9.81 ± 0.09
EC (dS m^{-1})	0.73 ± 0.03
Proximate analysis (%)	
Moisture	1.08 ± 0.10
Volatile matter	22.88 ± 1.37
Ash	30.25 ± 2.68
Resident matter	45.79 ± 3.48
Specific surface area (m^2 g^{-1})	201.39
Total pore volume (mL g^{-1})	0.2026
Average pore diameter (nm)	4.0240
EDTA-extractable metal(loid) concentration (mg kg^{-1})	
As	ND
Fe	112.59 ± 5.67
Mn	170.70 ± 4.38
Si	246.31 ± 9.37
Zn	1.71 ± 0.35
Mg	229.37 ± 10.6
K	53.62 ± 6.58
Ni	1.38 ± 0.21
Co	42.96 ± 4.39
Ca	1771.74 ± 24.6
Al	50.69 ± 3.47

Note: ND: not detectable.

recommended maximum value of 20 mg kg^{-1} of As to be present in agricultural soils (Shrivastava et al., 2017). The EDTA-extractable As concentration was 0.04 mg kg^{-1} which was 1.29% of the total As content in paddy soils. Total Fe and Mn concentrations in the paddy soil were 12,270.26 and 288.15 mg kg^{-1}, respectively. Extractable percentage of Mn (68.65%) was higher than that of Fe (9.02%). Extractable concentrations of Fe and Mn exceeded the critical concentrations (10 and 5 mg kg^{-1}, respectively) in paddy soils (Pirzadeh et al., 2010). Paddy soils also contained other essential elements, including Si, Zn, Mg, K, Ni, Co, and Ca, which are important for the growth of rice plants as well as for protection against biotic and abiotic stresses (Table 1).

3.2. *Biochar characterization*

Characteristics of the RBC were summarized in the Table 2. The produced RBC possesses an alkaline nature (9.81). The resident matter of RBC is greater than that of mobile matter indicating loss of volatile matter through thermal decomposition. The amount of mobile matter and resident matter in RBC (68.67%) is an important factor in terms of short- and long-term carbon availability in paddy soils. A relatively high percentage of ash (30.25%) in RBC demonstrates that inorganic minerals and residues remain after the pyrolysis of RBC. The SEM image (Fig. S1) and BET data demonstrate the well-developed pore structure and the development of mesopores (4.0240 nm) in RBC.

EDTA extractable elemental concentrations indicated that As is not detectable in RBC. Redox sensitive elements including Fe and Mn (112.59 and 170.70 mg kg^{-1}, respectively) have been incorporated in RBC. Most importantly, RBC contains 246.31 mg kg^{-1} of extractable Si. In addition, RBC comprises essential elements such as Zn, Mg, K, Co, Ca, and Al (Table 2). The presence of redox sensitive elements and other essential elements in RBC may influence the As dynamics in the rice paddy-soil system and subsequent accumulation of As in rice tissues, while improving the quality of rice grains.

3.3. *Analysis of soil solution*

After 30 days of transplantation, paddy soil surface turned into moderately reduced conditions, as shown in Fig. 1(a). The Eh of each treatment gradually decreased over time for the following 90 days when there was a slight increase in Eh at day 120. Flooded water management practices have indicated a decreased Eh value compared to the Eh values of intermittent water management practices. Highly reduced conditions in flooded water management favor reduction of redox sensitive elements such as Fe and Mn which could directly influence the mobility and bioavailability of As in the paddy soil-water system. The pHs of RBC-

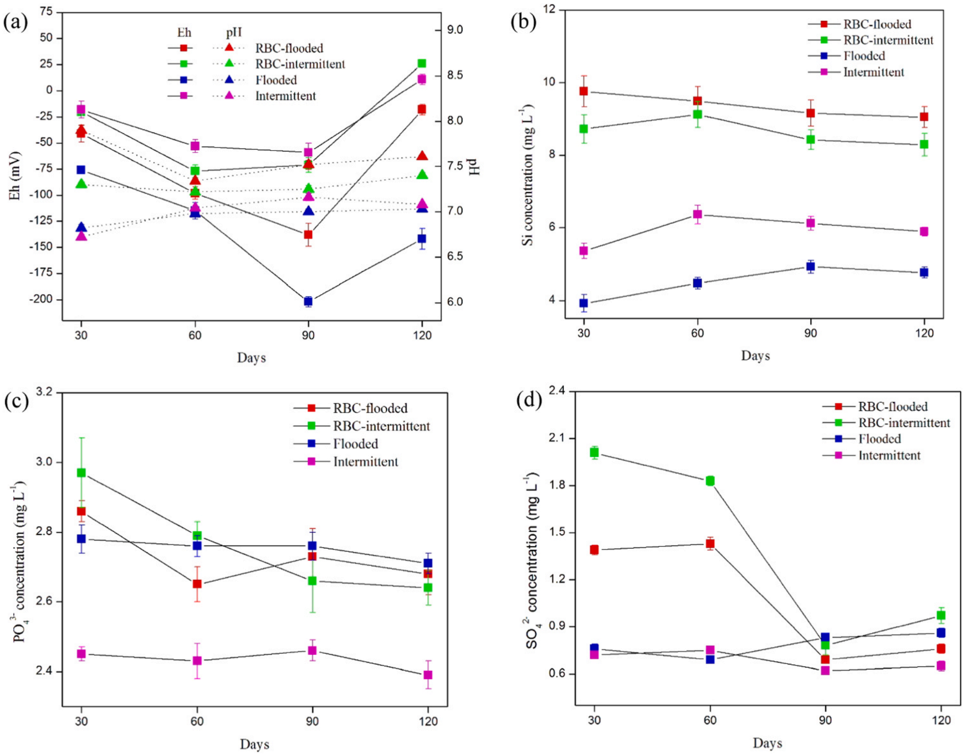

Fig. 1. Temporal variation of pH-Eh (a) and concentrations of Si (b), PO_4^{3-} (c), and SO_4^{2-} (d) in paddy water under different combination of RBC and water management methods.

Table 3
Plant growth parameters and water usage efficiencies per pot under different combination of RBC and water management approaches.

Parameter	RBC-flooded	RBC-intermittent	Flooded	Intermittent
Plant height (cm)				
30 days after transplanting	10.67 ± 0.11	11.39 ± 0.12	10.50 ± 0.09	10.17 ± 0.07
60 days after transplanting	15.61 ± 0.16	15.11 ± 0.08	16.00 ± 0.07	14.17 ± 0.11
90 days after transplanting	20.42 ± 0.14	18.39 ± 0.09	19.06 ± 0.10	18.50 ± 0.12
120 days after transplanting	41.26 ± 0.19	38.93 ± 0.22	38.47 ± 0.16	39.01 ± 0.36
At the harvest (142 days)	52.42 ± 0.32	47.33 ± 0.28	45.72 ± 0.37	47.28 ± 0.40
Grain weight (g)	11.32 ± 0.15	10.54 ± 0.27	9.49 ± 0.19	8.83 ± 0.23
Root length (cm)	27.72 ± 1.57	22.19 ± 1.37	21.17 ± 0.92	20.38 ± 1.12
Shoot dry weight (g)	16.61 ± 0.43	16.06 ± 0.27	15.33 ± 0.15	14.38 ± 0.17
Water usage (cm^3)	11,167	8815	13,300	8994

flooded and -intermittent treatments were higher than that of the flooded and intermittent treatments (Fig. 1(a)). The alkaline nature of RBC may have increased pH in RBC-flooded and -intermittent treatments.

Incorporation of RBC to paddy soils increased the Si concentration in paddy water (Fig. 1(b)). In comparison, RBC-flooded treatment indicated a 46.3% increment of Si concentration compared to the flooded treatment after 120 days of transplantation. Similarly, RBC-intermittent treatment showed a 27.3% increment of Si levels in the paddy water, which was greater than that of intermittent treatment. As summarized in Table 2, RBC contained 246.31 mg kg^{-1} of EDTA-extractable Si which might be continuously released into the paddy soil-water system. The availability of Si in the paddy soil–water system is an important factor for a reduced As(III) uptake by rice roots (Kumarathilaka et al., 2018b). Silicon can also increase the plant resistance to abiotic (i.e. wind and strong rain) and biotic stresses (i.e. insect and fungal infections). Due to the scarcity of the resource, as well as high cost, Si supplementation in As-contaminated paddy ecosystems is limited. Since rice hulls contain 246.31 mg kg^{-1} of EDTA-extractable Si, the incorporation of RBC may be a cost-effective method to introducing Si into As-contaminated paddy soil-water systems for decreasing As uptake by rice roots.

Anions such as PO_4^{3-} and SO_4^{2-} were detected in paddy water in all treatments (Fig. 1(c) and (d)). The PO_4^{3-} concentration in all the treatments slightly varied throughout the rice growing cycle (2.43–2.97 mg L^{-1}). The presence of PO_4^{3-} in paddy water led to a reduced uptake of inorganic As(V) by rice plants. The RBC-flooded and -intermittent treatments have shown approximately two times higher SO_4^{2-} concentrations in paddy water than in flooded and intermittent treatments. The SO_4^{2-} in the paddy water may also decrease the accumulation of As in rice grains by alternating the metabolism in rice plants (Zhao et al., 2009). Concentrations of NO_3^- and NO_2^- in paddy water in all treatments were below the limit of detection (LOD).

3.4. *Plant growth parameters*

Table 3 summarizes plant growth parameters and water usage in all treatments. Rice plants in the RBC-flooded treatment recorded the highest plant height, followed by the RBC-intermittent, intermittent, and flooded treatments. Similarly, highest grain weight per pot (11.32 g) was recorded in the RBC-flooded treatment, which was an approximately 19% greater increment of rice yield compared to the flooded treatment. Root length and shoot dry weight were also highest

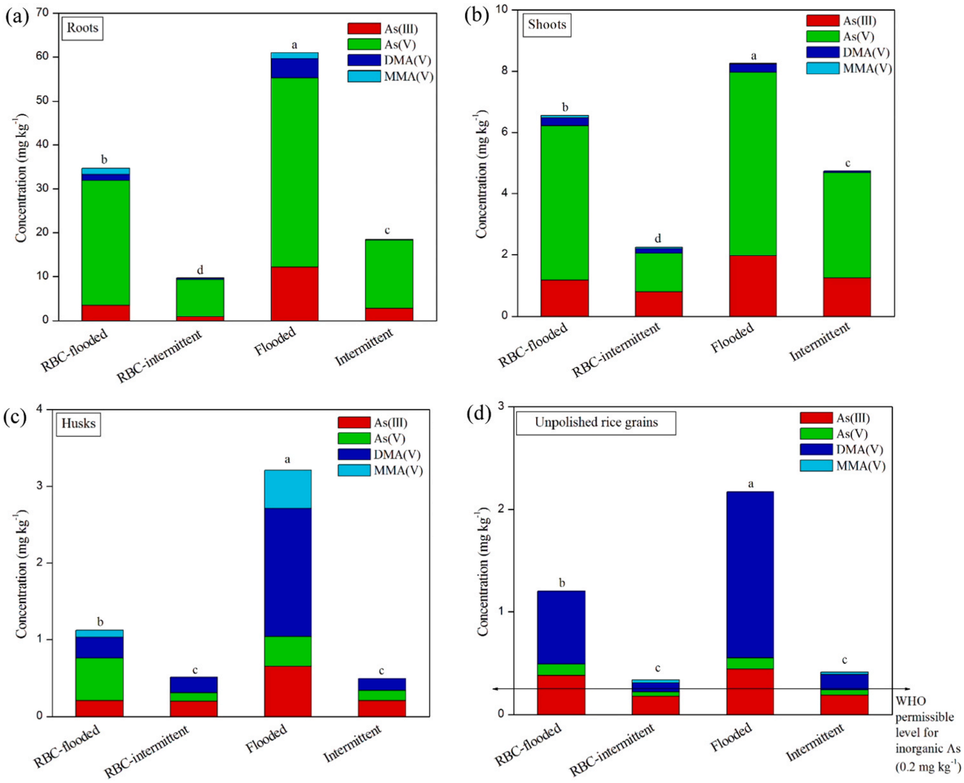

Fig. 2. The concentration of As species in rice roots (a), shoots (b), husks (c), and unpolished rice grains (d) under different combination of RBC and water management regimes. Treatments labelled with same letter are not significantly different from each other for the total As concentration (Duncan's multiple range test; $p < 0.05$).

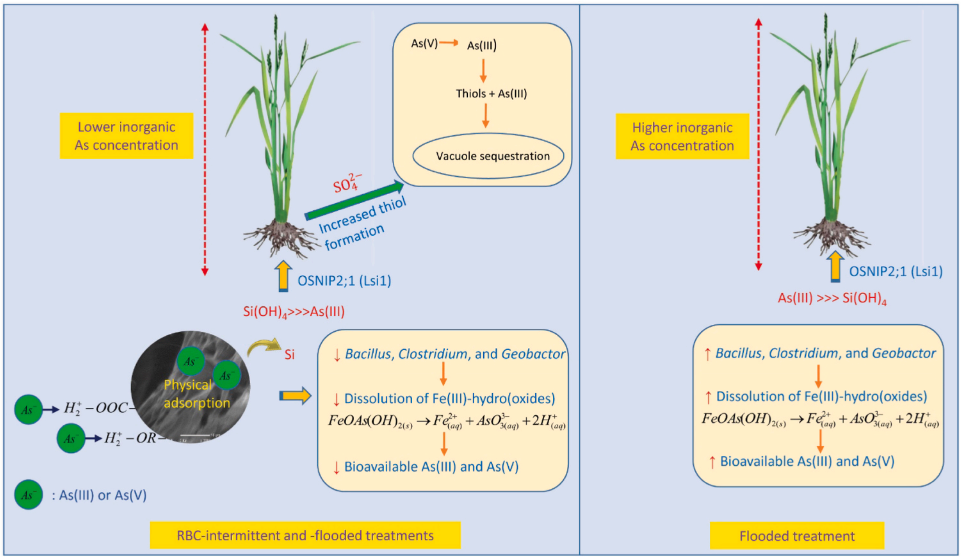

Fig. 3. Possible mechanisms for lowered As accumulation in rice tissues under RBC-intermittent and -flooded treatments compared to flooded treatment.

in the RBC-flooded treatment. The reductive conditions associated with flooded water management may prevent aerobic microbe-related disease damage (Minamikawa et al., 2015). The addition of RBC to paddy soils may also steadily release essential elements into paddy soil solution for plant uptake. As a result, RBC-flooded treatments have reported highest yield and other plant growth parameters such as plant height, root length, and shoot weight.

Water usage efficiency was higher in RBC-flooded and -intermittent treatments than in flooded and intermittent treatments. For instance, RBC-flooded treatment indicated an approximately 16% lower water consumption than that of flooded treatment. The supplementation of RBC to paddy soils may have improved the water holding capacity of paddy soils. However, RBC-intermittent treatment has shown only a 2% less water consumption over the intermittent treatment. During the intermittent irrigation practices, paddy soil is only flooded when the soil becomes dry. This may be a reason for only 2% difference between RBC-intermittent treatment and intermittent treatment. The RBC supplementation to paddy soils has decreased the volume of As-contaminated water required for irrigating rice plants. Consequently, the lower As levels in paddy soil-water systems cause decreased As concentrations in rice tissues. Therefore, an integrated approach of RBC amendment and intermittent irrigation practices would be a promising method to ensure water usage efficiency and to reduce the volume of As-contaminated water required for irrigating rice in As endemic areas worldwide.

3.5. *Arsenic speciation in rice tissues*

The supplementation of RBC to paddy soils has decreased the accumulation of As (sum of As(III), DMA(V), MMA(V), and As(V)) in rice tissues (i.e. roots, shoots, husks, and unpolished grains) (Fig. 2). In comparison, RBC-intermittent treatment achieved the least As content in rice tissues, followed by RBC-flooded, intermittent, and flooded treatments (Fig. 2). Total As concentration (sum of As(III), DMA(V), MMA (V), and As(V)) in rice roots decreased by approximately 84% in RBC-intermittent treatment when compared with conventional flooded treatment. This figure was 43% and 70%, respectively, in RBC-flooded and intermittent treatments. RBC-intermittent treatment reduced total As content in rice shoots by approximately 73% than that of the flooded treatment, whereas RBC-flooded and intermittent treatments have reduced total As content in rice shoots by 21% and 43%, respectively, compared to the flooded treatment. In rice husks, total As concentration was lowered by 89% in both RBC-intermittent and intermittent treatments in comparison to flooded treatment while this figure was 69% in the RBC-flooded treatment. Total As concentration in unpolished rice grains decreased by 81%, 45%, and 76% in RBC-intermittent, RBC-flooded, and intermittent treatments, respectively, in comparison to conventional flooded treatment. Total As concentrations in rice roots and shoots were significantly different among all different treatments (Fig. 2). In rice husks and grains, total As concentrations were also significantly different among different treatments except between RBC-intermittent and intermittent treatments.

Most importantly, RBC-intermittent treatment decreased the level of most toxic inorganic As species (i.e. As(III) and As(V)) in rice roots, shoots, husks, and unpolished grains (Fig. 2). The respective figures for the decrement of inorganic As levels in RBC-intermittent, RBC-flooded, and intermittent treatments compared to flooded treatment were as follows: roots (83%, 42%, and 67%), shoots (74%, 22%, and 41%), husks (74%, 27%, and 67%), and unpolished rice grains (46%, 10%, and 36%). In all the treatments, the percentage of inorganic As species in rice roots and shoots was higher (~90%) than the percentage of organic As species (i.e. MMA(V) and DMA(V)) in rice roots and shoots (~10%). However, in rice husks and unpolished grains, the percentages of organic As species increased to 30–75% of total As species.

Different metabolic activities in rice plants could involve varied As levels in rice tissues under different treatments (Fig. 3). The supplementation of RBC to paddy soils has increased the Si concentration in paddy water, as shown in Fig. 1(b). Both $Si(OH)_4$ and As(III) acquire nodulin 26-like intrinsic proteins (NIPs) such as OsNIP2;1 (Lsi1) by rice roots. The increased Si/As(III) ratio, due to the RBC supplementation in the paddy soil-water system could decrease the uptake of As(III) by rice roots. Therefore, RBC-intermittent and -flooded treatments have lower As concentrations in rice roots compared to those under the conventional flooded treatment. The concentration of inorganic As species in rice shoots, husks, and unpolished grains are lower compared to the concentration of inorganic As species in rice roots in this study. Following the uptake, both inorganic and organic As species undergo different metabolic activities. The reduction of As(V) to As(III) by As reductase enzymes such as OsHAC1;1, OsHAC1;2, and OsHAC4 occurs in rice roots (Shi et al., 2016). The efflux of As(III) back into the external environment by As(III) efflux transporters and the complexation of As (III) with thiol-rich peptides such as phytochelatins and glutathione may reduce the translocation of inorganic As species from rice roots to shoots (Tripathi et al., 2013). In addition, as shown in Fig. 1(d), relatively high concentrations of SO_4^{2-} in RBC-intermittent and -flooded treatments may enhance the formation of thiol-rich peptides which have a high

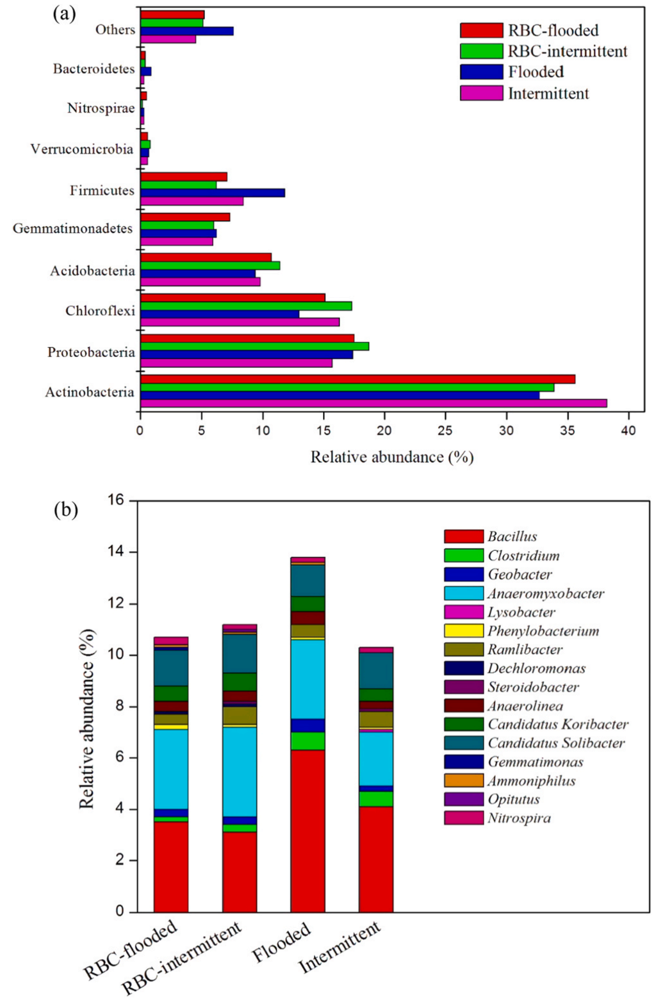

Fig. 4. Relative abundance of phylogenetic groups (a) and the dominated genera at genus level in the rhizosphere (b) under different combination of RBC and water management approaches.

affinity to As(III) in rice roots. Zhang et al. (2011) have also found that the formation of phytochelatins and glutathione increases due to the S in paddy soils. Therefore, the concentration of inorganic As species in rice shoots, husks, and unpolished grains are lower compared to the concentration of inorganic As species in rice roots. In this study, percentages of organic As species in rice husks and unpolished grains are higher than the percentages of organic As species in rice roots and shoots. The translocation efficiency of organic As species in rice plants has been found to be higher than the translocation efficiency of inorganic As species (Raab et al., 2007).

Different mechanisms in RBC lead to varied As accumulation in rice tissues (Fig. 3). The well-developed pore structure in RBC as shown in Fig. S1 may facilitate the diffusion of As species through the process of physical adsorption. Oxygenated functional groups in BC have also been found to minimize the availability of As in aqueous medium through the surface complexation process (Beiyuan et al., 2017). Moreover, supplementation of RBC might promote the abundance of microbial community which involves the oxidation, methylation and volatilization of As in the paddy soil-water system (discussed in detail in Section 3.6). Due to different metabolic activities in rice plants and different mechanisms in RBC, As concentrations in rice tissues in RBC-intermittent and -flooded treatments were lower than the As concentrations in rice tissues in conventional flooded treatment. Rice tissues in intermittent treatment have been shown to have a lower As content compared to rice tissues in RBC-flooded treatment. Comparatively less usage of As-contaminated water for irrigating rice would be the reason for less As content in rice tissues under intermittent treatment over RBC-flooded treatment.

3.6. Microbial diversity

Microorganisms in paddy soil-water systems are important factors in mobilization/immobilization and speciation of As. In this study, 20 different phyla, 66 classes, 116 orders, 180 families, and 228 genera have been identified. The phyla Actinobacteria dominated in the rhizosphere soil, comprising 35.6%, 33.9%, 32.7%, and 38.2% in RBC-flooded, RBC-intermittent, flooded, and intermittent treatments. Proteobacteria (15.7%–18.7%), Chloroflexi (13.0%–17.3%), Acidobacteria (9.4%–11.4%), Gemmatimonadetes (5.9%–7.3%), and Firmicutes (6.2%–11.8%) were other dominant phyla in this study (Fig. 4(a)), suggesting that members of those phyla may actively behave in the rhizosphere. Both Proteobacteria and Firmicutes have been found to comprise diverse genera which involve As-cycling in the paddy agroecosystem (Das et al., 2016).

The relative abundance of different genera in rhizosphere soils were influenced by different treatments, as shown in Fig. 4(b). The abundance of Fe(III) reducing bacteria such as *Bacillus*, *Clostridium*, and *Geobactor* was higher in the conventional flooded treatment than in other treatments. The abundance of *Bacillus* was lowered by 44%, 51%, and 35% in RBC-flooded, RBC-intermittent, and intermittent treatments when compared to conventional flooded treatment. The abundance of *Clostridium* decreased by 71%, 57%, and 14% in RBC-flooded, RBC-intermittent, and intermittent treatments in comparison to flooded treatment. In the case of *Geobactor*, this figure was 40% each for RBC-flooded and -intermittent treatments, and 60% for intermittent treatment. The genera *Anaeromyxobacter* was also found to reduce Fe(III) to Fe(II); however, the abundance of the *Anaeromyxobacter* has not shown a significant pattern in this study. The Fe(III)-(hydro)oxides in the paddy soil-water systems are important factors in governing the mobility of As. Both As(III) and As(V) can be complexed with Fe(III)-(hydro)oxides in the paddy soil-water system; therefore, the mobility and bioavailability of both As(III) and As(V) can be minimized (Kumarathilaka et al., 2018a). The reduction of Fe(III) to Fe(II) by *Bacillus*, *Clostridium*, and *Geobactor* in the conventional flooded treatment may lead to mobilization of more As(III) and As(V) into the soil solution and consequently to a higher As content in rice tissues than other treatments. In contrast, the genera *Lysobacter* was detected in the intermittent treatment. The *Lysobacter* was found to oxidize Fe(II) to Fe(III), therefore, minimizing the bioavailability of As(III) and As(V) for plant uptake. That may be one of the reasons for less As content in rice tissues in intermittent treatment than in the conventional flooded treatment. Overall, RBC amendment into paddy soils and intermittent irrigation practices have decreased the abundance of Fe(III) reducing bacteria and subsequently, lower As content in rice tissues is found compared to conventional flooded treatment. The supplementation of RBC and changes in water management can form new habitats for microbes by adsorbing nutrients and organic substrates on RBC surface and changing the redox chemistry of the rice rhizosphere, leading to a shift in the abundance of microorganisms and community composition. Therefore, long-term effect of the RBC and water management approaches on As-contaminated paddy soil-water systems should be further systematically examined.

3.7. Essential elements in rice grains

The nutritional value of rice grains is of particular importance to sustain rice quality for human consumption (Mwale et al., 2018). There are no reports on the concentration of essential elements in unpolished rice grains under different mitigation techniques used for reducing As content in rice grains. This study revealed that the supplementation of RBC into paddy soils has increased the elemental concentrations of Fe, Mn, Zn, Mg, and Ca in unpolished rice grains (Fig. 5). The supplementation of RBC to flooded and intermittent treatments has increased

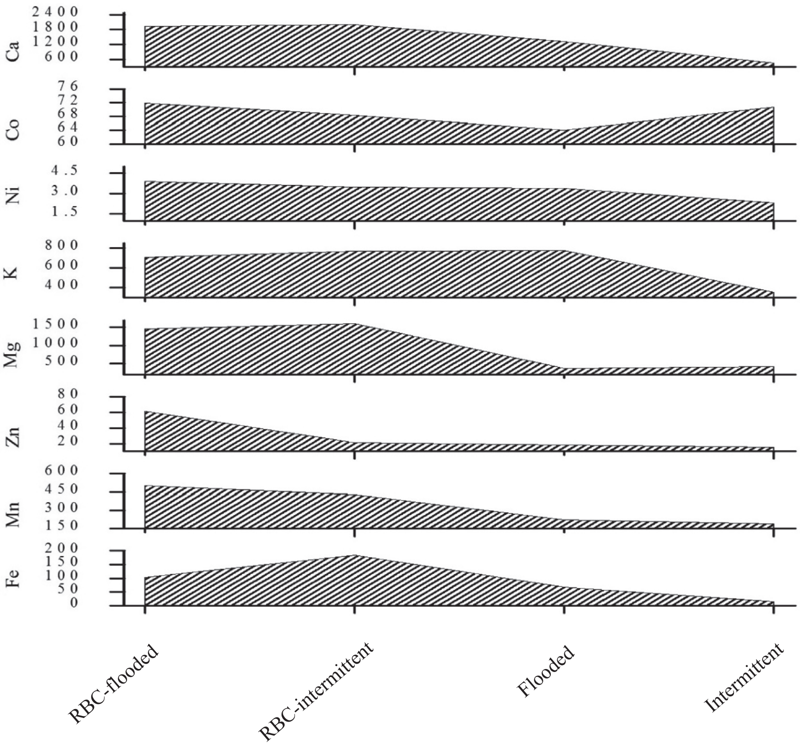

Fig. 5. The variation of essential elements in mg kg^{-1} in unpolished rice grains under different combinations of RBC and water management techniques.

elemental concentrations in unpolished rice grains in comparison to conventional flooded treatment as follows: (1) Fe: 57% and 178%, (2) Mn: 124% and 92%, (3) Zn: 239% and 15%, (4) Mg: 291% and 329%, and (5) Ca: 45% and 52%. However, there was no significant pattern for K, Ni, and Co in unpolished rice grains with the supplementation of RBC to paddy soils. Since RBC steadily releases essential elements into paddy soil solution, unpolished rice grains in RBC-flooded and -intermittent treatments have accumulated higher essential elements than in unpolished rice grains under flooded and intermittent treatments. Therefore, the addition of RBC to paddy soils leads to the enhancement of essential elemental concentrations in unpolished rice grains. Interestingly, the majority of the elemental concentration (i.e. Fe, Mn, Zn, K, Ni, and Ca) in unpolished rice grains was lowered in intermittent treatment compared to the unpolished rice grains in the flooded treatment. Wetting and drying cycles in the intermittent treatment may have lowered the bioavailability of essential elements in the rhizosphere zone compared to the flooded treatment. Even though intermittent treatment has decreased the As content in unpolished rice grains compared to that of RBC-flooded and flooded treatments, lower levels of essential elements in unpolished rice grains are required to be concerned. Overall, incorporation of RBC to paddy soils could sustain the level of essential elements in rice grains and could lead to minimizing food-related malnutrition in people worldwide.

4. Conclusions

The integrated approach of RBC amendment and intermittent water management practices has reduced inorganic As content in rice tissues and improved essential elemental content in unpolished rice grains, ensuring global food safety. Even though the integrated approach of RBC amendment, together with flooded water management practices, has increased rice yield and essential elemental content in unpolished rice grains, the inorganic As content in unpolished rice grains is higher compared to RBC-intermittent and intermittent treatments. The RBC in paddy soil-water system contributes to a reduced As accumulation and to an increased essential elemental content in unpolished rice grains through releasing Si and SO_4^{2-}, and essential nutrients, respectively, into the paddy water. In addition, RBC could adsorb As species through chemi- and physisorption processes to decrease the bioavailable As for plant uptake. The RBC in paddy soil has also reduced the abundance of Fe(III) reducing bacteria, therefore, the mobility and bioavailability of As(III) and As(V) in paddy soil-water systems has decreased. Further studies are required at glasshouse and field scales to optimize the effect of an integrated approach of BC amendment and alternative water management practices under different BC amendment rates, BC types (i. e. pristine BC and modified-BC), and range of As concentrations in irrigation water/paddy soils on decreased inorganic As content and to sustain the essential elemental levels in rice grains for safer human consumption.

CRediT authorship contribution statement

Prasanna Kumarathilaka: Conceptualization, Data curation, Investigation, Writing - original draft, Writing - review & editing, Formal analysis, Methodology, Resources. **Jochen Bundschuh**: Conceptualization, Writing - review & editing, Methodology, Resources, Supervision. **Saman Seneweera**: Resources, Supervision. **Yong Sik Ok**: Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124188.

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3.3 Concluding remarks

The integrated rice hull BC-intermittent water supply management strategy has significantly decreased the concentration of most toxic inorganic As in rice tissues (roots, shoots, husks, and unpolished rice grains) compared to other treatments. Different mechanisms could involve decreased As accumulation in rice tissues under the integrated rice hull BC-intermittent water supply management strategy. The application of Si-rich rice hull BC has increased Si concentration in the paddy pore water. Since both $Si(OH)_4$ and As(III) are acquired by the same Si transporters in rice roots (OsNIP2;1 (Lsi1)), increased Si concentration in the paddy pore water has decreased the uptake of As(III) by rice roots. The increased SO_4^{2-} concentration in the paddy pore water, following the supplementation of rice hull BC, could enhance the formation of thiol-rich peptides in rice roots which can complex with As(III) to limit the translocation of As(III) from roots to shoots. Arsenic species can also be adsorbed to the rice hull BC structure through physical and chemical adsorption processes. The abundance of Fe(III)-reducing bacteria such as *Bacillus*, *Clostridium*, and *Geobactor* has decreased under rice hull BC supplementation, therefore, the mobility and bioavailability of As can be reduced in the rice rhizosphere. Rice hull BC has released essential elements, which are required for plant growth, into the paddy pore water. As a result, plant growth parameters have been promoted under the rice hull BC supplementation. In addition, the concentration of essential elements in unpolished rice grains has increased, following the rice hull BC amendment. Therefore, an integrated approach incorporating rice hull BC-intermittent water supply management strategy can be suggested for rice grown with As-contaminated water to improve the quality of rice, as well as tackling food-related malnutrition in people.

CHAPTER 4

Rice genotype’s responses to arsenic stress and cancer risk: the effects of integrated birnessite-modified rice hull biochar-water management applications

Article V

Kumarathilaka, P., Bundschuh, J., Seneweera, S., Ok, Y.S., (2021). Rice genotype’s responses to arsenic stress and cancer risk: the effects of integrated birnessite-modified rice hull biochar-water management applications. Science of the Total Environment, 768, 144531. (Published).

4.1 Introduction

Millions of people around the world are at the cancer risk because of ingestion of As through the consumption of rice. More precisely, rice is a key source of most toxic inorganic As species. The accumulation of As in rice is genotype-dependent. The presence of MnO_2 in paddy rice soils is an important factor in decreasing mobility and bioavailability of As. Different physico-chemical, biological, and alternative water management techniques have been examined as mitigation measures to reduce As accumulation in rice grains. However, there are no reports on the role of modified BC in As-contaminated paddy rice soils under different water management practices. Moreover, no study has evaluated health risk assessments under the integrated approach of modified BC-water management regimes. Taking these facts into account, this study, for the first time, examined the integrated effects of birnessite modified rice hull BC application-water management approaches to As-contaminated paddy rice soils.

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Rice genotype's responses to arsenic stress and cancer risk: The effects of integrated birnessite-modified rice hull biochar-water management applications

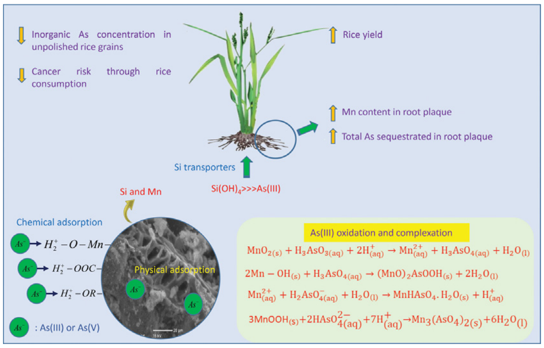
Prasanna Kumarathilaka [a], Jochen Bundschuh [a,b,*], Saman Seneweera [c,d], Yong Sik Ok [e]

[a] School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia
[b] UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia
[c] Centre for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia
[d] National Institute of Fundamental Studies, Hantana Road, Kandy 20000, Sri Lanka
[e] Korea Biochar Research Center & Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Korea

HIGHLIGHTS

- Birnessite modified rice hull biochar (Mn-RBC) has increased rice yield by 10%–34% in both rice varieties.
- Mn-RBC supplementation has increased Mn content in root plaque.
- There is no significant difference in total As in grains between rice varieties.
- Mn-RBC-water management has decreased cancer risks via rice consumption.
- Mn-RBC-intermittent treatment is proposed for producing safer rice grains.

GRAPHICAL ABSTRACT



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ABSTRACT

The health risks associated with ingestion of arsenic (As) via consumption of rice are a global concern. This study investigated the effects of integrated biochar (BC)-water management approaches to As stress and to associated health risks in rice. Rice cultivars, *Jayanthi* and *Ishikari*, were grown, irrigated with As-containing water (1 mg L^{-1}), under the following treatments: (1) birnessite-modified rice hull biochar (Mn-RBC)-flooded water management, (2) Mn-RBC-intermittent water management, (3) conventional flooded water management, and (4) intermittent water management. Rice yield in both rice varieties increased by 10%–34% under Mn-RBC-flooded and Mn-RBC-intermittent treatments compared to the conventional flooded treatment. In most cases, inorganic As concentration in rice roots, shoots, husks, and unpolished grains in both rice varieties was significantly ($p \leq 0.05$) lowered by 20%–81%, 6%–81%, 30%–75%, and 18%–44%, respectively, under Mn-RBC-flooded, Mn-RBC-intermittent, and intermittent treatments over flooded treatment. Incremental lifetime cancer risks associated with consumption of both rice varieties were also lowered from 18% to 44% under Mn-RBC-flooded, Mn-RBC-intermittent, and intermittent treatments compared to flooded treatment. Overall, the integrated Mn-RBC-intermittent approach can be applied to As-endemic areas to produce safer rice grains and reduce the incremental lifetime cancer risk through rice consumption.

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[*] Corresponding author at: UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia.
E-mail address: jochen.bundschuh@usq.edu.au (J. Bundschuh).

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0048-9697/

1. Introduction

Arsenic (As) is recognized as a class I carcinogen by the International Agency for Research on Cancer (IARC, 2004). Dietary exposure of As through numerous food types is a global health concern. It is reported that millions of people worldwide are at the risk due to ingestion of As through the consumption of rice and rice-base products (Kumarathilaka et al., 2019; Yin et al., 2019). Most importantly, rice is a major source of inorganic As species (i.e. arsenite (As(III)) and arsenate (As(V))) which are more toxic compared to organic As species (i.e. dimethylarsinic acid (DMA(V)) and monomethylarsonic acid (MMA(V))). Ingestion of inorganic As could increase the risk for cancers of lungs, skin, and urinary bladder and can cause cardiovascular, respiratory, neurological, and metabolic diseases (Karagas et al., 2019; Tchounwou et al., 2019). Recent health risk assessment studies demonstrated that infants, children, and pregnant women, in particular, could be at higher risk for cancers due to consumption of As-containing rice and rice-based products (González et al., 2020; Khan et al., 2020; Mondal et al., 2019). The World Health Organization (WHO) has recommended a permissible level of 200 μg kg^{-1} for inorganic As in rice grains for adult consumption (WHO, 2014). Moreover, the European Union (EU) has set a maximum value of 100 μg kg^{-1} for inorganic As in rice for consumption by young children (EC, 2015).

The conventional way of paddy management leads to an accumulation of high As levels in rice grains in comparison to other cereal crops. The accumulation of As species in rice tissues is genotype-dependent (Irem et al., 2019). A long period of flooded water management during the rice growing cycle decreases the redox potential (Eh) in the paddy soil-water system. Such lowered Eh values enhance the mobility and bioavailability of inorganic As species (i.e. As(III)) in the paddy soil-water system through different processes (i.e. dissolution of Fe hydro (oxides) and microbial As(V) reduction) (Islam et al., 2016; Kumarathilaka et al., 2018). The changes in redox potential in paddy agro-ecosystems also affect the behavior of redox-sensitive elements such as Mn in the paddy soils where MnO_2 can occur as fine-grained coating of soil particles or as nodules (Essington, 2015). The presence of MnO_2 in paddy soils could reduce the bioavailability of inorganic As through different processes such as oxidation of As(III) and adsorption of As(V) (Kumarathilaka et al., 2020). Moreover, formation of Mn plaque on rice roots may decrease the bioavailable concentration of As in the rice rhizosphere (Liu et al., 2005).

Various types of physico-chemical, biological, and alternative water management approaches have been examined as mitigation measures to reduce As accumulation in rice grains (Shri et al., 2019; Suriyagoda et al., 2018). Intermittent water management practice has been found to decrease the accumulation of As in rice grains, mainly due to an increased redox potential in the paddy soil-water system. However, loss of rice yield is associated with these intermittent irrigation practices (Basu et al., 2015). The supplementation of biochar (BC) as a pristine BC or BC-composite to As-contaminated rice ecosystems may increase the rice yield and rice quality, since BC contains essential elements which are required for the growth of plants. The BC can be produced by using a range of organic materials under an O_2 free environment (Kim et al., 2020; Mohan et al., 2018). Various physical and chemical activation processes can be used to make BC-composites (Frišták et al., 2018; Sajjadi et al., 2019).

In this study, an integrated approach of BC-composite-water management practices is proposed to promote rice yield and rice quality by decreasing As accumulation in rice grains. There are no reports on the behavior of BC-composites in As-contaminated rice ecosystems under different water management approaches. In addition, none of the previous studies have evaluated health risk assessments under the integrated approach of BC-composite-water management practices. Taking these facts into account, this study, for the first time, evaluated the effects of birnessite modified rice hull BC (Mn-RBC) supplementation to As-contaminated paddy soils under different water management practices (i.e. conventional flooded and intermittent water management practices). Two rice genotypes were selected in this study to examine the effects of rice genotypes under integrated Mn-RBC-water management practices. Health risk assessments were also performed to evaluate the best potential treatment/s.

2. Material and methods

2.1. Paddy soil collection

Soils were collected from a paddy land in Yanco, New South Wales, Australia (34° 35′ 53.5″ S, 146° 21′ 38.1″ E). The collected soils were air-dried for 5 days and sieved before mixing to obtain a composite sample. Physico-chemical characteristics of the soil were determined and summarized in Table S1.

2.2. Birnessite-modified rice hull biochar preparation and characterization

Air-dried rice hulls were pyrolyzed in a muffle furnace (RIO GRANDE) under flowing of N_2 gas. Ramping temperature was set at a rate of 7 °C min^{-1} while holding time at peak temperature of 600 °C was set at 2 h. The produced rice hull biochar (RBC) was washed with distilled deionized water several times followed by oven-drying (STERIDIUM) at 85 °C before use.

The Mn-RBC was prepared following the method described by Wang et al. (2015). Briefly, 3.15 g of $KMnO_4$ was dissolved in 50 mL distilled deionized water and 5 g of RBC was added to the solution and reacted for 2 h with a magnetic stirrer (BIBBY HB502). After that, the suspension was boiled for 20 min, followed by dropwise addition of 3.3 mL of concentrated HCl. The reaction was kept for an extra 10 min under continuous stirring and the mixture was allowed to cool down. Once the mixture reached room temperature (~25 °C), Mn-RBC was separated, rinsed thoroughly with distilled deionized water, dried in an oven at 80 °C overnight, and stored in a closed container until use.

The physico-chemical characteristics of Mn-RBC including pH, electrical conductivity (EC), proximate analysis, BET surface area, average pore size, and total pore volume were investigated by the methods described elsewhere (Ahmad et al., 2012). In addition, Scanning Electron Microscopy (SEM) (JCM-6000, JEOL) analysis was performed to examine the morphology Mn-RBC.

2.3. Pot experiment design

Seeds from two different rice varieties, *Jayanthi* and *Ishikari*, were surface sterilized with 10% H_2O_2 and germinated in moist compost. After three weeks, uniform size seedlings were transplanted in pots. Prior to transplantation, each pot was filled with 2.5 kg of paddy soil and flooded with 1 mg L^{-1} of As-containing water. This As concentration (1 mg L^{-1}) in water was selected based on the previously reported values in irrigation water which was used for rice cultivation (Biswas et al., 2014; Dahal et al., 2008; Huang et al., 2016). There were four different treatments: (1) flooded water management with Mn-RBC (1% w/w); Mn-RBC-flooded, (2) intermittent water management with Mn-RBC (1% w/w); Mn-RBC-intermittent, (3) conventional flooded water management without Mn-RBC; flooded, and (4) intermittent water management without Mn-RBC; intermittent. Two different water management practices were applied as follows: flooded: a water level of ~5 cm was maintained, intermittent: irrigated intermittently to 5 cm water level, particularly when the soil was found to have dried. The water levels in each treatment were maintained by adding 1 mg L^{-1} of As-containing water. All the treatments contained triplicates. Plant growth parameters including grain yield, shoot weight, root length, and plant height were recorded at the time of harvesting (growing periods for *Jayanthi* and *Ishikari* were 142 days and 128 days, respectively).

2.4. Chemical analysis

The plaque on root surfaces was extracted using the Dithionite-Citrate-Bicarbonate (DCB) solution, as described by Amaral et al. (2017). The DCB extract solution was analysed for total As and Mn using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer NexION™ 300×) and atomic absorption spectrophotometer (AAS-7000, Shimadzu).

Powdered rice tissue samples (roots, shoots, husks, and unpolished grains) were digested in a microwave digestion system (Multiwave 3000, Anton Paar) prior to As speciation analysis. The detailed parameters of microwave digestion are summarized in Table S2. Ultra-High Performance Liquid Chromatography - Inductively Coupled Plasma Mass Spectrometry (UHPLC-ICP-MS) (Flexar, PerkinElmer - PerkinElmer NexION™ 300×) was used for the analysis of As species. The UHPLC-ICP-MS operational conditions are summarized in Table S3. The certified reference material (CRM) for rice, ERM-BC211, was used to validate As speciation analysis.

2.5. Sequential and single extractions

Sequential extraction procedure developed by Wenzel et al. (2001) was followed to determine how As was bound to different fractions such as non-specifically sorbed, specifically sorbed, bound to non-crystalline and poorly crystalline Fe and Al hydrous oxides, bound to crystalline Fe and Al hydrous oxides, and residual. Table S4 summarizes extractant/s and extraction conditions used for each step. Each sample from sequential extraction steps was centrifuged and filtered through 0.22 μm membrane filter before analysis for As using ICP-MS.

2.6. Dietary intake and risk assessment

The estimation of daily intake of As via consumption of rice was obtained using the following equation (Li et al., 2011; Zheng et al., 2007; Zhuang et al., 2009).

$$\mathrm{EDI} = \frac{\mathrm{ED} \times \mathrm{EF} \times \mathrm{IR} \times \mathrm{C}}{\mathrm{BW} \times \mathrm{LE}}$$

where,

EDI = Estimated daily intake
ED = Exposure duration (70 years)
EF = Exposure frequency (365 days year^{-1})
IR = Rice intake rate (398.3 g adult^{-1})
C = As concentration in rice grains (mg kg^{-1})
BW = Average body weight (65 kg)
LE = Life expectancy (25,550 days)

The hazard quotient (HQ) indices for As were determined using the following equation detailed in USEPA (2010).

$$\mathrm{HQ} = \frac{\mathrm{EDI}}{\mathrm{RfD}}$$

where,

RfD = Oral reference dose for As (0.0003 mg kg^{-1} day^{-1})

The incremental lifetime cancer risk was calculated for the inorganic arsenic using the following equation (Li et al., 2009; USEPA, 2010).

$$\mathrm{ILTR} = \frac{\mathrm{ED} \times \mathrm{EF} \times \mathrm{IR} \times \mathrm{CiAs}}{\mathrm{BW} \times \mathrm{LE}} \times \mathrm{SF}$$

where,

CiAs = Inorganic As concentration in rice grains
SF = Cancer slope factor (1.5 mg kg^{-1} day^{-1})

2.7. Statistical analysis

Statistical graphing was performed using the Origin 6.0 software package. Kruskal-Wallis one way ANOVA method has been used to test the mean ranks of the data. The statistical significance of As content in rice tissues in two different rice genotypes under different combinations of Mn-RBC amendments and water management practices were determined by using Duncan's multiple range test ($p < 0.05$). The data represent the means of three replicates.

3. Results and discussion

3.1. Birnessite-modified rice hull biochar characterization

Table 1 summarizes physico-chemical properties of Mn-RBC. The Mn-RBC has a slightly acidic pH (5.83). The total amount of mobile matter and resident matter (78.6 w/w) in Mn-RBC demonstrated a potential of carbon availability in short- and long-term basis in the paddy environment. Mobile matter is the organic fraction of BC which can migrate into paddy soil-water system and become a source of food for soil microbes. Resident matter is the organic fraction of BC which is expected to remain stable in the paddy soil-water system for a very long time. The ash content (17.54 w/w) represented inorganic minerals and residues remaining in Mn-RBC. The BET data indicated a well-developed pore structure in Mn-RBC and the development of mesopores (6.0944 nm of average pore diameter). The SEM image (Fig. 1) of the Mn-RBC showed the successful incorporation of Mn into the RBC structure. Arsenic is not detected in the EDTA-extractable fraction of Mn-RBC. However, Mn-RBC contains 3146 mg kg^{-1} of EDTA-extractable Mn. The relatively higher Mn content in Mn-RBC likely resulted from the incorporation of Mn oxides into the RBC structure. Moreover, Mn-RBC contains 174 mg kg^{-1} of EDTA-extractable Si. The availability of Si in the paddy soil-water system could affect the uptake of highly toxic As(III) by rice roots (Fleck et al., 2013). Since both $Si(OH)_4$ and As(III) are acquired by the same uptake transporters in rice plants, the application of Mn-RBC could decrease the uptake of As(III) through competitive uptake with $Si(OH)_4$.

3.2. Plant growth parameters

Fig. 2 shows plant height, root length, shoot and grain weight per pot under different treatments in both rice varieties. The integrated Mn-RBC-flooded treatment has reported the highest value for plant height, root length, shoot and grain weight. Most importantly, integrated Mn-RBC-flooded and Mn-RBC-intermittent approaches have increased grain yield in variety *Jayanthi* by 34% and 16%, respectively, in comparison to the flooded treatment. In variety *Ishikari*, integrated

Table 1
Physicochemical characteristics of Mn-RBC.

Parameter	Value
pH	5.83 ± 0.07
EC (dS m^{-1})	2.05 ± 0.04
Proximate analysis	
Moisture (w/w)	3.78 ± 0.24
Mobile matter (w/w)	31.11 ± 2.49
Ash (w/w)	17.54 ± 1.25
Resident matter (w/w)	47.58 ± 3.12
Specific surface area (m^2 g^{-1})	116.3
Total pore volume (mL g^{-1})	0.1772
Average pore diameter (nm)	6.0944
As (mg kg^{-1})[a]	ND
Mn (mg kg^{-1})[a]	3146 ± 47
Si (mg kg^{-1})[a]	174.26 ± 11.68

ND: not detectable.
[a] EDTA-extractable fraction.

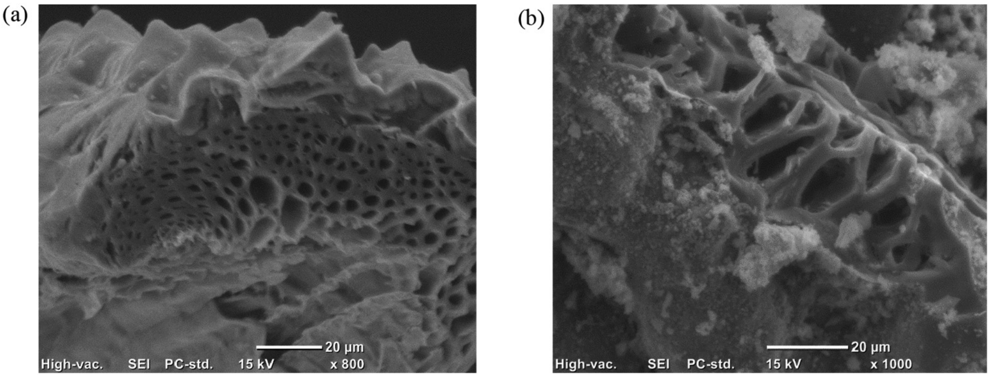

Fig. 1. Scanning electron microscopy images before (a) and after modification to Mn-RBC (b).

Mn-RBC-flooded and Mn-RBC-intermittent approaches have increased grain yield by 21% and 10%, respectively, compared to the flooded treatment. The highest rice yield and other growth parameters (plant height, root length, and shoot weight) in Mn-RBC-flooded treatment could correspond to the prevention of microbe-mediated disease damage under flooded water management. In addition, Mn-RBC could release essential nutrients into the paddy soil-water system. The uptake of essential nutrients by rice roots has promoted rice growth parameters following the Mn-RBC supplementation. Previous studies by Lin et al. (2017) and Yu et al. (2017) also demonstrated that the amendment of ferromanganese oxide impregnated BC and manganese oxide-modified BC to As-contaminated paddy soils has improved the growth parameters in rice plants. Therefore, integrated Mn-RBC-flooded and Mn-RBC-intermittent approaches can be used to improve rice growth parameters, in particular, in As-contaminated rice fields.

3.3. Arsenic fractionations in paddy soils

Fig. 3 shows As in different fractions in paddy soils under different treatments in both rice varieties. The As concentration in the non-specifically sorbed fraction in each treatment was relatively low, ranging from 0.06–0.16 mg kg^{-1}. The specifically sorbed fraction increased in Mn-RBC amended treatments in both rice varieties. For example, in variety *Jayanthi*, the specifically sorbed fractions in Mn-RBC-flooded and Mn-RBC-intermittent treatments increased by approximately 122% and 83%, respectively, compared to the flooded treatment. In variety *Ishikari*, these figures were 124% and 194%, respectively, in comparison to the flooded treatment. Adsorption of As species to Mn-RBC through physical and chemical adsorption processes may have increased the As concentration in the specifically sorbed faction in Mn-RBC-flooded and Mn-RBC-intermittent treatments. Arsenic bound to non-crystalline and poorly crystalline Fe and hydrous oxides fraction ranged from 1.44–8.00 mg kg^{-1} in each treatment. In contrast, As bound to crystalline Fe and Al hydrous oxides in each treatment ranged from 0.87 to 3.15 mg kg^{-1}. There was no significant pattern in the concentration of As bound to non-crystalline and poorly crystalline Fe and hydrous oxides and to the fractions of non-crystalline and poorly crystalline Fe and hydrous oxides. Arsenic bound to the residual fraction in Mn-RBC amended treatments increased in both rice varieties. For instance, in variety *Jayanthi*, As bound to the residual faction in Mn-RBC-flooded and Mn-RBC-intermittent treatments increased by approximately 6%–25% compared to the flooded treatment. In variety *Ishikari*, As bound to the residual fraction in Mn-RBC-flooded and Mn-RBC-intermittent treatments increased by 0.7%–29% in comparison to the flooded treatment. Yin et al. (2017) also reported that incorporation of rice straw and Fe-impregnated BC into As-contaminated paddy soils has increased As concentration associated with the residual fraction. The increased As concentration in residual faction in RBC-amended treatment indicated the adsorption of As to Mn-RBC through strong electrostatic attractions. Therefore, supplementation of Mn-RBC into paddy soils could increase the As retention in paddy soil, while decreasing the bioavailability of As for uptake by rice roots. As a result, the accumulation of As in rice tissues could be decreased.

3.4. Role of root plaque on arsenic retention

The supplementation of Mn-RBC has increased Mn concentration in root plaque in both rice varieties as shown in the Fig. 4. Liu et al. (2005)

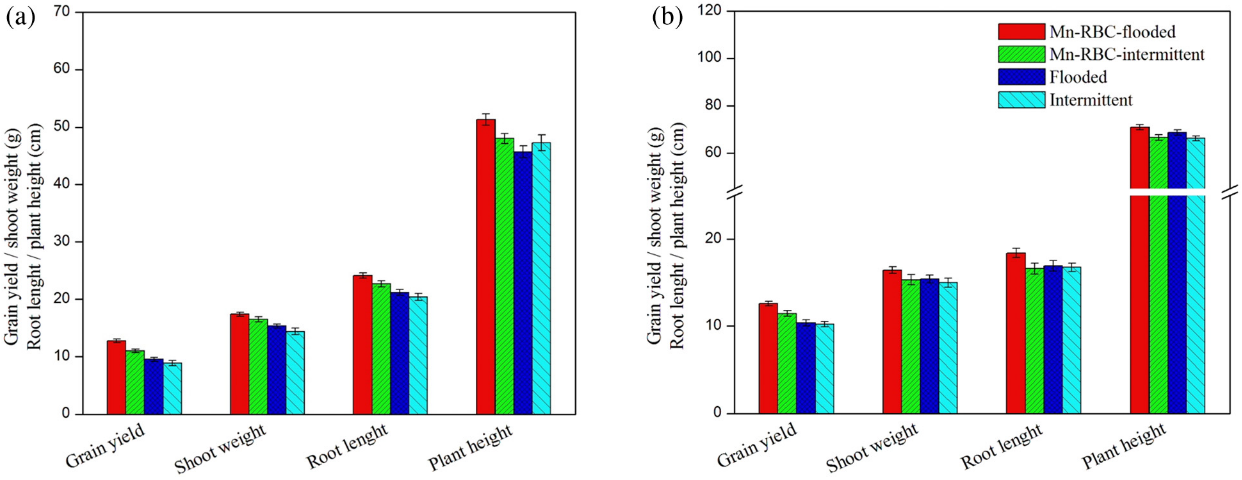

Fig. 2. Plant growth parameters under different treatments in two different rice varieties (a) *Jayanthi*, (b) *Ishikari*.

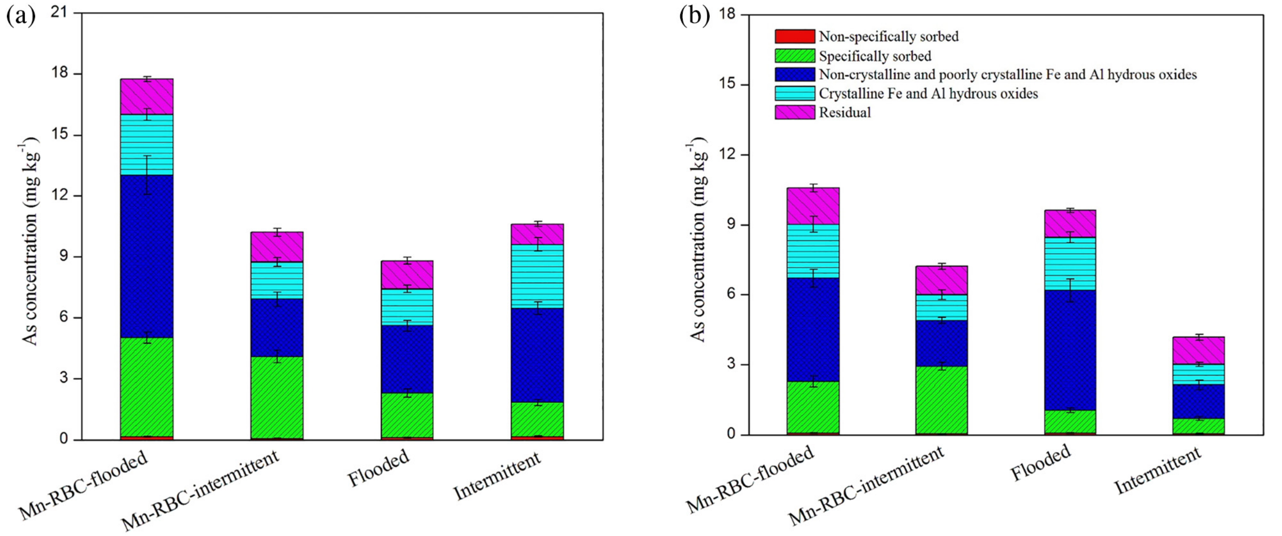

Fig. 3. Arsenic bound to different fractions (non-specifically sorbed, specifically sorbed, bound to non-crystalline and poorly crystalline Fe and Al hydrous oxides, bound to crystalline Fe and Al hydrous oxides, and residual) under different combination of Mn-RBC and water management approaches (a) *Jayanthi*, (b) *Ishikari*.

demonstrated that the formation of Mn plaque could reduce the bioavailability of As in the rice rhizosphere. In variety *Jayanthi*, Mn concentration in root plaque increased by 203% and 92%, respectively, in Mn-RBC-flooded and Mn-RBC-intermittent treatments when compared to the conventional flooded treatment. Similarly, in variety *Ishikari*, Mn content in root plaque increased in Mn-RBC-flooded and Mn-RBC-intermittent treatments by 167% and 136%, respectively, in comparison to the flooded treatment. The increased levels of Mn, in particular in the Mn-RBC-flooded treatment, have retained higher As content in root plaque compared to the conventional flooded treatment. For example, in variety *Jayanthi*, As retained in the root plaque was approximately 52% higher compared to the flooded treatment. In variety *Ishikari*, the Mn-RBC-flooded treatment retained 16% more As in root plaque in comparison to the conventional flooded treatment. Therefore, supplementation of Mn-RBC could decrease the bioavailability of As in the rhizosphere through sequestration of As in root plaque. The reduced bioavailability of As in the paddy soil-water leads to decreased As concentration in rice tissues. Relatively lower As concentration in root plaque in Mn-RBC-intermittent treatments (7.14–7.36 mg kg^{-1}) in both rice varieties compared to flooded treatments (17.17–24.85 mg kg^{-1}) corresponded to lower supplementation of As-contaminated water for irrigating rice.

3.5. Arsenic in rice tissues

The Mn-RBC in the paddy soils has decreased total As (sum of As(III), DMA(V), MMA(V), and As(V)) and most toxic inorganic As (sum of As (III) and As(V)) concentrations in rice roots, shoots, husks, and unpolished grains in both rice varieties (Fig. 5). In variety *Jayanthi*, inorganic As content in rice roots decreased by approximately 46%, 81%, and 70%, respectively, under Mn-RBC-flooded, Mn-RBC-intermittent, and intermittent treatments compared to conventional flooded treatment. In variety *Ishikari*, Mn-RBC-flooded, Mn-RBC-intermittent, and intermittent treatments have lowered inorganic As concentration in rice roots by 20%, 76%, and 65%, respectively, compared to the flooded treatment. In variety *Jayanthi*, Mn-RBC-intermittent treatment has reduced inorganic As levels in rice shoots by 81% in comparison to the flooded treatment. These figures were 9% and 41%, respectively, of Mn-RBC-flooded and intermittent treatments. Inorganic As concentration in rice shoots in variety *Ishikari* decreased in different treatments when compared to the flooded treatment as follows: (1) Mn-RBC-intermittent: 20%, (2) Mn-RBC-flooded: 9%, and (3) intermittent: 6%. Inorganic As content in rice husks in variety *Jayanthi* decreased by approximately 70% in Mn-RBC-intermittent in comparison to the conventional flooded treatment, whereas Mn-RBC-flooded and intermittent

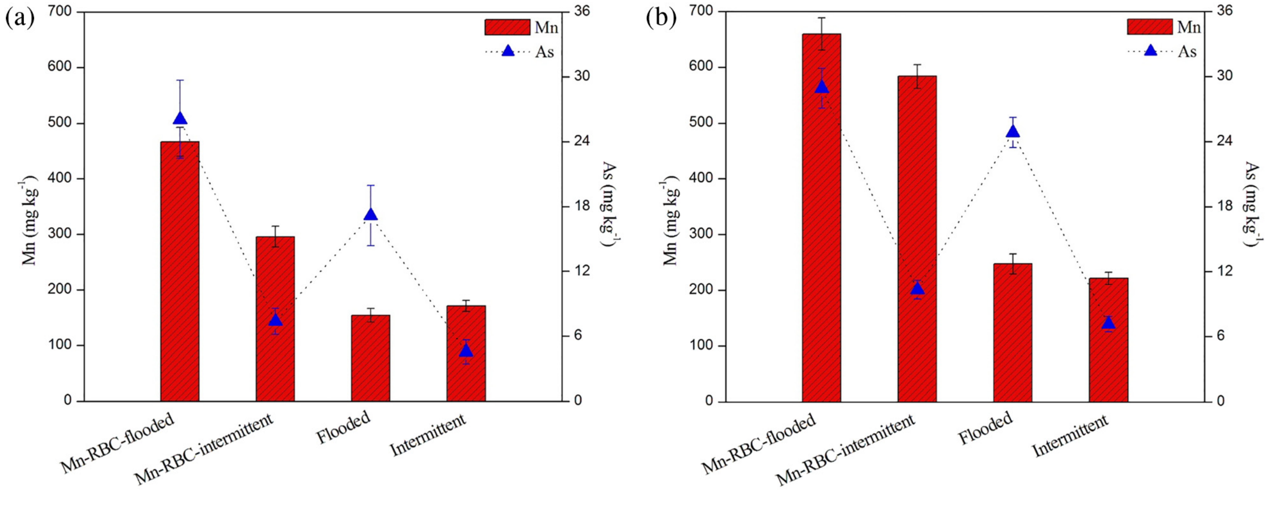

Fig. 4. Manganese and As concentration in root plaque in different combinations of Mn-RBC and water management regimes (a) *Jayanthi*, (b) *Ishikari*.

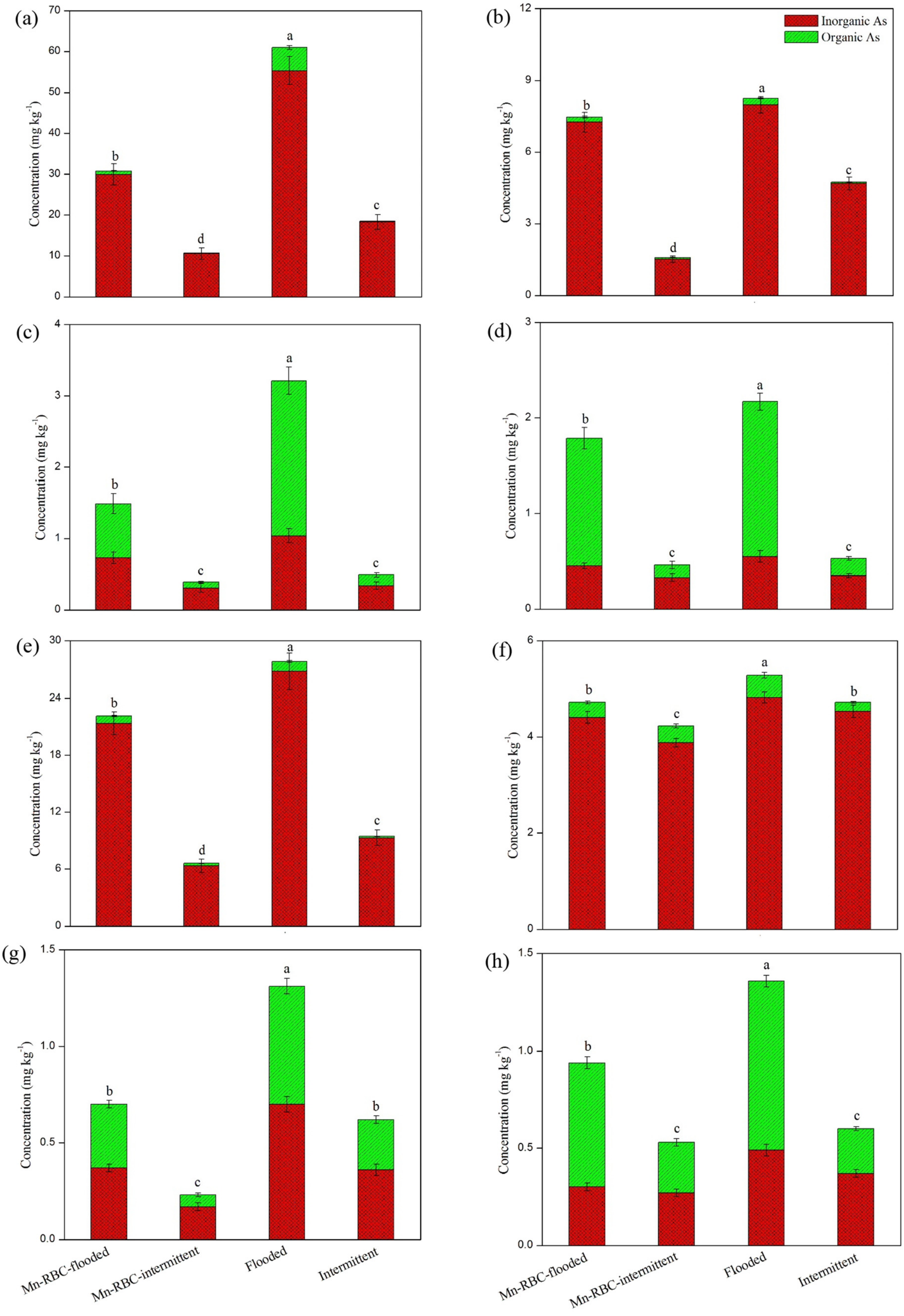

Fig. 5. Arsenic content in rice tissues under different combinations of Mn-RBC and water management approaches; *Jayanthi*: (a) roots, (b) shoots, (c) husks, and (d) unpolished grains, *Ishikari*: (e) roots, (f) shoots, (g) husks, and (h) unpolished grains. Treatments labelled with the same letter are not significantly different from each other for the total As concentration (Duncan's multiple range test; $p \leq 0.05$).

treatments have decreased inorganic As content in rice husks by 30% and 67%, respectively, compared to the flooded treatment. In variety *Ishikari*, inorganic As concentration in rice husks decreased by 75%, 47%, and 48%, respectively, in Mn-RBC-intermittent, Mn-RBC-flooded, and intermittent treatments compared to the conventional flooded treatment. Inorganic As concentration in unpolished rice grains in variety *Jayanthi* decreased by 41% in Mn-RBC-intermittent in comparison to the flooded treatment. These figure were 18% and 36%, respectively, in Mn-RBC-flooded and intermittent treatments. In variety *Ishikari*, inorganic As concentration in unpolished rice grains decreased by 40%, 44%, and 25%, respectively, in Mn-RBC-intermittent, Mn-RBC-flooded, and intermittent treatments compared to the conventional flooded treatment.

In both rice varieties, inorganic As species dominated in rice roots and shoots ranging from 91% to 99% in all treatments (Fig. 5). However, the percentage of inorganic As species in rice husks and unpolished rice grains gradually lowered in all the treatments. For instance, inorganic As percentages in rice husks and unpolished grains in two selected rice varieties decreased as follows: husks (*Jayanthi*: 32%–80%, *Ishikari*: 53%–75%), unpolished rice grains (*Jayanthi*: 25%–71%, *Ishikari*: 30%–61%). Even though the percentage of organic As species (DMA(V) and MMA (V)) lowered in rice roots and shoots compared to inorganic As percentages in both selected rice varieties, the percentage of organic As species increased in rice husks (*Jayanthi*: 20%–68%, *Ishikari*: 26%–47%) and unpolished rice grains (*Jayanthi*: 29%–75%, *Ishikari*: 26%–47%). In both rice varieties, total As concentrations in rice roots and shoots were significantly different ($p \le 0.05$) between each treatment. In variety *Jayanthi*, total As concentrations in husks and unpolished rice grains were significantly different ($p \le 0.05$) in each treatment except between Mn-RBC-intermittent and intermittent treatments. In variety *Ishikari*, the total As concentration in husks was significantly different ($p \le 0.05$) in each treatment whereas total As concentration in unpolished rice grains was not significantly different ($p \le 0.05$) between Mn-RBC-intermittent and intermittent treatments. Moreover, total As contents in unpolished rice grains were not significantly different ($p \le 0.05$) between *Jayanthi* and *Ishikari* rice varieties.

Different mechanisms can be involved in decreasing As concentrations in rice tissues under Mn-RBC supplementation to paddy soils. The presence of Mn in the paddy soils leads to oxidation of As(III) and subsequent complexation of As(V) as shown in the Fig. 6. Previous studies have demonstrated that As(III) oxidation and consequent As (V) co-precipitation/complexation in As-contaminated soils under Mn supplementation could reduce bioavailability of As in the soil-water system (Komárek et al., 2013). The Mn concentration in root plaques in Mn-RBC-flooded and Mn-RBC-intermittent treatments increased as shown in Fig. 4. Subsequently, the sequestration of As in root plaque has increased under Mn-RBC supplementation to paddy soils. Silicon in Mn-RBC structure can also be released into the paddy soil-water system. Since both $Si(OH)_4$ and As(III) are conveyed by the same transporter, the presence of $Si(OH)_4$ in the paddy pore water could decrease the uptake of As(III) by rice roots (Fig. 6). Wu et al. (2015) and Fleck et al. (2013) also revealed that Si fertilization in As-contaminated paddy environment could decrease As(III) accumulation in rice grains. Moreover, As species could be diffused into the well-developed pore structure of Mn-RBC through a physical adsorption process. Furthermore, both As(III) and As(V) can be complexed with oxygenated surface functional groups (i.e. carboxylic, alcoholic, and phenolic) on the Mn-RBC surface (Mohan et al., 2014). As a result, the bioavailability of As in the paddy soil-water has decreased in Mn-RBC-flooded and Mn-RBC-intermittent treatments compared to the flooded treatment. Therefore, the uptake of As by rice roots and consequent accumulation of As in rice roots, shoots, husks, and unpolished rice grains decreased under the supplementation of Mn-RBC into paddy soils. Relatively less supplementation of As-contaminated water may be the reason for lowered As concentration in rice tissues in the intermittent treatment in comparison to RBC-flooded treatment.

3.6. Health risk assessment

Health risks associated with As intake through rice consumption under different treatments were calculated by using different indexes such as EDI, HQ, ILTR, and cancer risk for 100,000 people. Those indexes are widely used for assessing potential health risks as well as adverse health effects due to the ingestion of pollutants (Khan et al., 2014). The integrated effects of Mn-RBC amendment and water management approaches on different health risk indexes are shown in Fig. 7. The EDI lowered following the addition of Mn-RBC in both rice varieties. For instance, in variety *Jayanthi*, EDI decreased by 79%, 18%, and 75%,

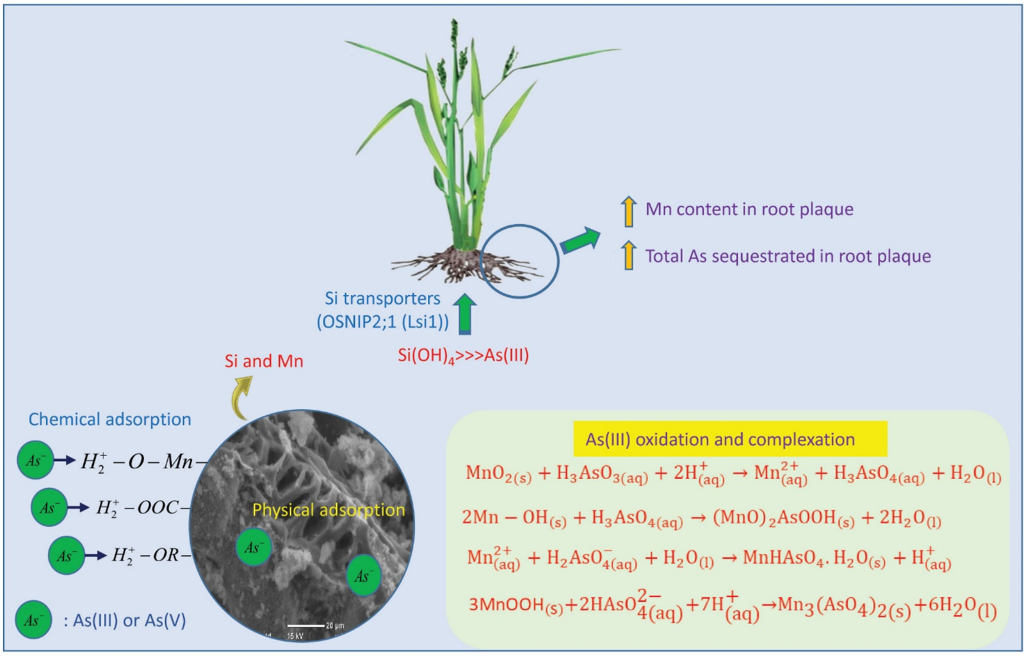

Fig. 6. Potential mechanisms involved in decreasing As bioavailability in the paddy soil-water system following the application of Mn-RBC.

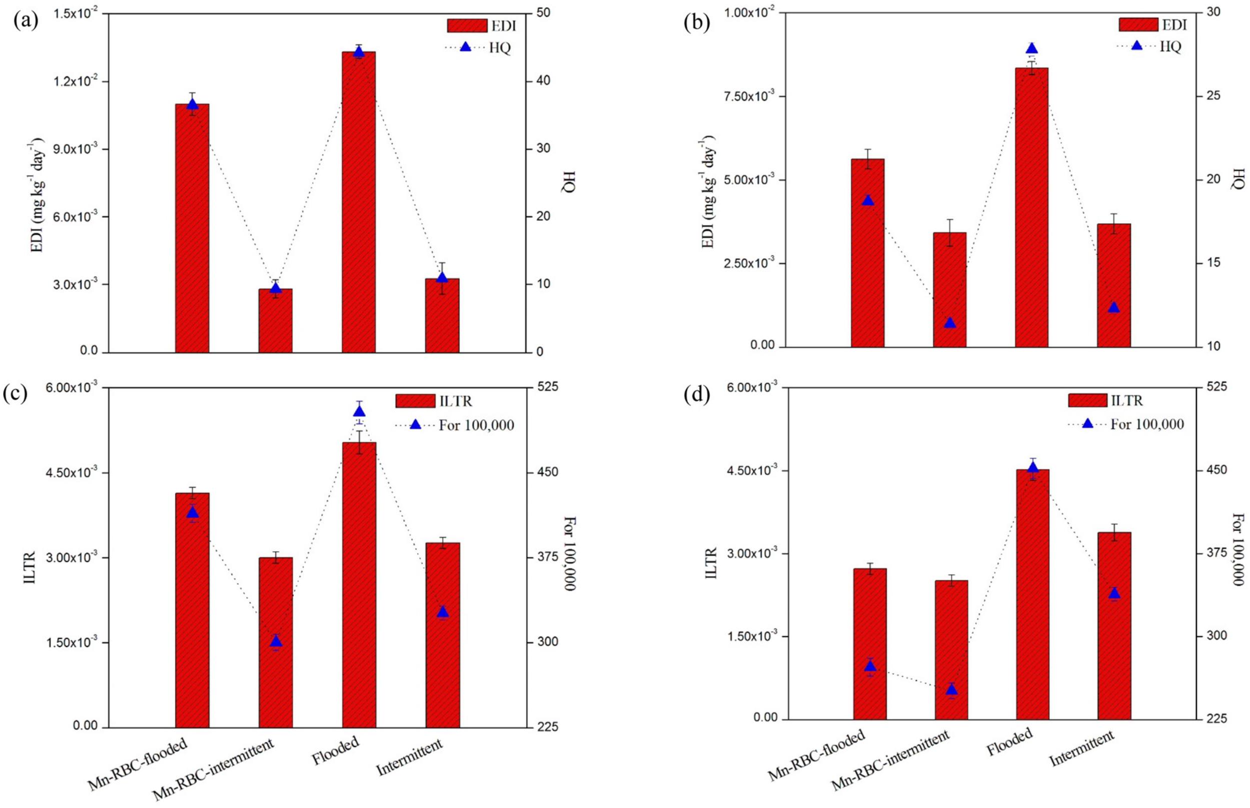

Fig. 7. Health risk indexes (EDI, HQ, ILTR, and cancer risk for 100,000 people) under different combination of Mn-RBC and water management techniques in *Jayanthi* (a and c) and *Ishikari* (b and d).

respectively, in Mn-RBC-intermittent, Mn-RBC-flooded, and intermittent treatments compared to conventional flooded treatment. In variety *Ishikari*, EDI decreased by 59%, 33%, and 56%, respectively, in Mn-RBC-intermittent, Mn-RBC-flooded, and intermittent treatments in comparison to conventional flooded treatment. The lowered EDI corresponded to the reduced As concentration in unpolished rice grains under integrated Mn-RBC-water management techniques. The HQ also reduced under the supplementation of Mn-RBC in paddy soils. In variety *Jayanthi*, the Mn-RBC-intermittent, Mn-RBC-flooded, and intermittent treatments decreased HQ by 79%, 17%, and 75%, respectively, compared to the flooded treatment. Similarly, in variety *Ishikari*, HQ lowered by 59%, 33%, and 56%, respectively, in Mn-RBC-intermittent, Mn-RBC-flooded, and intermittent treatments in comparison to the flooded treatment. Moreover, the value of ILTR associated with inorganic As decreased in both rice varieties following the incorporation of Mn-RBC into paddy soils. For example, in variety *Jayanthi*, ILTR decreased by 18%–40% in Mn-RBC-intermittent, Mn-RBC-flooded, and intermittent treatments in comparison to the flooded treatment. This figure ranged from 25% to 44% in variety *Ishikari*. The calculated lifetime cancer risks in the flooded treatment in variety *Jayanthi* was 503 per 100,000. However, Mn-RBC-intermittent, Mn-RBC-flooded, and intermittent treatments have decreased calculated lifetime cancer risks to 326–414 per 100,000. Similarly, in variety *Ishikari*, calculated lifetime cancer risks of 452 per 100,000 in the flooded treatment decreased to 251–338 per 100,000 following Mn-RBC-intermittent, Mn-RBC-flooded, and intermittent treatments. The decreased ILTR under integrated Mn-RBC-water management approaches were attributed to reduced ingestion of inorganic As through rice consumption. Therefore, all the tested indexes (i.e. EDI, HQ, ILTR, and cancer risk for 100,000 people) in this study demonstrated that integrated Mn-RBC-water management approaches in As-contaminated paddy environments could be used to reduce cancer risk in human through rice consumption.

4. Conclusions

The application of Mn-RBC to paddy soils has increased rice yield in both rice varieties. The integrated approach of Mn-RBC supplementation and intermittent water management regimes has decreased inorganic As concentration in rice roots, shoots, husks, and unpolished rice grains compared to other treatments. The Mn-RBC in the paddy soil-water system has increased root plaque Mn concentrations which could decrease the bioavailable As concentration in the rhizosphere. Different mechanisms such as As(III) oxidation and subsequent As(V) sequestration, physi- and chemisorption processes could also contribute for lowered inorganic As concentrations in rice tissues following Mn-RBC supplementation to paddy soils. Integrated Mn-RBC-intermittent water management approach also decreased the cancer risk via rice consumption in comparison to other treatments. Further studies in field scales are required to optimize the Mn-RBC amendment rates to paddy soils which are irrigated with As-contaminated water, to decrease mostly toxic inorganic As concentrations in rice grains. It is also important to assess Mn concentration in paddy pore water and rice grains under different Mn-RBC amendment rates, since Mn at higher concentration could be toxic to aquatic organisms in the paddy environment, as well as to humans through rice consumption. Moreover, an economic feasibility analysis needs to be performed to evaluate the applicability of Mn-RBC-alternative water management approaches in As-contaminated rice ecosystems.

CRediT authorship contribution statement

Prasanna Kumarathilaka: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing, Formal analysis, Methodology, Resources. **Jochen Bundschuh:** Conceptualization, Writing – review & editing, Methodology, Resources, Supervision.

Saman Seneweera: Resources, Supervision. **Yong Sik Ok:** Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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4.3 Concluding remarks

The integrated birnessite modified rice hull BC-intermittent water supply management strategy have significantly decreased the accumulation of As in rice tissues in both selected rice genotypes compared to other treatments. Various mechanisms can be involved in reducing As accumulation in rice tissues under the integrated birnessite modified rice hull BC-intermittent water supply management strategy. The Mn in the paddy soil-water system causes oxidation of As(III) and consequent complexation of As(V), thereby, limiting the mobility and bioavailability of inorganic As species. The Mn can be sequestrated on root plaque which can also sequestrate As in the rice rhizosphere as well. In addition, As species can be adsorbed to birnessite modified rice hull BC through chemical and physical adsorption processes. The release of Si from birnessite modified rice hull BC to the paddy pore water also limits the uptake of As(III) by rice roots, through competitive uptake with $Si(OH)_4$. The supplementation of birnessite modified rice hull BC has increased rice yield in both selected rice varieties. Moreover, incremental lifetime cancer risks associated with consumption of both rice varieties decreased by ~ 44% under the integrated birnessite modified rice hull BC-intermittent water supply management strategy compared to the conventional flooded water management practices. Therefore, the integrated birnessite modified rice hull BC-intermittent water supply management strategy can be suggested for As-contaminated areas to produce safer rice grains and reduce the incremental lifetime cancer risk in humans through rice consumption.

CHAPTER 5

Iron modification to silicon-rich biochar and alternative water management to decrease arsenic accumulation in rice (*Oryza sativa* L.)

Article VI

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5.1 Introduction

Production of rice grains at non-toxic levels of As to meet the demand of an ever-growing population is a worldwide challenge. Recent studies revealed that the use of As-contaminated groundwater for irrigating rice has contributed to increased As concentrations in paddy rice soils. The availability of Fe in the paddy agro eco-system plays a key role in sequestrating inorganic As species through surface complexation. However, phase conversion from poorly crystalline ferrihydrite to crystalline Fe oxides decreases the number of adsorption sites available for the inorganic As species over time. The external supplementation of Fe(III)-hydro(oxides) to As-contaminated paddy agro eco-systems may enhance the number of adsorption sites available for As. In literature, Fe-modified BC produced by using different feedstocks has been applied to As-contaminated paddy rice soils to investigate the As phyto-availability. However, none of the studies has evaluated the effects of Fe-modified rice hull BC in As-contaminated paddy environments. Moreover, this is the first study evaluating the integrated effects of Fe-modified rice hull BC and water management practices on As phyto-availability in the paddy rice soil.

Iron modification to silicon-rich biochar and alternative water management to decrease arsenic accumulation in rice (*Oryza sativa* L.)

Prasanna Kumarathilaka[a], Jochen Bundschuh[a,b,]*, Saman Seneweera[c,d], Alla Marchuk[e], Yong Sik Ok[f]

[a]*School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[b]*UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[c]*Centre for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[d]*National Institute of Fundamental Studies, Hantana Road, Kandy 20000, Sri Lanka*
[e]*Institute for Life Sciences and the Environment, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[f]*Korea Biochar Research Center & Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Republic of Korea*

*Corresponding author at: UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia

E-mail address: jochen.bundschuh@usq.edu.au (Jochen Bundschuh)

ABSTRACT

Production of rice grains at non-toxic levels of arsenic (As) to meet the demands of an ever-increasing population is a global challenge. There is currently a lack of investigation into integrated approaches for decreasing As levels in paddy rice soils. By examining the integrated iron-modified rice hull biochar (Fe-RBC) and water management approaches on As phyto-availability in the paddy rice soil, this study aims to reduce As accumulation in rice grains. The rice cultivar, *Ishikari*, was grown and irrigated with As-containing water (1 mg L^{-1} of As(V)), under the following treatments: (1) Fe-RBC-flooded water management, (2) Fe-RBC-intermittent water supply management strategy, (3) conventional flooded water management, and (4) intermittent water supply management strategy. Compared to the conventional flooded water management, rice yield per pot and Fe and Si concentrations in the paddy pore water under Fe-RBC-intermittent and Fe-RBC-flooded treatments increased by 24%-39%, 100%-142%, and 93%-184%, respectively. The supplementation of Fe-RBC decreased the As/Fe ratio and the abundance of Fe(III) reducing bacteria (i.e. *Bacillus*, *Clostridium*, *Geobacter*, and *Anaeromyxobacter*) by 57%-88% and 24%-64%, respectively, in Fe-RBC-flooded and Fe-RBC-intermittent treatments compared to the conventional flooded treatment. Most importantly, Fe-RBC-intermittent treatment significantly ($p \leq 0.05$) decreased As accumulation in rice roots, shoots, husks, and unpolished rice grains by 62%, 37%, 79%, and 59%, respectively, compared to the conventional flooded treatment. Overall, integrated Fe-RBC-intermittent treatment could be proposed for As endemic areas to produce rice grains with safer As levels, while sustaining rice yields to meet the demands of growing populations.

Keywords: Rice; Inorganic arsenic; Iron-biochar; Microorganisms; Silicon

1. Introduction

Arsenic (As) in paddy rice soils is a global environmental and health concern (Bhattacharya et al., 2012, Zavala and Duxbury, 2008). Throughout the full rice growing cycle, a volume of 500 to 3000 mm of water needs to be supplied, and this figure depends on the rice genotype, climatic conditions, and soil type (Kumarathilaka et al., 2018a). Particularly in dryer periods, rice cultivation is dependent on both groundwater and surface water (i.e. water from lake, river, and dam) sources. Use of

As-contaminated groundwater for irrigating rice is the major source of As to paddy rice soils. Studies have demonstrated that the use of As-contaminated groundwater for irrigating rice has contributed to increased As levels in the paddy soil-water system as well as in rice grains (Brammer and Ravenscroft, 2009, Rahman and Hasegawa, 2011, Vicky-Singh et al., 2010).

Different physico-chemical and biological factors affect As speciation in the paddy soil-water system (Kumarathilaka et al., 2020a). Arsenite (As(III)) and arsenate (As(V)) are inorganic As species found in paddy rice soils. Dimethylarsinic acid (DMA(V)) and monomethylarsonic acid (MMA(V)) are the commonly detected organic As species in paddy rice soils (Bakhat et al., 2017). In terms of toxicity, inorganic As species are more toxic than the mentioned organic As species. Arsenic (III) is mainly acquired through nodulin 26-like intrinsic proteins (NIPs) such as OsNIP2;1 (Lsi1) which is also responsible for Si uptake in rice plants (Zhao et al., 2009). Phosphate transporters share the same pathway for PO_4^{3-} and As(V) in rice. It is suggested that both DMA(V) and MMA(V) are acquired by $Si(OH)_4$ transporters (Li et al., 2009).

The method of rice cultivation (i.e. long-term flooded water management) results in a higher inorganic As concentrations in rice grains than in other cereal crops (Su et al., 2010). The availability of iron (Fe) in the paddy-soil water system plays a major role in sequestrating both As(III) and As(V) through surface complexation (Liu et al., 2006). Even though paddy soils comprise indigenous Fe(III)-hydro(oxides) (i.e. ferrihydrite, hematite, lepidocrocite, and goethite), phase conversion from poorly crystalline ferrihydrite to crystalline Fe oxides such as goethite and hematite diminishes the number of adsorption sites available for the sorption of inorganic As species (Komárek et al., 2013). The external supplementation of Fe(III)-hydro(oxides) to As-contaminated paddy environments may enhance the number of adsorption sites available for As.

Intermittent water supply management strategy has been recognized as a potential way of decreasing As accumulation in paddy rice soils (Mukherjee et al., 2017). During intermittent water supply management strategy, the rice field is only flooded to desired levels (~3 - 5 cm) when the soil is dry and cracks are found (P. Hu et al., 2013).

Therefore, intermittent water supply management strategy has been found to cut down the volume of irrigation water required for rice cultivation. Even though intermittent water supply management strategy has reduced As accumulation in rice grains, loss of rice yield is one of the concerns associated with this practice (Basu et al., 2015). The loss of rice yield could adversely affect both farmers' incomes and the demand for rice by ever-increasing populations around the world.

Application of biochar (BC) in agricultural ecosystems has been found to increase crop productivity since BC releases essential nutrients into soil pore water (Jeffery et al., 2011). Biochar is produced under an O_2 free environment at higher temperatures (~400 - 800 ºC) by using organic feedstocks (Cha et al., 2016, Jayawardhana et al., 2018). Biochar production by using waste organic feedstocks is a technically feasible and economically viable option. The BC in agricultural soil-water systems can also adsorb contaminants through physical and chemical adsorption processes (Jayawardhana et al., 2019, Kumarathilaka et al., 2018c). In addition, BC in agricultural soil-water systems can promote C sequestration and enhance the water holding capacity (Ghani et al., 2013). The BC can be modified through physical and chemical activation processes to enhance the properties of BC to improve contaminant removal and nutrient supplementation (Sizmur et al., 2017, Wang and Wang, 2019).

In this study, an integrated approach was performed to examine As accumulation in rice tissues as well as rice yield. For that, Fe-modified rice hull BC (Fe-RBC) was applied to As-contaminated paddy soil-water systems under different water management practices (i.e. conventional flooded water management and intermittent water supply management strategy). In literature, Fe-modified BC produced by using different organic feedstocks has been applied to As-contaminated rice ecosystems to examine As phyto-availability. To the author's knowledge, this is the first study of supplementation of Fe-RBC to As-contaminated paddy soils. In addition, this is the first study that evaluates the integrated effects of Fe-modified BC and water management practices on As phyto-availability in paddy rice soils.

2. Materials and methods

2.1. Collection and characterization of paddy soils

Paddy soils were collected from New South Wales, Australia (34º 35′ 53.5″ S, 146º 21′ 38.1″ E), air-dried, sieved, and then thoroughly mixed to obtain a composite sample. Physico-chemical properties of the soil such as pH, electrical conductivity (EC), moisture content, cation exchange capacity, and total and EDTA (ethylene-diamine-tetra-acetic acid)-extractable concentrations of metal(loids) were determined. Table S1 summarizes methods used for the determination of physico-chemical characteristics of paddy soils and obtained values for each parameter.

2.2. Preparation and characterization of iron modified biochar

Biochar samples were prepared and characterized according to the methods described elsewhere (Ahmad et al., 2012, Kumarathilaka and Vithanage, 2017). The rice hull biomass was pyrolyzed at 600 ºC for 2 h by using a muffle furnace (RIO GRANDE) at a heating rate of 7 ºC min^{-1} to obtain rice hull biochar (RBC). The produced RBC was washed with deionized water several times, oven-dried at 85 ºC, and placed in a sealed container before use. Fe-modified RBC (Fe-RBC) was prepared through direct hydrolysis of iron salt. Solutions of Fe salt were prepared by dissolving 7.23 g of $Fe(NO_3)_3.9H_2O$ in 40 mL of deionized water. A weight of 10 g of RBC was added to the Fe solution and the mixture was stirred for 12 h using a magnetic stirrer (BIBBY HB502). The mixture was then dried at 105 ºC in an oven (STERIDIUM). The dried composites were washed with deionized water several times for removing excess Fe(III)-hydro(oxides). After the washing step, the composite was again dried at 80 ºC and resulting product was the Fe-RBC.

The physico-chemical properties of biochar such as pH, EC, proximate analysis (moisture content, mobile matter, ash content, and resident matter), Brunauer-Emmett-Teller (BET surface area), total pore volume and average pore size were determined according to methods reported by Ahmad et al. (2012). The EDTA extraction solution was used to determine bioavailable As, Fe, and Si contents in Fe-RBC. Spectroscopic analyses including Scanning Electron Microscopy (SEM) (JCM-6000, JEOL) and Fourier Transform Infrared Spectroscopy (FTIR) (IRAffinity-1S, Shimadzu) analysis were also performed to examine the morphology and spectral characteristics of Fe-RBC before and after the modification.

2.3. Pot experiment of rice

A pot experiment was designed to assess the effects of flooded/intermittent irrigation and Fe-RBC supplementation on the accumulation of As species in rice tissues as well as on the rice growth parameters. Rice seeds (*Ishikari*) were surface sterilized with 10% H_2O_2 before germinating them in moist compost for three weeks. Subsequently, uniform size and healthy seedlings were transplanted in pots which contained 2.5 kg of paddy soils. There were four different treatments: (1) continuous flooding with Fe-RBC (1% w/w); Fe-RBC-flooded, (2) intermittent irrigation with Fe-RBC (1% w/w); Fe-RBC-intermittent, (3) conventional flooding without Fe-RBC; flooded, and (4) intermittent irrigation without Fe-RBC; intermittent. Two different water management regimes were designed as follows: (1) flooded water management: a ~5 cm water level was maintained, (2) intermittent water supply management strategy: irrigated intermittently to 5 cm water level, in particular when the soil has dried. The water levels in each treatment were maintained by adding 1 mg L^{-1} of As(V)-containing water. All the treatments consisted of triplicates. The water supplementation throughout the rice growing season under each treatment was calculated. Plant growth and yield parameters including plant height, root length, shoot biomass, and grain production per pot were also measured.

2.4. Chemical analysis

Soil solution samples were collected until grain maturity at 30-day intervals. The Fe and Si concentrations were determined by atomic absorption spectrophotometer (AA-7000, Shimadzu). The levels of total carbon (TC) and total organic carbon (TOC) were measured by total organic carbon analyser (TOC-VCSH, Shimadzu).

Iron plaque on root surfaces was extracted using a Dithionite-Citrate-Bicarbonate (DCB) solution. After harvesting, roots were thoroughly washed with tap water to remove soil attached to the roots. Then roots were rewashed with distilled deionized water several times. A 10 g of fresh roots was agitated with DCB solution for 3 h. The DCB solution comprised 40 mL of 0.3 M sodium citrate ($Na_3C_6H_5O_7.2H_2O$), 5 mL of 1.0 M sodium bicarbonate ($NaHCO_3$), and 3 g of sodium dithionite ($Na_2S_2O_4$). After 3 h agitation, roots were removed from the DCB solution and rinsed with distilled deionized water, with the rinse solution added to the DCB solution. Then, DCB extracted solution was bought to the volume of 100 mL. The concentrations of As and

Fe in the extracted solution were analysed using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer NexION^{TM} 300X) and AAS.

To determine As species in rice tissues (roots, shoots, husks, and unpolished rice grains), powdered rice tissue samples were digested in a microwave digestion system (Multiwave 3000, Anton Paar) following a method described by Herath et al. (2020). The concentrations of As species in rice tissues were measured by Ultra-High Performance Liquid Chromatography - Inductively Coupled Plasma Mass Spectrometry (UHPLC-ICP-MS) (Flexar, PerkinElmer - PerkinElmer NexION^{TM} 300X). For accuracy and precision, As speciation analysis was validated using a certified reference material (CRM) for rice. The ERM-BC211 from the European Commission was used as the CRM for As speciation. Reagent blanks and continuing calibration verification (CCV) were included in each batch. The optimized operational conditions for UHPLC-ICP-MS system to analyse As species is described elsewhere (Herath et al., 2020).

2.5. Microbial diversity analysis

Rhizosphere soils collected at the flowering stage of the rice were used for microbial diversity analysis. DNeasy® PowerSoil® Pro kit (QIAGEN) was used for extracting DNA, according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) amplicons were generated using the primers and conditions summarized in Table S2. Thermocycling was completed with an applied biosystem (Veriti^{TM} 384) and a Platinum SuperFi II PCR master mix (Invitrogen, Australia) was used for the primary PCR. The first stage PCR was cleaned using magnetic beads, and samples were visualised on 2% Sybr Egel (Thermo Fisher Scientific). A secondary PCR was carried out to index the amplicons with the same polymerase master mix. The resulting amplicons were cleaned again using magnetic beads, quantified by fluorometry (QuantiFluor®dsDNA System, Promega), and normalized. The equimolar pool was cleaned for a final time using magnetic beads to concentrate the pool and then measured using a High-Sensitivity D1000 Tape on an Agilent 2200 TapeStation system. The pool was diluted to 5 nM and molarity was confirmed again using a Qubit^{TM} dsDNA high sensitivity assay kit (Thermo Fisher Scientific). This process was followed by sequencing on an Illumina MiSeq (San Diego, CA, USA) with a V3, 600 cycle kit (2 x 300 base pairs paired-end). Paired-end reads were assembled by aligning the forward and reverse reads using PEAR software (version 0.9.5). Primers were identified and trimmed. Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8) software.

2.6. Data analysis

Reported values were the means of three replicates. The statistical significance of As/Fe in root plaque and As concentration in rice roots, shoots, husks, and unpolished grains under different combinations of Fe-RBC supplementation and water management regimes were evaluated by using Duncan's multiple range test with $p<0.05$ in SAS 9.1 statistical package. Origin 6.0 software package was used for making graphs.

3. Results and discussion

3.1. Characterization of iron-modified rice hull biochar

Table S3 summarizes physico-chemical properties of Fe-RBC. The pH of the Fe-RBC possesses a slightly acidic pH (5.33). Relatively high percentages of resident matter and mobile matter in Fe-RBC, 41.36 w/w and 23.24 w/w, respectively, indicated that the application of Fe-RBC to paddy soils could enhance the carbon availability in these soils. Resident matter is the organic fraction of BC that could remain stable in the paddy rice soil for a very long time. Mobile matter is the organic fraction of BC that migrates into the paddy rice soil and becomes a food source for soil microorganisms. A 31.33% of ash content in Fe-RBC demonstrated that inorganic minerals and residues remained in the Fe-RBC structure after Fe modification. The BET results demonstrated a well-developed pore structure in Fe-RBC with a 142.60 m^2 g^{-1} of surface area and 0.1650 mL g^{-1} of pore volume. According to the average pore diameter value (4.6285 nm), it can be revealed that mesopores have been developed in Fe-RBC. As shown in the SEM image (Figure 1(b)), Fe has been successfully incorporated into the RBC structure. Figures 1(c) and 1(d) show FTIR spectra before and after modification to Fe-RBC. The broad and strong band at 3261 cm^{-1} was due to the hydroxyl groups (-OH) of the RBC. The bands at 1049 and 1056 cm^{-1} corresponded to C-OH stretching of carboxylic acids and alcohols. New peaks have appeared at 1695 and 1321 cm^{-1} after modification of RBC with Fe. These findings were consistent with the results reported by X. Hu et al. (2015). The band at 1695 cm^{-1} in Fe-RBC can be attributed to stretching vibration of the carboxyl groups which could complex with Fe(III) (Pehlivan et al., 2013).

The EDTA-extractable As in Fe-RBC was not detectable whereas EDTA-extractable Fe and Si concentrations in Fe-RBC were 1396.5 and 196.27 mg kg^{-1}, respectively. The presence of Fe in the paddy soil-water system could decrease the bioavailability of inorganic As for rice plant uptake. Similarly, the presence of Si in the paddy soil-water system can also reduce As(III) uptake by rice roots. Since both Si and As(III) are acquired by the same Si transporter in rice roots, high Si concentration in the rice rhizosphere decreases the uptake of As(III) by rice roots. Moreover, uptake of Si by rice plants increases the rice plant's resistance to both biotic (fungal infections) and abiotic stresses (strong wind, heavy rain, and salinity).

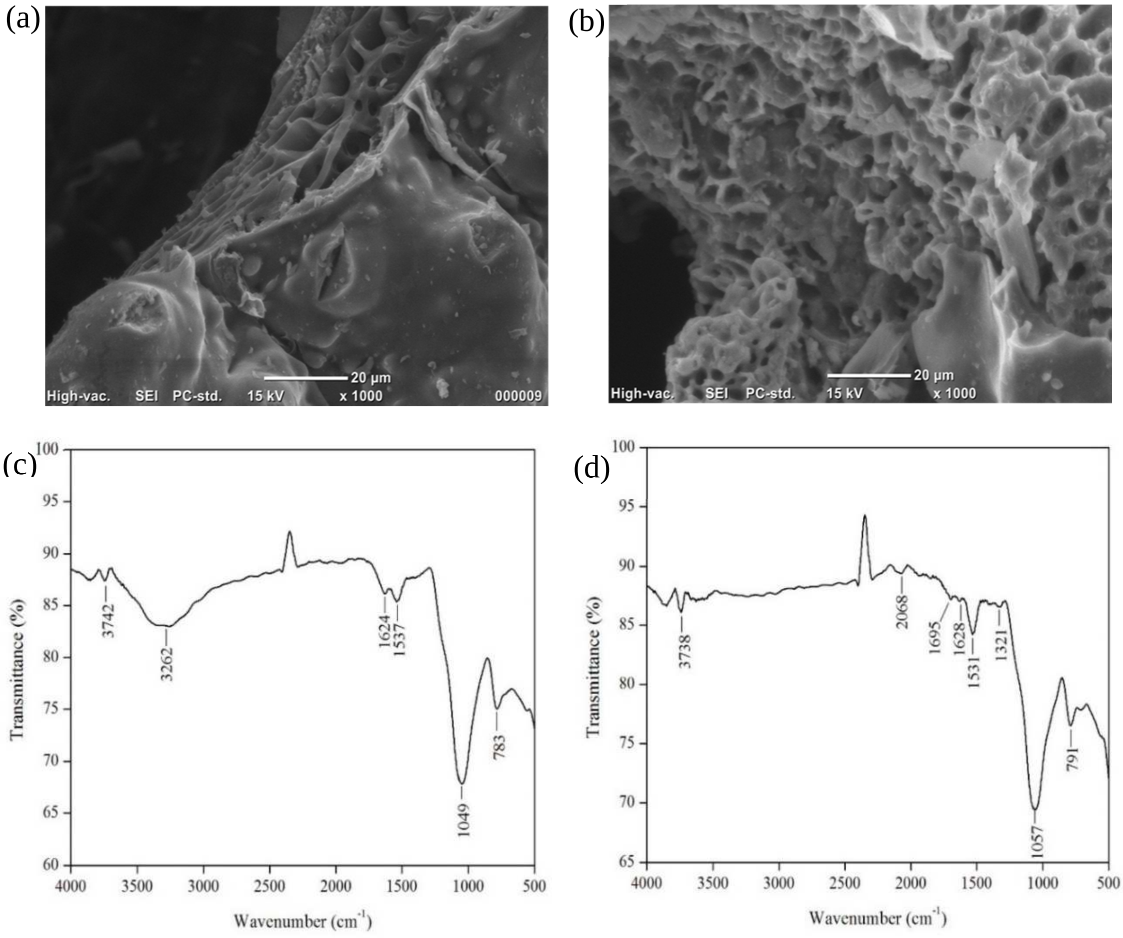


Fig. 1. Scanning electron microscopy images and FTIR spectrums; before (a and c) and after modification to Fe-RBC (b and d).

3.2. Plant growth parameters and water usage

Plant growth parameters such as plant height, root length, shoot weight, and rice yield per pot under different treatments are summarized in Table 1. The application of Fe-RBC to paddy soils increased plant height, root length, shoot weight, and rice yield per pot compared to the conventional flooded treatment. There was a slight increase in plant height (1% - 4%) in Fe-RBC-intermittent and Fe-RBC-flooded treatments in comparison to the flooded treatment. In the case of root length and shoot weight, Fe-RBC-intermittent and Fe-RBC-flooded treatments recorded the highest growth parameters (6% - 14% increment for root length and 6% - 13% increment for shoot weight) over the flooded treatment. Most importantly, rice yield per pot increased in Fe-RBC-intermittent and Fe-RBC-flooded treatments by 24% and 39%, respectively, compared to the conventional flooded treatment. Overall, the highest plant growth parameters were recorded under the Fe-RBC-flooded treatment compared to other treatments. Flooded water management could prevent microbial-induced disease damage in rice plants. Rice plants could also acquire essential elements released from Fe-RBC into paddy pore water. Therefore, Fe-RBC-flooded treatment possessed the highest plant growth parameters compared with other treatments.

The application of Fe-RBC increased water usage efficiency in the paddy soil-water system. For instance, water usage in RBC-flooded treatment decreased by approximately 17% in comparison to the conventional flooded treatment. This figure for Fe-RBC-intermittent treatment was 32%. Since BC could enhance the water holding capacity in agricultural soils, supplementation of Fe-RBC increased water retention in paddy soils while minimizing the water requirement for irrigating rice. Therefore, application of Fe-RBC in paddy rice soils could reduce the cost for irrigation water supplementation. Moreover, Fe-RBC reduced the introduction of As-contaminated water in paddy rice soils.

Table 1

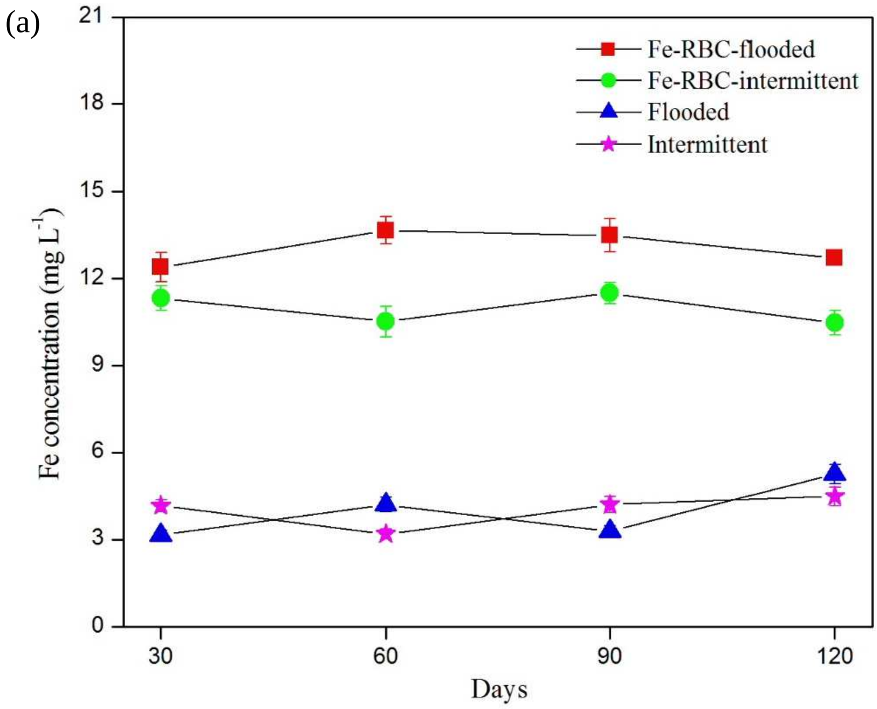
Plant growth parameters and water usage efficiencies per pot under different combinations of Fe-RBC and water management practices. Treatments labelled with same letter mean no significant differences in row.

Parameter	Fe-RBC-Flooded	Fe-RBC-Intermittent	Flooded	Intermittent
Plant height (cm)	71.11 ± 0.83a	69.44 ± 0.76b	68.67 ± 0.72b	66.25 ± 0.66c
Root length (cm)	19.26 ± 0. 93a	17.94 ± 0.86a	16.92 ± 1.10b	16.75 ± 0.78b
Shoot dry weight (g)	17.37 ± 0.69a	16.36 ± 0.57a	15.39 ± 0.85a	14.98 ± 0.72a
Grain weight (g)	14.39 ± 0.24a	12.83 ± 0.37b	10.38 ± 0.28c	10.23 ± 0.17c
Water usage (cm^3)	7411	6065	8925	6327

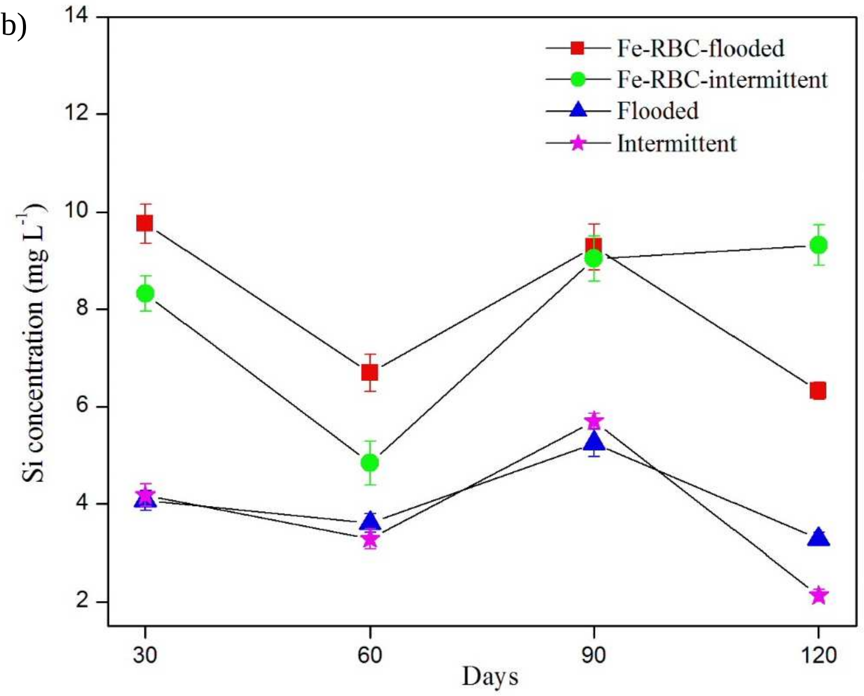
3.3. Analysis of paddy pore water and root plaque

Figure 2 shows the temporal variation of Fe, Si, TC and TOC concentrations in paddy pore water under different treatments. The Fe-RBC in paddy soils increased Fe concentrations in the paddy pore water. For instance, Fe concentrations in Fe-RBC-flooded and Fe-RBC-intermittent treatments increased by 100% - 142% in comparison to the conventional flooded treatment after 120 days of the transplantation. The higher Fe concentration in paddy pore water under Fe-RBC-flooded and Fe-RBC-intermittent treatments corresponded to the release of Fe into paddy pore water from Fe-RBC. The availability of Fe in the paddy soil-water system is an important factor to a decrease bioavailability of As. Since both As(III) and As(V) are complexed with Fe(III)-hydro(oxides), the bioavailability of inorganic As species for rice plant uptake is reduced. In addition, Fe could retain in rice roots, forming Fe plaque, which also decreases As uptake by rice roots. The supplementation of Fe-RBC has also increased Si concentration in paddy pore water in Fe-RBC-flooded and Fe-RBC-intermittent treatments by 93% and 184% (after 120 days of the transplantation), respectively, compared to the conventional flooded treatment. The availability of Si in the paddy soil-water system plays a major role in decreasing As(III) uptake by rice roots. As discussed in section 3.1, both $Si(OH)_4$ and As(III) are acquired by Si transporters in rice roots. Therefore, higher Si concentration in the paddy pore water decreases As(III) uptake by rice roots.

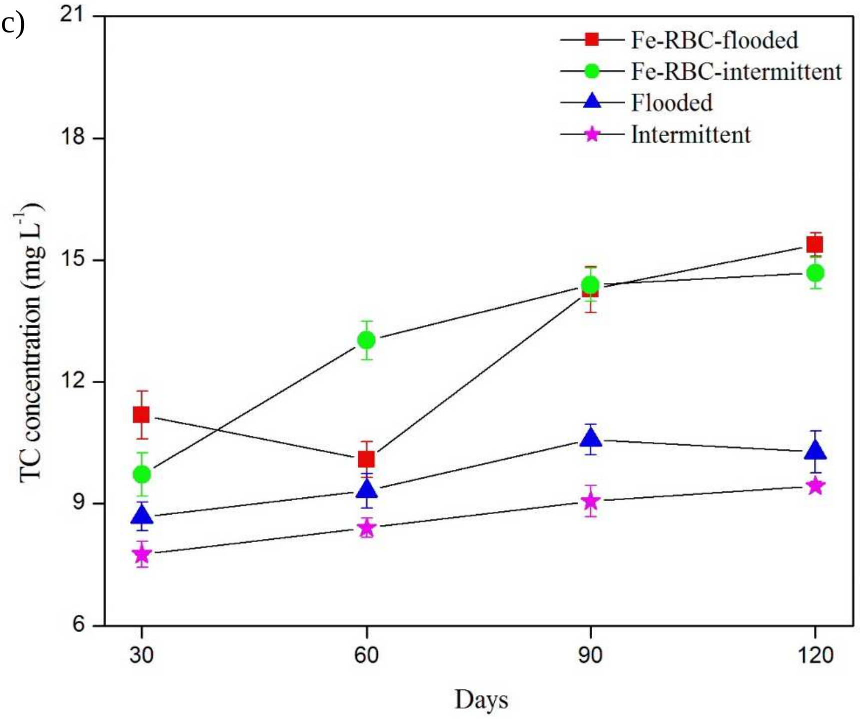
The TC and TOC concentrations in the paddy pore water increased following the application of Fe-RBC in paddy soils (Fig. 2(c) and 2(d)). In Fe-RBC-flooded and Fe-RBC-intermittent treatments, TC increased by 43% - 50% compared to the conventional flooded treatment. In addition, TOC concentration in Fe-RBC-flooded and Fe-RBC-intermittent treatments increased by 72% and 62%, respectively, in comparison to the flooded treatment. As discussed in the section 3.1, Fe-RBC contained 74.6 w/w of mobile matter and resident matter which has increased the C availability in the paddy soil-water system. The availability of C in the paddy soil-water system could promote the activity of microbial community which is responsible for the transformation of As in direct and indirect ways.



(b)


Fe-RBC-flooded
Fe-RBC-intermittent
Flooded
Intermittent
Si concentration (mg L^{-1})
Days
14
12
10
8
6
4
2
30
60
90
120

(c)


Fe-RBC-flooded
Fe-RBC-intermittent
Flooded
Intermittent
TC concentration (mg L^{-1})
Days
21
18
15
12
9
6
30
60
90
120

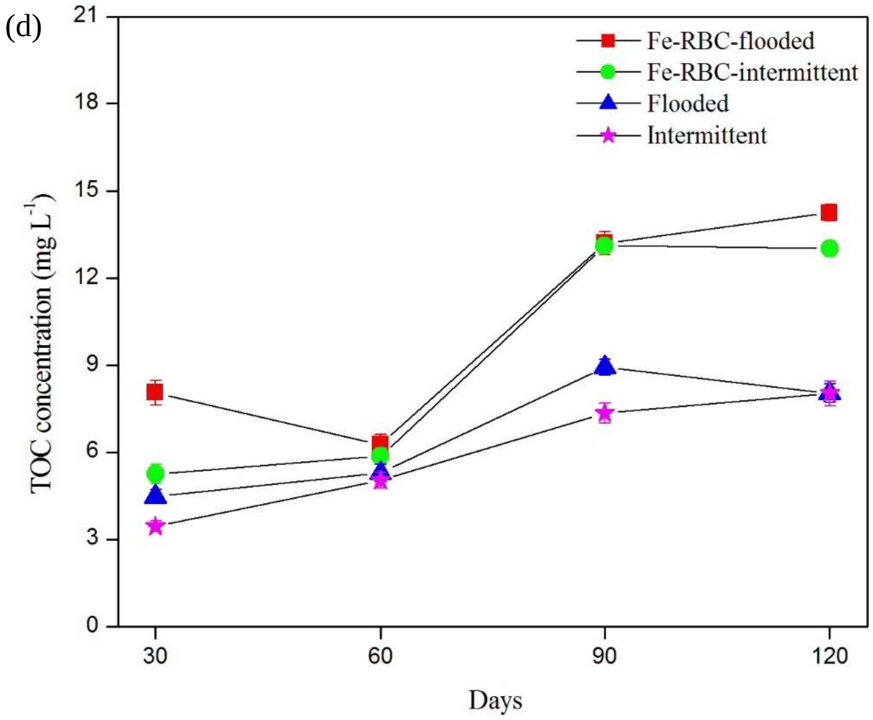


Fig. 2. Variation of Fe (a), Si (b), TC (c), and TOC (d) concentrations in paddy water under different combination of Fe-RBC and water management approaches.

Figure 3 shows the As/Fe ratio in root plaque under different treatments. The As/Fe ratio in Fe-RBC-flooded, Fe-RBC-intermittent, and intermittent treatments significantly ($p \leq 0.05$) decreased by 57%, 88%, and 69%, respectively, in comparison to the conventional flooded treatment. The decreased As/Fe in rice roots indicated the retention of Fe in the root plaque, as well as sequestration of As in Fe(III)-hydro(oxides) in the root plaque. Therefore, Fe-RBC has increased As retention in root plaque, minimizing As uptake by rice roots.

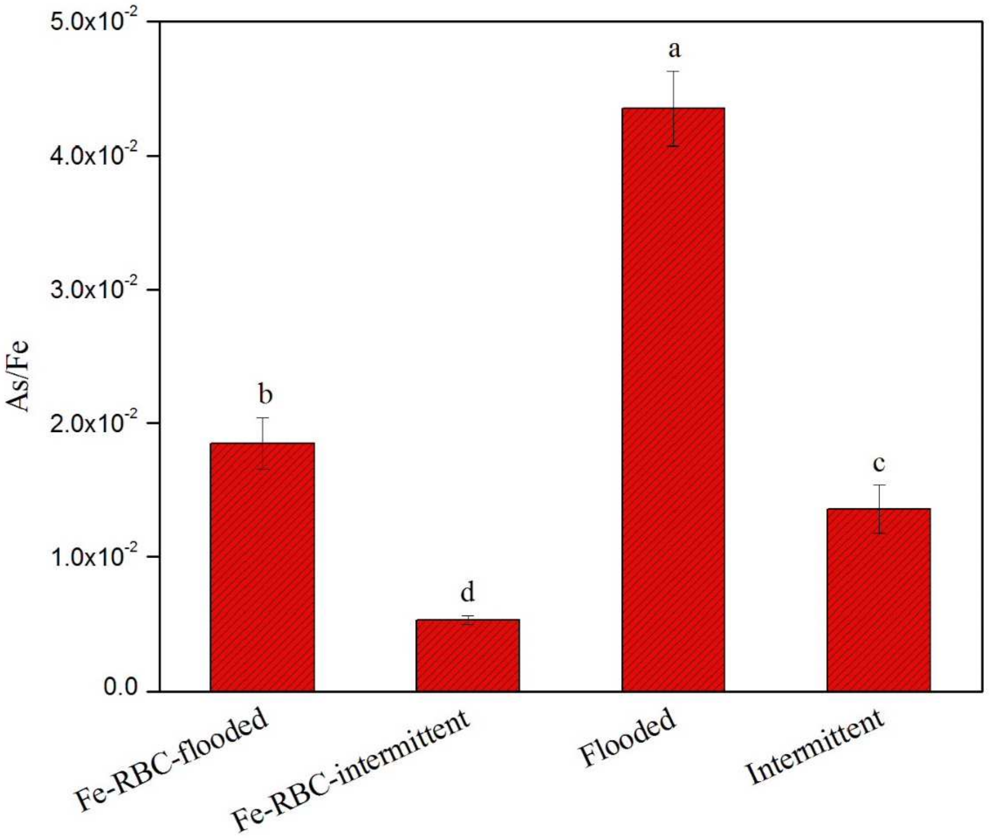
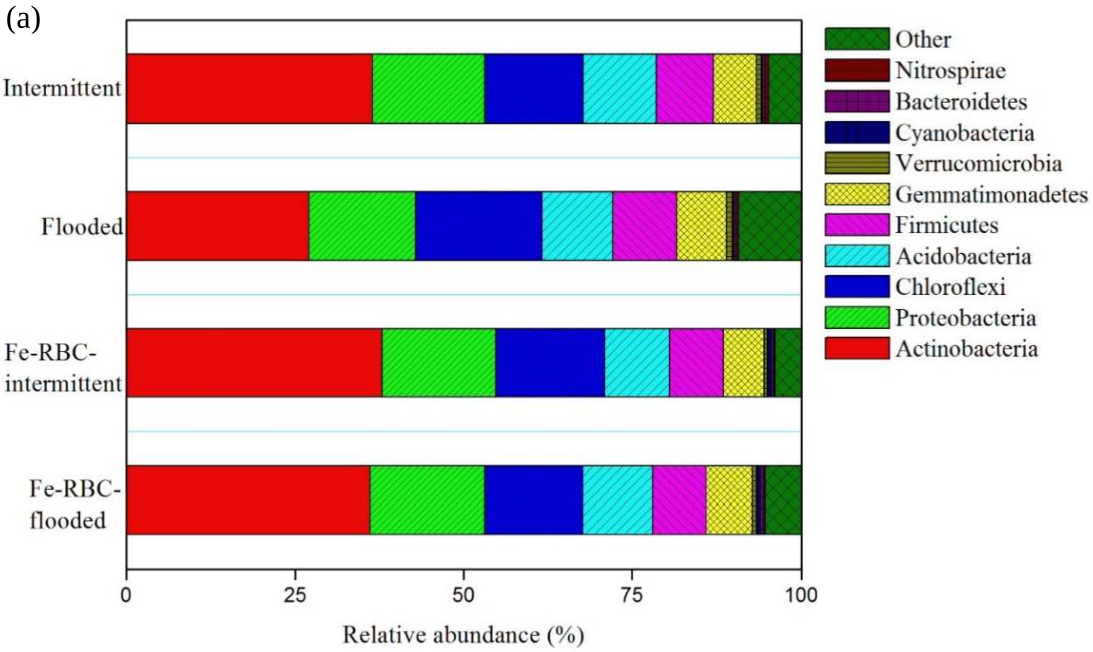


Fig. 3. The ratio of As/Fe in root plaque under different combinations of Fe-RBC and water management techniques. Treatments labelled with different letters are significantly different from each other for the As/Fe in root plaque (Duncan's multiple range test; $p < 0.05$).

3.4. Diversity and abundance of microorganisms

Microbial diversity and abundance in paddy rice soils are important factors since microorganisms directly and indirectly affect the mobility and bioavailability of As in paddy soil-water systems. In this study, 21 different phyla, 67 classes, 110 orders, 170 families, and 221 genera were recorded. The phyla Actinobacteria was prominent in all treatments, containing 36.1%, 37.9%, 27.0%, and 36.5% in Fe-RBC-flooded, Fe-RBC-intermittent, flooded, and intermittent treatments. Following the Actinobacteria, Proteobacteria (15.9% - 17.0%), Chloroflexi (14.6% - 18.7%), Acidobacteria (9.6% - 10.8%), Firmicutes (7.8% - 9.5%), and Gemmatimonadetes (6.0% - 7.3%) dominated in all treatments (Fig. 4(a)). Das et al. (2016) reported that both Proteobacteria and Firmicutes involve the transformation of As in the rice rhizosphere.

Figure 4b shows the relative abundance of different genera in the rice rhizosphere under different treatments. More importantly, the relative abundance of Fe(III) reducing genera such as *Bacillus* and *Clostridium* (belongs to Firmicutes) and *Geobacter* and *Anaeromyxobacter* (belongs to Protobacteria) was higher in the conventional flooded treatment in comparison to other treatments. The abundance of *Bacillus* decreased by 24%, 29%, and 2%, respectively, under Fe-RBC-flooded, Fe-RBC-intermittent, and intermittent treatments compared to the conventional flooded treatment. In addition, the abundance of *Clostridium*, *Geobacter*, and *Anaeromyxobacter* in Fe-RBC-flooded, Fe-RBC-intermittent, and intermittent treatments, respectively, decreased as follows in comparison to the conventional flooded treatment: *Clostridium*: 36%, 64%, and 45%, *Geobacter*: 40%, 60%, and 40%, and *Anaeromyxobacter*: 6%, 15%, and 3%. The availability of Fe(III)-hydro(oxides) in the paddy soil-water system is an important factor to reduce the mobility and bioavailability of As since both As(III) and As(V) could complex with Fe(III)-hydro(oxides). Iron (III) reduction to Fe(II) by Fe(III) reducing bacteria could release inorganic As species complexed with Fe(III) into the paddy soil-water system. Therefore, bioavailability of As in the rice rhizosphere under conventional flooded treatment increased compared to other treatments.



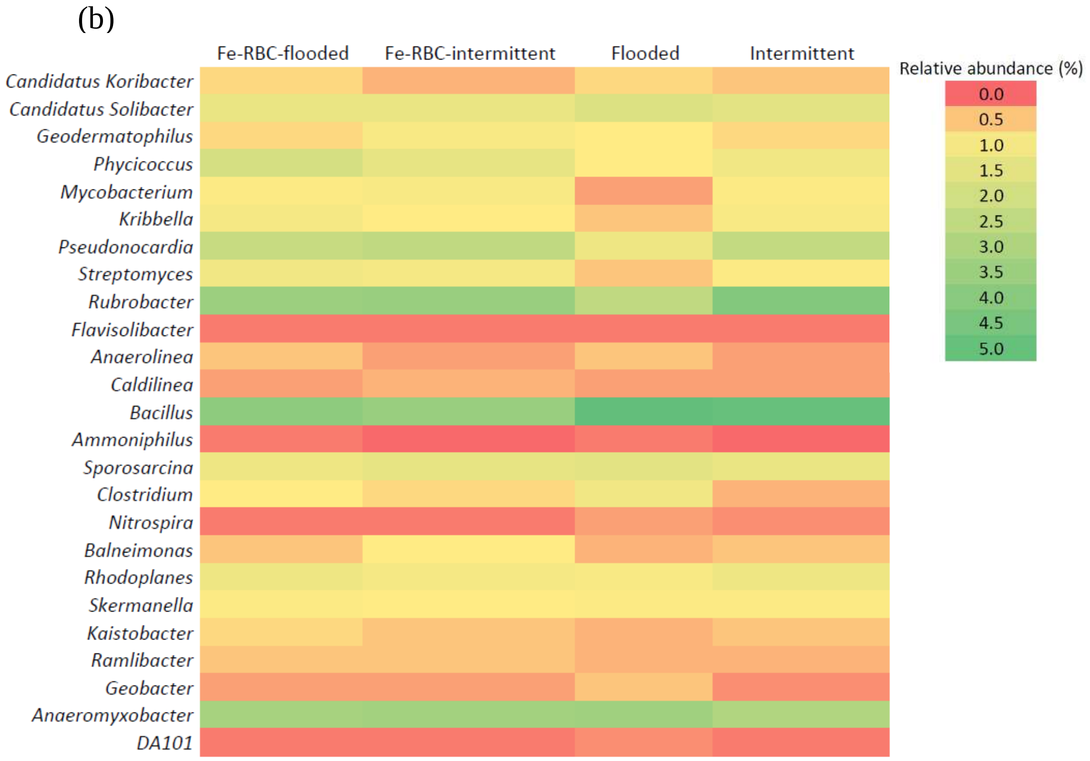
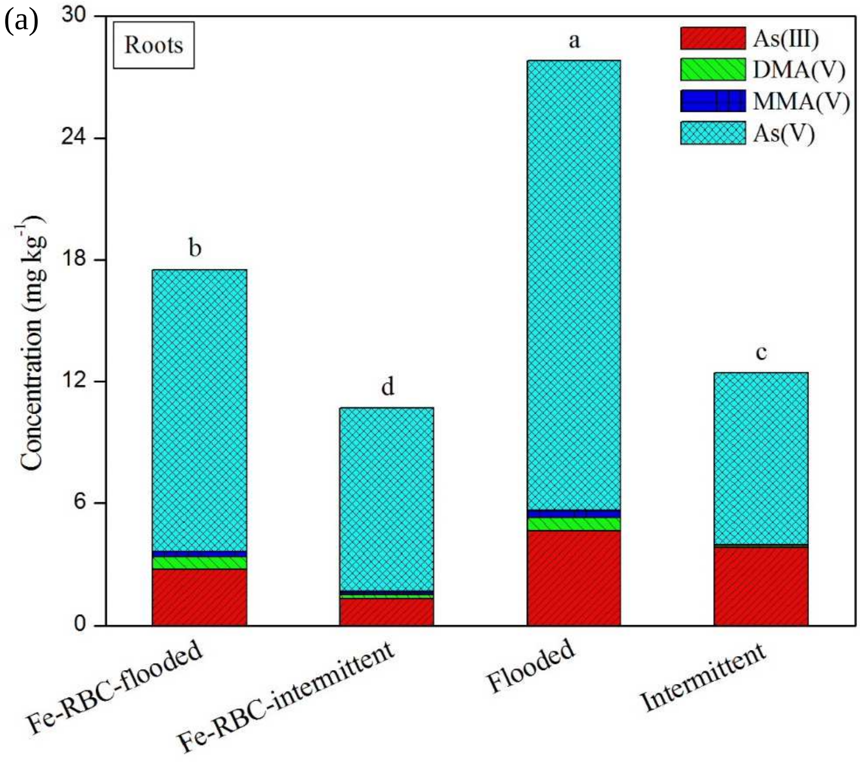


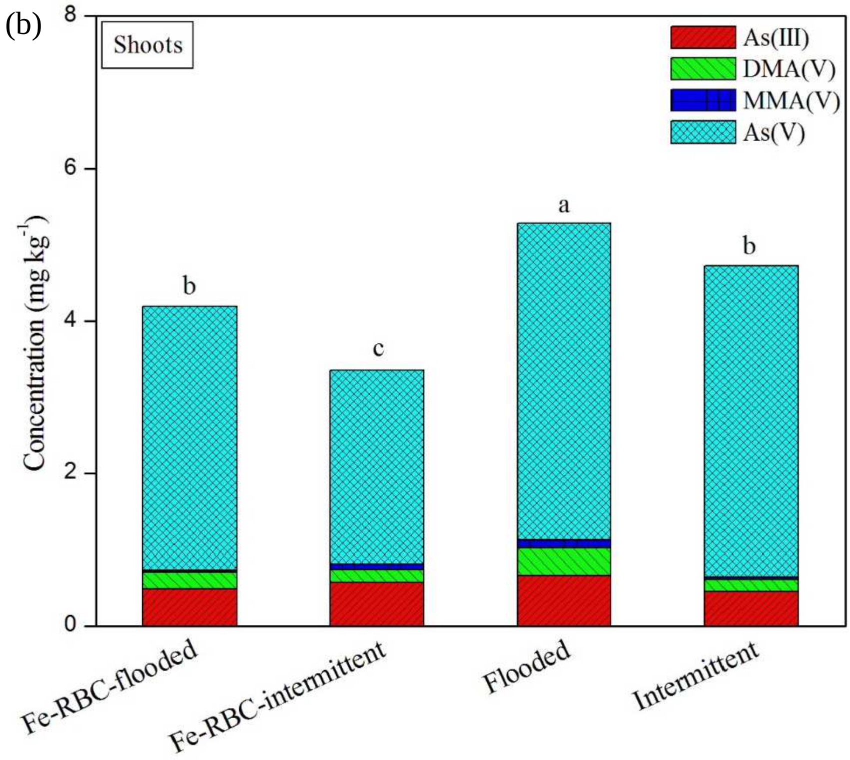
Fig. 4. Relative abundance of phylogenetic groups (a) and prominent genera at genus level (b) in the rice rhizosphere under different combinations of Fe-RBC and water management regimes.

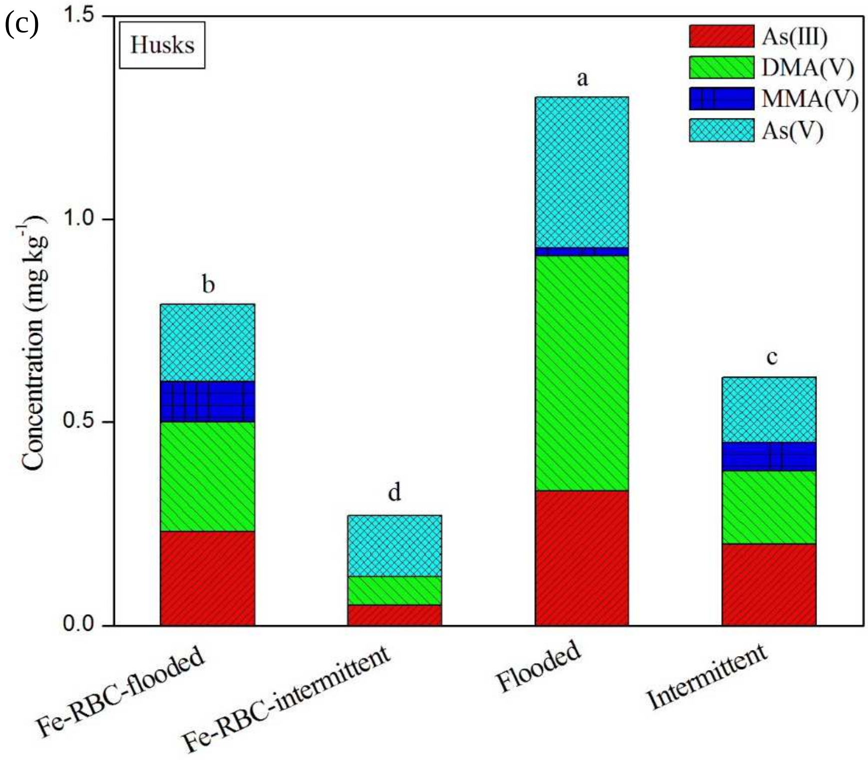
3.5. Arsenic speciation in rice plant tissues with respect to potential mechanisms

Total As concentrations (sum of As(III), DMA(V), MMA(V), and As(V)) in rice roots, shoots, husks, and unpolished rice grains decreased following the addition of Fe-RBC to paddy soils (Fig. 5). In comparison to the conventional flooded treatment, total As content in rice tissues in Fe-RBC-flooded, Fe-RBC-intermittent, and intermittent treatments, respectively, significantly ($p \leq 0.05$) decreased as follows: rice roots (37%, 62%, and 55), shoots (21%, 37%, and 11%), husks (40%, 71%, and 48%), and unpolished rice grains (10%, 44%, and 17%). Most importantly, integrated Fe-RBC-intermittent treatment has decreased most toxic inorganic As concentrations in rice tissues in comparison to conventional flooded treatment. For instance, inorganic As concentration in rice roots, shoots, husks, and unpolished rice grains under Fe-RBC-intermittent treatment was reduced by 61%, 36%, 71%, and 44%, respectively, compared to flooded treatment. This figure under Fe-RBC-flooded treatment was 38%, 18%, 40%, and 10%. Moreover, inorganic As concentration in rice roots, shoots, husks, and unpolished rice grains under intermittent treatment decreased by 55%, 11%, 53%, and 43%, respectively, in comparison to conventional flooded treatment.

The translocation efficiency of inorganic As species from root to shoot in rice plants under each treatment decreased as indicated in Figure 5. The percentages of inorganic As species in rice roots (95% - 99%) and shoots (91% - 96%) were higher than the percentages of organic As species in rice roots (1% - 5%) and shoots (4% - 9%). However, the percentage of inorganic As species in rice husks and unpolished grains decreased to 53% - 74% and 36% - 53%, respectively. In contrast, percentage of organic As species in rice husks (26% - 47%) and unpolished rice grains (47% - 64%) increased compared to the percentage of organic As species in rice roots and shoots.




(b)
Shoots
As(III)
DMA(V)
MMA(V)
As(V)
Concentration (mg kg^{-1})
0
2
4
6
8
b
c
a
b
Fe-RBC-flooded
Fe-RBC-intermittent
Flooded
Intermittent


(c)
Husks
As(III)
DMA(V)
MMA(V)
As(V)
Concentration (mg kg^{-1})
0.0
0.5
1.0
1.5
b
d
a
c
Fe-RBC-flooded
Fe-RBC-intermittent
Flooded
Intermittent

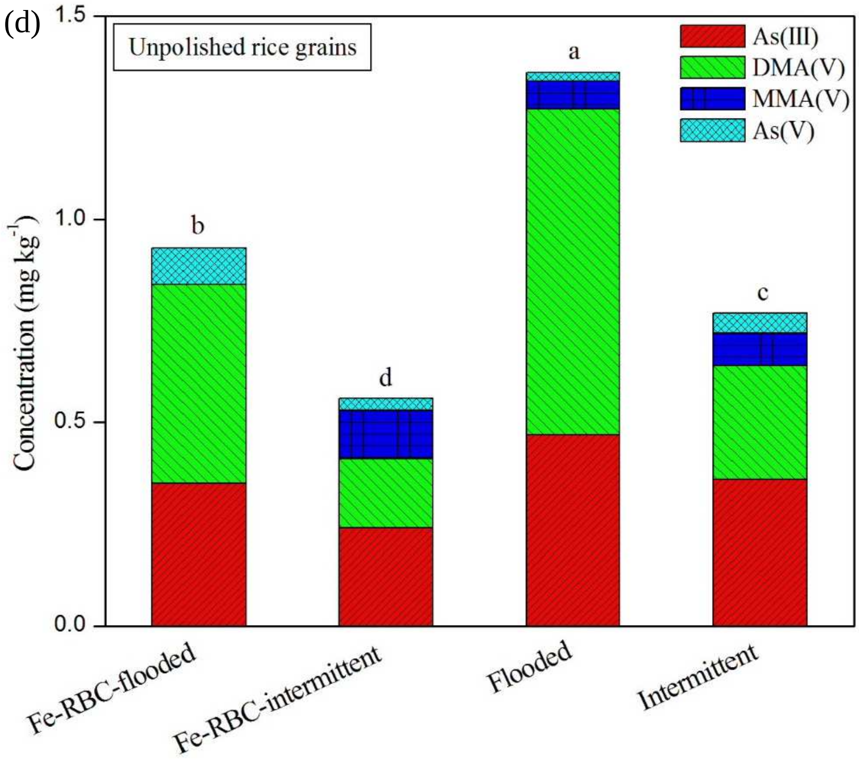


Fig. 5. The concentration of As(III), DMA(V), MMA(V) and As(V) in rice roots (a), shoots (b), husks (c), and unpolished rice grains (d) under different combinations of Fe-RBC and water management approaches. Treatments labelled with the same letter are not significantly different from each other for the total As concentration (Duncan's multiple range test; $p < 0.05$).

Different mechanisms could involve decreasing As accumulation in rice tissues under Fe-RBC and alternative water management conditions (Fig. 6). As shown in Figure 1(b), Fe-RBC possessed a well-developed pore structure. Arsenic species could diffuse into the pores of Fe-RBC through physical adsorption processes. As indicated in Figure 1(d), oxygenated functional groups (i.e. alcoholic and carboxylic) could complex with inorganic As species through the surface complexation. The modification of RBC with Fe could also minimize the bioavailability of inorganic As species in the rice rhizosphere, since inorganic As species could complex with Fe(III)-hydro(oxides) on the RBC surface (Fig. 6). Supplementation of Fe-RBC to paddy soils had increased the Fe concentration in the paddy soil-water system (Fig. 2(a)). The presence of Fe in the rice rhizosphere is an important factor to sequestrate inorganic

As species. Even during the flooded water management conditions, rice roots diffuse O_2 into the rhizosphere through aerenchyma cells in a process called redial oxygen loss (ROL). As a result, more oxidative conditions develop in the rhizosphere zone compared to the bulk soils (Wu et al., 2012). Oxidative conditions in the rice rhizosphere promote the oxidation of Fe(II) to Fe(III) which has high affinity with both As(III) and As(V) (Fig. 6). In addition, oxidative conditions in the rice rhizosphere enhance the formation of Fe(III) plaque on rice roots. As shown in Figure 3, Fe plaque has sequestrated As in the rhizosphere, limiting the bioavailability of As for rice root uptake. Moreover, Figure 4(b) shows decreased abundance of Fe(III), reducing bacteria in the rice rhizosphere, following the supplementation of Fe-RBC in paddy soils. Therefore, the tendency to release As(III) and As(V), which are complexed to Fe(III) hydro(oxides), into the paddy soil-water system decreases, due to the supplementation of Fe-RBC in paddy soils.

The supplementation of Fe-RBC has increased the TOC concentration in the paddy soil-water system (Fig. 2(d)). Inorganic As species could complex with the dissolved organic compounds in the paddy soil-water system, limiting the bioavailability of As. The increased TOC following the application of Fe-RBC could also increase the abundance of microorganisms which are responsible for Fe(II) oxidation. As shown in Figure 2(b), supplementation of Fe-RBC has increased the Si concentration in paddy pore water. Both $Si(OH)_4$ and As(III) are acquired by the same Si transporter, OsNIP2;1 (Lsi1). This could be attributed to relatively similar dissociation constants of $Si(OH)_4$ and $As(OH)_3$, (pK_a of 9.2 and 9.3, respectively), and their similar sizes with tetrahedral orientation. Therefore, higher availability of Si in the paddy soil-water system could increase Si uptake while decreasing the uptake of As(III) through competitive uptake.

An intermittent water supply management strategy rather than the conventional flooded water management could increase the redox potential of the paddy soil-water system. The mobility and bioavailability of inorganic As species decrease under intermittent water supply management strategy. Therefore, Fe-RBC-intermittent treatment has significantly ($p \leq 0.05$) decreased As accumulation in rice tissues in comparison to other treatments. Relatively less As accumulation in rice tissues under intermittent treatment compared to the Fe-RBC-flooded treatment could correspond to less As introduction to paddy soil systems through intermittent water supply management strategy.

Once inorganic As species are acquired by rice roots, there are different As metabolic activities taking place in the rice roots such as reduction of As(V) to As(III), complexation of As(III) with thiol-rich peptides, sequestration of As(III)-thiol-rich peptides in vacuoles, and As(III) efflux back into the external environment (Kumarathilaka et al., 2018b). These different processes are mediated by various enzymes and transporters present in the rice root. As a result, translocation of inorganic As species from rice roots to above ground tissues such as shoots, husks, and unpolished rice grains gradually decreased. On the contrary, translocation of organic As species from rice roots to above ground tissues has increased. This could correspond to the dissociation of both MMA(V) and DMA(V) at the cytoplasmic pH. Moreover, unlike inorganic As species, lack of complexation of organic As species with thiol-rich peptides promoted organic As species to be readily translocated from rice roots to above ground tissues.

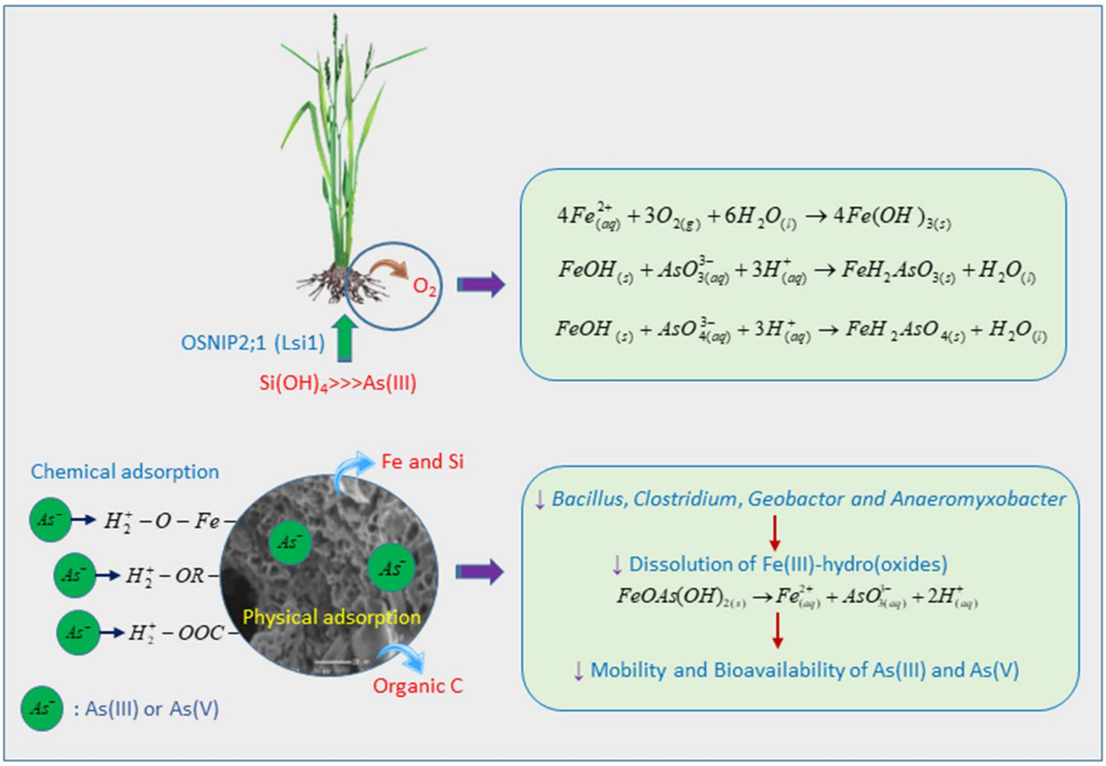


Fig. 6. Potential mechanisms for decreased As mobility and bioavailability in the paddy rice soil under the supplementation of Fe-RBC in paddy soils.

4. Conclusions

Supplementation of Fe-RBC to paddy soils has increased rice yield per pot. Even though Fe-RBC-flooded treatment recorded the highest yield increment per pot compared to the conventional flooded treatment, As concentration in rice tissues under Fe-RBC-flooded treatment was higher in comparison to the Fe-RBC-intermittent and intermittent treatments. The Fe-RBC in the paddy soil-water system has increased Fe, Si, and TOC levels in the paddy pore water and Fe concentrations in the root plaque. The integrated Fe-RBC-intermittent treatment recorded the lowest level of As in rice roots, shoots, husks, unpolished rice grains in comparison to other treatments. Different processes and mechanisms including chemi- and physisorption of As species into Fe-RBC, sequestration of As on Fe plaque, decreased As(III) uptake by rice roots due to the competitive uptake with $Si(OH)_4$ could involve less As accumulation in rice tissues following the supplementation of Fe-RBC in paddy soils. The less abundance of Fe(III) reducing bacteria, following the Fe-RBC supplementation, could also minimize the reduction of Fe(III) which decreases mobility and bioavailability of inorganic As species in the rice rhizosphere. Further research could be focused on examining the As phyto-availability in paddy rice soils under different Fe-RBC supplementation rates, Fe and RBC ratios, and As levels.

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CRediT authorship contribution statement

Prasanna Kumarathilaka: Conceptualization, Data curation, Investigation, Writing - original draft, Writing - review and editing, Formal analysis, Methodology, Resources. **Jochen Bundschuh:** Conceptualization, Writing - review and editing, Methodology, Resources, Supervision. **Saman Seneweera:** Resources, Supervision. **Alla Marchuk:** Resources, Supervision. **Yong Sik Ok:** Resources, Supervision.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary Materials

Iron modification to silicon-rich biochar and alternative water management to decrease arsenic accumulation in rice (*Oryza sativa* L.)

Prasanna Kumarathilaka[a], Jochen Bundschuh[a,b,*], Saman Seneweera[c,d], Alla Marchuk[e], Yong Sik Ok[f]

[a]*School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[b]*UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[c]*Centre for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[d]*National Institute of Fundamental Studies, Hantana Road, Kandy 20000, Sri Lanka*
[e]*Institute for Life Sciences and the Environment, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[f]*Korea Biochar Research Center & Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Republic of Korea*

Table S1

Methods used for physico-chemical characterization of paddy soils and obtained values for each analysed parameters (Kumarathilaka et al. 2020b).

Parameter	Method	Value
pH EC (dS m^{-1})	1:10 suspensions of soil-to-distilled water were agitated for 4 h. A digital pH and conductivity meter (PC2700, EUTECH instruments) was used to take readings	6.59 ± 0.06 0.04 ± 0.01
Moisture content (%)	A moisture meter (ISSCO, MB 120, OHAUS Corporation) was used to measure the moisture content	5.79 ± 0.21
Total organic carbon content	Through a loss-on-ignition method described by Rayment and Lyons (2014)	7.22 ± 0.36
Cation exchange capacity	1M / ammonium extraction (pH adjust to 7.0) method described by Rayment and Lyons (2014)	14.37 ± 1.16
Total concentrations of metal(loids)	Soil samples (0.25 g) were digested in a microwave digestion system (Multiwave 3000, Anton Paar). Arsenic concentration in paddy soils was measured using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer NexIONTM 300X). The concentration of other elements was determined using an atomic absorption spectrophotometer (AAS) (AAS-7000, Shimadzu)	As - 3.10 ± 0.27 Fe - 12720.26 ± 130 Mn - 288.15 ± 9.56 Si - 2190.10 ± 24.3 Zn - 32.55 ± 2.58 Mg - 877.05 ± 26.8 K - 1284.50 ± 33.4 Ni - 23.99 ± 1.27 Co - 119.05 ± 7.92 Ca - 5531.58 ± 29.6 Al - 43718.86 ± 147
Bioavailable concentrations of metal(loids)	20 mL of 0.05 M / Ethylene-diamine-tetra-acetic acid (EDTA) is added to 1 g of soil. Then the mixture is stirred	As - 0.04 ± 0.01 Fe - 1147.74 ± 22.5 Mn - 197.82 ± 12.3

	for 3 h, centrifuged, and the supernatant is filtered through the membrane filtration. The concentration of As was determined through ICP-MS. The concentration of other elements was determined using AAS.	Si - 153.43 ± 14.6 Zn - 2.23 ± 0.29 Mg - 37.78 ± 5.31 K - 66.65 ± 4.68 Ni - 2.35 ± 0.62 Co - 42.5 ± 4.93 Ca - 513.64 ± 15.6 Al - 563.88 ± 13.8

Table S2

Conditions optimized for microbial diversity analysis in the rice rhizosphere.

Parameter	Value
Target	16S: V3-V4
Cycle	29
Initial	95 ºC for 7 min
Disassociate	94 ºC for 30 sec
Anneal	50 ºC for 60 sec
Extension	72 ºC for 60 sec
Finish	72 ºC for 7 min

Target	341F-806R
Forward primer (341F)	CCTAYGGGRBGCASCAG
Reverse primer (806R)	GGACTACNNGGGTATCTAAT

Table S3

Physico-chemical properties of Fe-RBC.

Parameter	Value
pH	5.33 ± 0.07
EC (dS m^{-1})	5.20 ± 0.09
Proximate analysis	
Moisture (w/w)	4.05 ± 0.31
Mobile matter (w/w)	23.24 ± 2.08
Ash (w/w)	31.33 ± 3.94
Resident matter (w/w)	41.36 ± 3.67
Specific surface area (m^2 g^{-1})	142.60
Total pore volume (mL g^{-1})	0.1650
Average pore diameter (nm)	4.6285
As (mg kg^{-1})[a]	ND
Fe (mg kg^{-1})[a]	1396.5 ± 43.2
Si (mg kg^{-1})[a]	196.27 ± 12.3

[a] EDTA-extractable fraction

ND: not detectable

5.3 Concluding remarks

The integrated Fe-modified rice hull BC-intermittent water supply management strategy has significantly decreased the accumulation of As in rice tissues in comparison to other treatments. Numerous mechanisms could be involved in minimizing the accumulation of As in rice roots, shoots, husks, and unpolished rice grains under the integrated Fe-modified rice hull BC-intermittent condition. Arsenic species can be complexed with oxygenated functional groups in the Fe-modified rice hull BC. Arsenic species can also be diffused into the well-developed pore structure of the Fe-modified rice hull BC. The supplementation of Fe-modified rice hull BC has promoted the formation of Fe plaque on rice roots which could sequestrate both As(III) and As(V), limiting the mobility and bioavailability of As in the rice rhizosphere. Since Fe-modified rice hull BC has released Si into the paddy pore water, the uptake of As(III) by rice roots has decreased in Fe-modified rice hull BC amended treatments. The supplementation of Fe-modified rice hull BC has reduced the abundance of Fe(III) reducing bacteria such as *Bacillus*, *Clostridium*, *Geobacter*, and *Anaeromyxobacter*. Therefore, the bioavailability of highly toxic As(III) can be reduced in the rice rhizosphere. Moreover, intermittent water supply management strategy can increase the redox potential of the paddy soil-water system, thereby, decreasing the bioavailability of As(III) in the paddy soil-water system. Interestingly, incorporation of Fe-modified rice hull BC in paddy soils has increased rice yield. Therefore, an integrated Fe-modified rice hull BC-intermittent water supply management strategy can be applied for As prone areas to produce rice grains with safer As concentrations for human consumption, while sustaining rice yield for the demand of increasing populations.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

This chapter represents a comprehensive overview of the key findings of this PhD research project and provides recommendations for future research directions.

6.1 Conclusions

This PhD research project initially investigated the current knowledge and research gaps in the field of As phyto-availability in paddy rice soils, uptake and metabolism of As species in rice plants, and the physico-chemical and biological approaches to decrease the accumulation of As in rice plants. The new insight and research gaps obtained from the above mentioned fields have been used to design and implement scientific-based, cost-effective, practically employable, and sustainable approach/es to decrease As accumulation in paddy rice soils and to increase rice yields to meet the demands of ever-increasing populations around the world.

The conventional way of rice cultivation (long-term flooded condition and short-term non flooded conditions) has increased As accumulation in paddy soil-water systems and in rice tissues. Alternative water management practices such as intermittent water supply management strategy could decrease the bioavailability of As in the rice rhizosphere. However, loss of rice yield is the major concern related to intermittent water supply management strategy in As-contaminated paddy rice soils. Therefore, incorporation of BC in the form of pristine or modified states into the As-contaminated paddy rice soils would be a cost-effective and sustainable way of decreasing the bioavailability of As in the paddy soil-water system as well as increasing the rice yield for the demand of growing populations worldwide. Taking these facts into account, this PhD research project, for the first time, evaluated the integrated effect of pristine/modified BC-water management approaches on As phyto-availability in the paddy rice soil and plant growth parameters with respect to potential mechanisms. The research findings obtained from this PhD research project can be applied to As-contaminated paddy rice soils to decrease As accumulation in rice grains and to increase rice yield in rice genotypes.

In the first phase, integrated pristine rice hull BC-water management approaches were investigated on As phyto-availability in paddy rice soils and plant growth parameters. The integrated approach of rice hull BC supplementation and intermittent water supply management strategy significantly decreased both inorganic and organic As concentrations and enhanced essential elemental concentrations (i.e. Fe, Mn, Zn, Mg, and Ca) in unpolished rice grains. Even though integrated rice hull BC-flooded water management practices have increased rice yield, the concentration of inorganic As in unpolished rice grains was higher in comparison to the integrated rice hull BC-intermittent water supply management strategy. The prevention of microbe-mediated disease damage to rice plants under flooded water management could be a reason for an increased rice yield under the integrated rice hull BC-flooded water management practice. The supplementation of rice hull BC to paddy soils has increased Si and SO_4^{2-} concentrations in the paddy soil-water system. Since both $Si(OH)_4$ and As(III) are acquired by Si transporters present in the rice roots, higher Si availability in the paddy pore water decreases the uptake of As(III), due to the competitive uptake with $Si(OH)_4$. In this sense, the supplementation of Si-rich rice hull BC to As-contaminated paddy agro eco-systems is a sustainable way of Si fertilization. The presence of SO_4^{2-} in the paddy pore water, following the supplementation of rice hull BC, could enhance the formation of thiol-rich peptides which have high affinity with As(III). Therefore, translocation of As(III) from the rice root to shoots can be decreased. Arsenic species can be adsorbed to the well-developed pore structure of rice hull BC through the physical adsorption process. In addition, As species could be complexed with oxygenated functional groups on rice hull BC through the chemical adsorption process. Moreover, the presence of rice hull BC in the paddy soil-water system has decreased the abundance of Fe(III)-reducing bacteria. The less abundance of Fe(III) reducing bacteria in the rice rhizosphere leads to minimization of the dissolution of Fe(III)-(hydro)oxides which have high affinity with both As(III) and As(V). Therefore, mobility and bioavailability of As in the paddy soil-water system decreased under rice hull BC supplementation. Taking these facts into account, integrated approach of rice hull BC-intermittent water supply management strategy can be used as a promising tool to decrease As accumulation and sustain essential element concentrations in rice grains.

In the second phase, integrated modified BC-water management approaches were investigated on plant growth parameters, As accumulation in rice tissues, and health risks associated with the consumption of rice, in different rice genotypes. Birnessite-modified rice hull BC was used for this second phase. The supplementation of birnessite-modified rice hull BC has increased the rice yield in both selected rice varieties in this study. The integrated approach of birnessite-modified rice hull BC-intermittent water supply management strategy significantly decreased both inorganic and organic As concentrations in rice roots, shoots, husks, and unpolished rice grains in comparison to other treatments. Birnessite-modified rice hull BC in the paddy soil-water system can adsorb As species through physical and chemical adsorption processes. Arsenic species could also be adsorbed to oxygenated functional groups and MnO_2 present on the rice hull BC structure. Sequential extraction results demonstrated that As bound to specifically sorbed fraction has increased following the incorporation of birnessite-modified rice hull BC in paddy soils. The presence of MnO_2, following the addition of birnessite-modified rice hull BC, can oxidize As(III) to As(V) which is subsequently complexed with Mn minerals. Therefore, mobility and bioavailability of highly toxic inorganic As decreased in the paddy soil-water system. More interestingly, health risk indexes such as estimated daily intake, hazard quotient, incremental lifetime cancer risk, and cancer risk for 100,000 people was reduced under the integrated birnessite-modified rice hull BC-intermittent water supply management strategy. Therefore, integrated birnessite-modified rice hull BC-intermittent water supply management strategy can be adapted to As-contaminated paddy rice soils to produce rice grains at non-toxic As levels for safer consumption of growing populations worldwide.

In the third phase, an integrated Fe-modified rice hull BC-water management approach has been examined regarding plant growth parameters, accumulation of As species in rice tissues and microbial diversity in the rice rhizosphere. The supplementation of Fe-modified rice hull BC has increased rice yield. The presence of Fe-modified rice hull BC has increased the Fe, Si, and TOC concentrations in paddy soil pore water. Increased Fe concentration in the paddy soil-water system leads to complexation of both As(III) and As(V) with Fe(III)-(hydro)oxides. Therefore, bioavailability of inorganic As species in the paddy soil-water system decreased. The presence of Si in the paddy soil-water system causes reduced As(III) uptake by rice roots since both $Si(OH)_4$ and As(III) are acquired by the same Si transporters. The increased TOC concentrations in the paddy pore water could enhance the microbial growth in the rice rhizosphere. The increased Fe concentrations on root plaque, following the addition of Fe-modified rice hull BC, also leads to sequestration of As, thereby limiting the bioavailability of As in the paddy soil-water system. Moreover, the incorporation of Fe-modified rice hull BC in paddy soils has decreased the abundance of Fe(III) reducing bacteria such as *Bacillus*, *Clostridium*, *Geobacter*, and *Anaeromyxobacter* in the rice rhizosphere. The availability of Fe(III)-hydro(oxides) in the paddy soil-water system is a key factor to reduce the mobility and bioavailability of As since both As(III) and As(V) could complex with Fe(III)-hydro(oxides). Iron (III) reduction to Fe(II) by Fe(III) reducing bacteria could release inorganic As species complexed with Fe(III) into the paddy soil-water system. Therefore, decreased abundance of Fe(III) reducing bacteria under the supplementation of Fe-modified rice hull BC leads to decreased bioavailability of inorganic As species in the paddy-water system. Taking these facts into account, Fe-modified rice hull BC-intermittent water supply management strategy can be proposed for As-contaminated paddy rice soils to decrease the accumulation of As and increase the rice yield to meet the demand of increasing populations.

When comparing the performance of three different BC types used in this PhD research, rice hull BC has mostly decreased As accumulation in rice grains. Overall, this PhD research provides sustainable and more scientific-based approaches and directions to produce rice grains for safer human consumption, to meet the demand for rice by an ever-growing population worldwide.

6.2 Recommendations

This PhD research project successfully investigated, for the first time, the effects of a pristine/modified BC-water management approach on plant growth parameters and As phyto-availability in paddy rice soils. In future research focused on decreasing As accumulation in paddy rice soils the following aspects will need to be investigated.

1. In this PhD research, rice hull BC was produced only at 600 ºC. Therefore, it is worth studying both the integrated effects of rice hull BC supplementation, which

is produced at different temperatures, and water management approaches on rice yield and As phyto-availability in paddy rice soils.

2. The pristine/modified BC amendment rate in this PhD research was 1 w/w. Therefore, investigation of the integrated effects of the rice hull BC-water management approach on rice yield and As phyto-availability in paddy rice soils with the pristine/modified rice hull BC at varying rates can be designed in future research.

3. In this PhD research, 1 mg L^{-1} of As-contaminated water was used to irrigate rice. Therefore, the integrated effects of the rice hull BC-water management approach on rice yield and As phyto-availability in paddy rice soils, which are contaminated with varying As concentrations, can be examined.

4. Production of birnessite-modified rice hull BC in different MnO_2: BC ratios and investigation of the integrated effects of birnessite-modified rice hull BC-water management approach on rice yield and As phyto-availability in paddy rice soils can be performed in future research to optimize the MnO_2: BC ratio and to prevent any secondary contamination.

5. Production of Fe-modified rice hull BC in different Fe: BC ratios and investigation of the integrated effects of the Fe-modified rice hull BC-water management approach on rice yield and As phyto-availability in paddy rice soils can be developed in future research to optimize the Fe: BC ratio and to prevent any secondary contamination.

6. This PhD research did not perform gene expression analysis. Performing a qualitative analysis of different genes involved in As species uptake, reduction, efflux, transformation, complexation, and sequestration in rice plants under different pristine/modified BC amendments-water management regimes and in different rice cultivars will provide a better understanding of As metabolism in rice plants.

7. This PhD research was conducted on a glasshouse scale. Therefore, implementation of a field scale experiment to investigate the long-term effects of the integrated pristine/modified rice hull BC-water management approaches on rice yield and As phyto-availability in paddy rice soils should be done in future research.

8. Silicon-rich rice hull BC was used for this PhD study. Therefore, it is important to find out other Si-rich organic feedstocks to produce Si-rich BC in applying As-contaminated paddy rice soils as a cost-effective alternative to expensive Si fertilization in future research.

9. It is worth studying the pristine/modified RBC-related increase of As accumulation in the rice rhizosphere and the expected stability of pristine/modified RBC to safely and permanently sequester As.

10. An economic feasibility analysis is required to be performed to evaluate the applicability of pristine/modified RBC in As-contaminated paddy rice soils.

6.3 Future research directions: how to decrease arsenic transfer through food chain?

Article VII

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6.3.1 Introduction

It is essential to prepare cooked rice at non-toxic levels of As for safer human consumption. This review discusses the major causes of high inorganic As content in cooked rice foods. Very limited numbers of studies have focused on the development of measures to reduce As content in cooked rice foods. The effects of post-harvesting technologies (i.e. polishing and storage of rice grains, washing of raw rice, cooking rice in excess water, and use of continual stream of percolating water) on As concentration in cooked rice foods have been discussed in detail in this review. The use of rainwater, collected through rainwater harvesting systems, as an alternative water source for preparing rice in As endemic areas is also evaluated. Furthermore, the importance of in vivo and in vitro methods which can be utilized for accessing the bioavailability and bioaccessibility of As species through rice consumption has been discussed in this review, to minimize As contamination through the food chain.

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Review article

Arsenic in cooked rice foods: Assessing health risks and mitigation options

Prasanna Kumarathilaka[a], Saman Seneweera[b,c], Yong Sik Ok[d], Andrew Meharg[e], Jochen Bundschuh[a,f,*]

[a] School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350, Australia
[b] Centre for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350, Australia
[c] National Institute of Fundamental Studies, Hantana Road, Kandy, 20000, Sri Lanka
[d] Korea Biochar Research Center & Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Republic of Korea
[e] Institute for Global Food Security, Queen's University Belfast, David Keir Building, Malone Road, Belfast, BT9 5BN, United Kingdom
[f] UNESCO Chair on Groundwater Arsenic within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350, Australia

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ABSTRACT

Human exposure to arsenic (As) through the consumption of rice (*Oryza sativa* L.) is a worldwide health concern. In this paper, we evaluated the major causes for high inorganic As levels in cooked rice foods, and the potential of post-harvesting and cooking options for decreasing inorganic As content in cooked rice, focusing particularly on As endemic areas. The key factors for high As concentration in cooked rice in As endemic areas are: (1) rice cultivation on As-contaminated paddy soils; (2) use of raw rice grains which exceed 200 μg kg^{-1} of inorganic As to cook rice; and (3) use of As-contaminated water for cooking rice. In vitro and in vivo methods can provide useful information regarding the bioaccessibility of As in the gastrointestinal tract. Urinary levels of As can also be used as a valid measure of As exposure in humans. Polishing of raw rice grains has been found to be a method to decrease total As content in cooked rice. Sequential washing of raw rice grains and use of an excess volume of water for cooking also decrease As content in cooked rice. The major concern with those methods (i.e. polishing of raw rice, sequential washing of raw rice, and use of excess volume of water for cooking rice) is the decreased nutrient content in the cooked rice. Cooking rice in percolating water has recently gained significant attention as a way to decrease As content in cooked rice. Introducing and promoting rainwater harvesting systems in As endemic areas may be a sustainable way of reducing the use of As-contaminated water for cooking purposes. In conclusion, post-harvesting methods and changes in cooking practices could reduce As content in cooked rice to a greater extent. Research gaps and directions for future studies in relation to different post-harvesting and cooking practices, and rainwater harvesting systems are also discussed in this review.

1. Introduction

Arsenic (As) ingestion in humans through various food sources is a worldwide health issue. Millions of consumers around the world may have high As ingestion from imported rice and rice-based foods, due to the rapid expansion of the global food trade (Heitkemper et al. 2009; Islam et al., 2017a; Nachman et al. 2018). Rice is a major dietary source of inorganic As, particularly for people in As endemic areas (Huang et al. 2015; Kwon et al. 2017; Meharg et al. 2008). Numerous investigations have demonstrated that rice grains in As endemic areas contain over 90% of inorganic As (i.e. arsenite (As(III)) and arsenate (As(V))), and the rest is the organic As species (dimethylarsinic acid (DMA(V)) and monomethylarsonic acid (MMA(V))) which are less toxic than the inorganic As species (Halder et al. 2014; Meharg and Rahman 2003). In the case of As uptake mechanisms in rice plants, both As(III) and As(V) are acquired by $Si(OH)_4$ and PO_4^{3-} transporters, respectively (Ma et al. 2008; Zhao et al. 2009). It has been suggested that DMA(V) and MMA(V) are also taken up by $Si(OH)_4$ transporters (Li et al. 2009).

Intake of inorganic As is a recognized cause of cancers of the skin, lungs, and bladder and a potential cause of non-cancerous health outcomes including respiratory, cardiovascular, neurological, and metabolic diseases (Sanchez et al. 2016). Recent health risk assessments reported that the consumption of As-containing rice and rice-based foods (e.g. cakes, cereals, crackers, and noodles) led to increased cancer

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[*] Corresponding author at: School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350 Australia.

E-mail address: jochen.bundschuh@usq.edu.au (J. Bundschuh).

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risks, especially in subpopulations such as infants and children (Fakhri et al. 2018; Islam et al. 2016; Lin et al. 2015; Rahman et al. 2014; Sofuoglu et al. 2014). In order to minimize inorganic As exposure during the transition stage to solid foods, infants should not consume rice products unless they are specifically mentioned as being safe for consumption (Carey et al. 2018; Signes-Pastor et al. 2018). As a result of these health issues, the European Union (EU) has recommended a maximum level of 100 μg kg^{-1} for inorganic As in rice-based products intended for young children (EC 2015). The World Health Organization (WHO) has also set a permissible level of inorganic As in polished rice grains for adults at 200 μg kg^{-1} (WHO 2014). Apart from the public health impacts of As-related chronic diseases, As ingestion in humans may also create socio-economic consequences for the victims, as well as their families (Meharg et al. 2009; Rahman et al. 2018).

Recent review articles have shown that the total As content in rice grains is dependent on paddy soil properties, microbial activities, and rice genotypes (Chen et al. 2017; Kumarathilaka et al. 2018a; Kumarathilaka et al. 2018b; Senanayake and Mukherji 2014; Zhao et al. 2009). The effects of different physico-chemical and biological methods on reduced As levels in rice grains have been extensively investigated over the past decade. For example, alternative water management practices such as intermittent and aerobic irrigation regimes have been found to decrease the availability of As in the paddy soil-water system (Mukherjee et al. 2017; Rahaman and Sinha 2013). Supplementation of nutrients (i.e. Si, PO_4^{3-}, S, and N) and amendments (i.e. Fe and Mn) into As-contaminated paddy soils may decrease the accumulation of As in rice grains (Farrow et al. 2015; Li et al. 2019; Senanayake and Mukherji 2014; Seyfferth et al. 2016). In addition, biological methods (i.e. inoculation of microorganisms and transgenic approaches) can be used to decrease the As accumulation in rice grains (Gustave et al. 2018; Li et al. 2016; Meng et al. 2011). However, only a limited number of studies have focused on the effects of post-harvesting technologies and cooking methods to decrease As content in raw and cooked rice grains in As endemic areas. Taking this into account, this review article discusses possible causes for high levels of As in cooked rice and potential post-harvesting and cooking techniques to decrease As levels in cooked rice. In addition, in vivo and in vitro methods which are used for evaluating the bioavailability and bioaccessibility of As species in cooked rice will be discussed. Research gaps and future research directions to reduce As ingestion in humans through cooked rice are also highlighted.

2. Major causes for high As levels in cooked rice

The key causes related to high As levels in cooked rice in As endemic areas are use of As-contaminated water for cooking and As contaminated rice grains. The source of cooking water has gained significant attention with regard to high As levels in cooked rice (Ackerman et al. 2005; Devesa et al. 2008; Signes–Pastor and Carbonell–Barrachina, 2012). People living in As endemic areas, particularly in Asian regions, largely rely on As-contaminated groundwater as their cooking water (Bae et al. 2002; Ohno et al. 2007; Pal et al. 2009; Rahman and Hasegawa 2011). O'Neill et al. (2013) estimated that the community using As-contaminated water (> 50 μg L^{-1}) for cooking rice in Prey Veng, Cambodia, consumed inorganic As up to 24 times more than the previous provisional tolerable daily intake value (PTDI) of 2.1 μg kg^{-1} of body weight day^{-1} which was withdrawn in 2010 by the joint FAO/WHO expert committee on food additives (JECFA). Roychowdhury (2008) demonstrated that in the Murshidabad and Naida districts in India, the use of As-contaminated cooking water (0.001–0.200 mg L^{-1}) has led to an approximate two-fold increase in As content in cooked rice compared to raw rice.

The As level in cooked rice is also dependent on the As concentration in raw rice grains. As stated in the introduction, consumption of cooked rice may increase the potential harm to human health if the raw rice exceeds the recommended maximum level for inorganic As (Liang et al. 2010; Roychowdhury et al. 2003; Smith et al. 2006). Worldwide field and market-based surveys have also pointed out that raw rice containing high As content is available in the global market and therefore, people who have a higher rate of rice consumption may be vulnerable to As related health risks (Adomako et al. 2011; Liang et al. 2010; Signes-Pastor et al., 2016). While it has been stated that the major causes for high As content in raw rice are the use of As-contaminated water for irrigating rice fields and mining activities which may increase the As concentration in nearby paddy soils due to the dry and wet depositions of As (Kwon et al. 2017; Shrivastava et al. 2017).

3. Accessing the health risks via consumption of cooked rice

The bioaccessibility of As in humans refers to the fraction of dissolved As, due to gastrointestinal digestion (Zhuang et al. 2016). Reproducible and cost-effective in vitro digestive methods are commonly used to assess As bioaccessibility of cooked rice in humans (Laparra et al. 2005; Sun et al. 2012; Zhuang et al. 2016). Basically, in vitro digestive methods can mimic both enzymatic and physico-chemical processes of the human digestive tract. Studies related to the simulated gastrointestinal digestion (i.e. using artificial gastrointestinal digestion) have shown that inorganic As species in cooked rice were largely bioaccessible in humans (63–99%) (Laparra et al. 2005). The undigested fraction of fiber in rice may undergo a microbial fermentation process in the colon. Sun et al. (2012) observed a decreased As bioaccessibility in the simulated colon (i.e. simulator of the human intestinal microbial ecosystem (SHIME) reactor). Calatayud et al. (2018) also found that the percentage of As bioaccessible fraction decreased from 36% to 14% (oral) and from 117% to 89% (colon), due to the presence of salivary bacteria along the gastrointestinal digestion.

There is very limited information available regarding the changes in As speciation in the human digestive system. Human gut microorganisms have previously been found to transform soil-derived As into methylated As species (Van de Wiele et al. 2010). Monomethylarsonate (MMA(III)), which is an intermediate product of As methylation, accounted for 10–14% in the simulated colon (Sun et al. 2012). The lethal dose (LD_{50}) of MMA(III) is 12 times lower than the LD_{50} value of the As (III) (Petrick et al. 2001; Tseng 2009). However, MMA(III) is more membrane permeable than both inorganic As species and the pentavalent organic As species (Drobná et al. 2005). Newly formed MMA(III) may become available for colon epithelial transport which increases health risks in humans (i.e. damage to DNA and enzyme inhabitation) (Drobná et al. 2005).

Urinary levels of As are also considered to be a valid measure of As exposure (i.e. through mass balance approach) via rice consumption (Banerjee et al. 2013; Davis et al. 2012; Meharg et al. 2014). After being ingested, As excretes through the kidneys as inorganic As and metabolites of methylated As species (i.e. MMA(V) and DMA(V)). Several studies have revealed that 40–60% of rice-derived total As excretes through the urine (He and Zheng 2010; Meharg et al. 2014). Correlation analyses have also confirmed that the consumption of As rich rice causes high As excretion in humans (Gilbert–Diamond et al. 2011; He and Zheng 2010). For example, Meharg et al. (2014) found that intake of 300 g of rice per day increased total As in urinary excretion by 730% in adults. In the case of As speciation, Meharg et al. (2014) demonstrated that DMA(V) was the dominant As species (90%) in the urine of individuals who has consumed rice containing inorganic As and DMA(V) at a ratio of 1:1. The end metabolite of As transformation in humans is DMA(V) (Li et al. 2013). Therefore, it is possible to have DMA(V) as the most prominent As species in human urine (Li et al. 2013). However, As excreted in urine does not reflect total bioavailability of As since As species can remain in the body but can also be excreted as feces.

A limited number of in vivo bioassays have been conducted as a surrogate of human exposure to assess the bioavailability and speciation of As by using animals such as swine and mice (Islam et al. 2017a;

Juhasz et al. 2006; Li et al. 2017). Islam et al. (2017b) administered As contained cooked rice (i.e. orally and via injection) to swine to measure the bioavailability of As in the swine blood. The results showed that approximately 90% As(III) and 85% As(V) were absorbed from the gastrointestinal tract. In contrast, organic As species had low bioavailability (~ 20% and 31% of MMA(V) and DMA(V), respectively) resulting in poor absorption by the gastrointestinal tract (Islam et al. 2017b). Similarly, Li et al. (2017) provided mice with a rice diet which was spiked with As species (2.5–15 μg of As(III), As(V), and DMA(V) per mouse) orally to evaluate the bioavailability of As and observed a strong positive correlation ($R^2 = 0.99$) between As levels in urinary excretion and cumulative As intake by mice. From a practical point of view, it is difficult to extend absolute bioavailability data of As to humans since the metabolism of As in humans and animals varies considerably. However, an estimation of As relative bioavailability may overcome this drawback to a certain extent.

4. Reducing As content in cooked rice

4.1. Post-harvesting and cooking practices

4.1.1. Polishing and storage of rice grains

Rice processing technologies may affect As levels in rice grains. Understanding of As accumulation and grain filling mechanisms is the most important aspect in this regard. Both inorganic and organic As species tend to accumulate in the bran layer of developing rice grains (Sun et al. 2008; Williams et al. 2009). Polishing of rice, by removing the bran layer, may thus reduce total As content in rice grains. Naito et al. (2015) demonstrated that total and inorganic As concentrations in white rice polished by removing 10% of bran by weight decreased total As contents by 61–66% and 51–70%, respectively, compared to those in brown rice. However, polishing of brown rice leads to the loss of important nutrients in the bran (Table 1). Liu et al. (2017) demonstrated that thiamine and riboflavin levels in rice grains decreased linearly when the degree of milling increased. During the 30 s of milling (ca. 9%) period, 58–65% of thiamine and 40–46% of riboflavin were lost from rice grains. Moreover, Mg, Mn, and Fe content decreased as the degree of milling increased (Liu et al. 2017). Paiva et al. (2016) also revealed that polishing of rice grains removed 90% of free phenolics from rice grains. Therefore, it is essential to study the optimum degree of milling and milling time to decrease As content and to maintain the level of nutrients in various rice genotypes.

Very limited studies have investigated the effects of storage conditions (i.e. storage time and storage temperature) on As content in rice grains. However, there is no conclusive evidence that storage temperature and storage time can influence the As concentration in rice grains. Naito et al. (2015) found that the concentration of total As, inorganic As, and DMA(V) in brown rice did not change when grains were kept at 15–25 °C for one year. Pizarro et al. (2003) also demonstrated that the concentration of total As and As species remained constant when rice grains were in the form of grain for more than six months at −20 °C. However, rice grains in the form of milled rice led to a reduced concentration of As(III), As(V), and MMA(V) when milled rice was stored for three months (Pizarro et al. 2003). Thus, further investigations are required to confirm whether storage conditions affect As concentrations in rice grains. In addition, it is worth studying the possible changes in As speciation in rice grains under varying storage conditions.

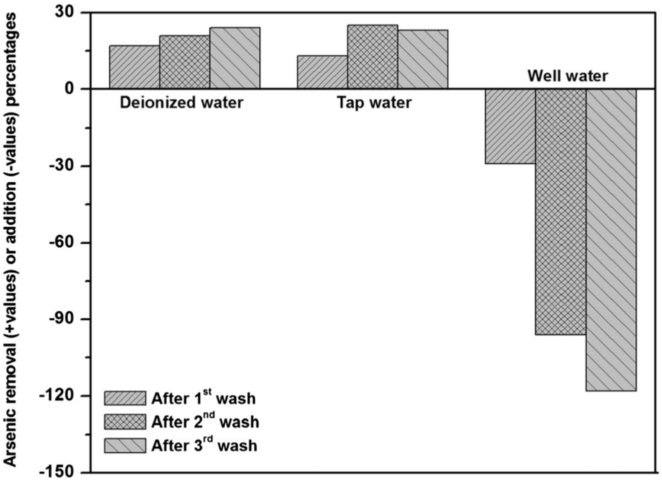

Fig. 1. The percentage removal (+ value) or addition (− value) of total As in sequentially washed rice in deionized water, tap water ($28\,\mu g\,L^{-1}$), and well water ($792\,\mu g\,L^{-1}$) compared to raw rice. Data adapted from Jaafar et al. (2018) and Naito et al. (2015).

4.1.2. Washing of raw rice

Modification of the practices at the kitchen level may reduce As content in cooked rice grains to a greater extent. Washing of rice before cooking has been recommended in numerous studies to reduce total As content in cooked rice (Liu et al. 2018; Raab et al. 2009). Naito et al. (2015) demonstrated that total As content in white rice and brown rice decreased to 81–84% and 71–83%, respectively, after washing 3 times with deionized water. Raab et al. (2009) also found that washing reduced total As content in basmati rice grains by 13–15%. However, Halder et al. (2014) demonstrated that washing of rice grains (i.e. at least 2–4 times) in the field experimental site in Tehatta-II block of Nadia District, West Bengal, India had a negligible effect on grain As content. According to Fig. 1, As free water or mildly As contaminated water can be used for sequential washing of rice to decrease As content of washed rice grains. Highly As-contaminated water leads to increased As content in washed rice grains, even after sequential washing (Fig. 1). The efficiency of As removal in respective sequential steps seems to be varied. The second and third washing steps under As free water had less effect on As removal from washed rice grains than that of the first washing step (Fig. 1).

Both positive and negative effects of washing of rice can be attributed to As concentrations in water and raw rice grains, the number of washing steps, and sample sizes. Washing time and mode of washing

Table 1
A comparison of As removing efficiencies and limitations of different post-harvesting and cooking practices.

Practice/method	Arsenic removing efficiency from cooked rice	Influence on nutrients	References
Polishing rice grains	~ 50–70% (in raw rice by removing 10% of bran)	Loss of nutrients	Naito et al. (2015)
Washing of raw rice	~ 13–84% (washing up to 2–4 times)	Loss of nutrients	Naito et al. (2015), Raab et al. (2009)
Cooking rice in excess water	~ 28–66% (when the ratio of deionized water: rice is 10–12:1)	Loss of nutrients	Carey et al. (2015), Gray et al. (2016)
Continual stream of percolating water	~ 96% of inorganic As from rice bran	Minimal effect on trace and macro nutrients elements. Neither vitamins or bioactive compounds were removed	Signes-Pastor, 2017

(i.e. mechanical or manual) may also influence the As content of washed rice grains. Further studies are needed to examine the effects of washing time and mode of washing on As content of washed rice grains. One negative effect of washing rice is that increasing the number of washing steps has led to a loss of minerals and vitamins in washed rice (Table 1). Jaafar et al. (2018) demonstrated that 3 step sequential washing removed 70% and 25% of Fe and Zn, respectively, from rice grains. Gray et al. (2016) also found that washing decreased folate, niacin, and thiamin by 77%, 57%, and 54%, respectively, from polished and parboiled rice, but not from whole grain brown rice. Therefore, the optimum number of washing steps and washing time for various rice varieties need to be set to decrease As content while reducing the loss of nutrients in washed rice grains.

4.1.3. *Cooking rice in excess water*

Arsenic content in cooked rice depends on the method of cooking (Mihucz et al. 2010; Perelló et al. 2008; Sengupta et al. 2006; Torres-Escribano et al., 2008). Rice is typically prepared by using a small ratio of water: rice (1–2:2) until no discarded water remains. However, a low volume of water for cooking of rice has been found to cause an increase in As levels in cooked rice. Evaporation of water under low volume cooking could be a potential reason for increasing As content in cooked rice. High ratio cooking (water: rice) of rice followed by discarding excess water has been found to effectively decrease As content in cooked rice as summarized in Table 1. For example, Raab et al. (2009) demonstrated that high ratio water (deionized water): rice (6:1) decreased total and inorganic As concentrations in long-grain and basmati rice by 35% and 45%, respectively, compared to uncooked rice. Gray et al. (2016) found that cooking of rice with excess deionized water (10:1 water: rice ratio) decreased inorganic As content in long grain polished, parboiled, and brown rice by 40%, 60%, and 50%, respectively. A relatively higher ratio of cooking water (deionized water): rice (12:1) has also been found to remove 57% inorganic As in cooked rice compared to uncooked wholegrain and polished rice samples (Carey et al. 2015). Mildly As-contaminated cooking water has been tested as a way to reduce As content in the cooked rice. For example, Signes et al. (2008) found that cooking rice in an excess volume of mildly As containing water (40 μg L^{-1}) has reduced total As concentration of cooked rice by 12.7% in comparison with raw rice. Overall, cooking water to rice ratio of 6:1 seems to decrease total As content of cooked rice to < 0.2 mg kg^{-1} in most cases if the cooking water is free from As (Table 2). If the cooking water is highly contaminated with As, high cooking water to rice ratios do not decrease As content in cooked rice to < 0.2 mg kg^{-1} (Table 2). The possible mechanism related to low As content in cooked rice prepared in excess water may be the release of As species from rice grains into cooking water. Different removal rates in As content in cooked rice under excess cooking water could be seen to correspond to the As concentration in cooking water and raw rice grains, cooking water: rice ratio, cooking time, and rice variety. Another important concern is that most of the studies related to the use of excess water for cooking rice were performed under laboratory conditions by using deionized water and have focused particularly on total As content in cooked rice. Thus, realistic field experiments and a clearer focus on As speciation analysis in cooked rice are required to assess the As behaviour in cooked rice under excess cooking water.

Recent findings revealed that cooking rice with excess water has led to decreased nutrients in the cooked rice grains, in addition to As (Table 1). For instance, Gray et al. (2016) demonstrated that cooking rice in excess water: rice (10:1) has led to decreased level of Fe, folate, niacin, and thiamin in polished and parboiled rice by 50–70%. Mwale et al. (2018) also demonstrated that water: rice ratio of 6:1 has led to significant loss of P (50%), Ni (44.6%), Mo (38.5%), Mg (22.4%), Co (21.2%), Mn (16.5%), Ca (14.5%), and Zn (7.7%). Even though cooking in excess water may reduce As content in the cooked rice, the rice has become a poor source of essential elements. Thus, further research is needed to optimize the water: rice ratio to decrease As content in cooked rice and to prevent any loss of essential nutrients from cooked rice. Another important concern is whether the appearance (i.e. texture) and flavour of cooked rice are affected, due to cooking with excess water. Since cooked rice is one of the most popular market products, changes in the texture (i.e. fluffy, slightly dry, and sticky rice) and flavour of rice may seriously affect the market value of rice. Therefore, further studies are required to ensure the texture and flavour in cooked rice when cooking with excess water.

The excess volume of cooking water may be used as gruel (i.e. discarded starch water) (Mandal et al. 2019; Rahman et al. 2006). Hot gruel is a popular drink in rural villages and the habit of eating gruel is believed to act against lack of nutrient content in rural diets. Mandal et al. (2019) demonstrated that gruel, after the traditional cooking in West Bengal, India, contains a median As concentration of 144 μg kg^{-1}. Thus, consumption of gruel may be an important route of As exposure in rural areas and risk assessments related to the consumption of gruel in rural areas need to be implemented.

The material and the type of the cooking vessel can also be important aspects in the As content in cooked rice. A study by Sengupta et al. (2006) revealed that total As content in cooked rice is not significantly influenced by the material of the cooking vessel (i.e. aluminum, steel, earthenware and glass). However, the total As content in rice cooked in a glass vessel was lower than that cooked in aluminum, steel, or earthenware vessels (Sengupta et al. 2006). Recent findings revealed that the type of the cooking vessel has a negligible effect on As content in cooked rice. For example, Liu et al. (2018) demonstrated that there is no significant relationship between types of cooking vessels (i.e. steamer, pressure cooker and microwave oven) and total As content in cooked rice when rice is cooked in the ratio of 1.8 water: 1 rice. Liao et al. (2018) also found that cooking rice in the stainless steel pot and the pressure cooker (water: rice ratio of 2:1, 4:1, and 6:1) has no significant influence on As concentration in cooked rice. However, it is worth studying the effect of the material and the type of the cooking vessel on As content in different rice varieties cooked in different ratios of water: rice.

4.1.4. *Continual stream of percolating water*

A novel method of rice cooking, “rice preparation using a continual stream of percolating near boiling water”, for decreasing As content in cooked rice has been tested recently by Carey et al. (2015). A coffee-maker, which provides a continual stream of percolating water (i.e. near boiling water) through a filter unit, has been used to test this novel method at the laboratory scale. In this method, rice is placed in the filter unit instead of coffee and near boiling water is passed through the filter unit continuously. As a result, it has been found to remove 59% and 69% of inorganic As compared to raw rice for polished and wholegrain rice, respectively (Carey et al. 2015). Cooking rice bran in percolating As free boiling water has also reduced up to 96% of inorganic As in the cooked bran compared to controls (Signes-Pastor, 2017). Therefore, it seems obvious that use of a continual stream of percolating water leads to decreased As to a greater extent in cooked rice. Advantages of the use of percolating water for cooking rice may be as follows: (1) rice grains are not exposed to a large volume of cooking water; (2) inorganic As species may leach into the water receiving vessel. Since the boiling water does not retain longer time in the filter unit, leached As species from rice grains may be removed quickly into the water receiving vessel. This may be a reason for higher As removal through this method in comparison to other discussed cooking practices. Scientific-based mechanistic investigations would provide better understandings on how a continual stream of percolating water decreases As content in cooked rice. In comparison with other post-harvesting and cooking practices, use of a continual stream of percolating water has been found to have a minimal effect on the trace and macro nutrient elements in the rice bran (Signes-Pastor, 2017). This method did not remove either vitamins or bioactive compounds from the rice bran (Table 1). However, further studies are also needed to assess

Table 2
Effects of different cooking water: rice ratios on As content in cooked rice. Inorganic As content of raw rice grains and cooked rice are shown in parenthesis.

Rice variety/type	Cooking method (water: rice)	Total As content in cooking water (mg L^{-1})	Total As content in raw rice grain (mg kg^{-1})	Total As content in the cooked rice (mg kg^{-1})	Reference
Brown long grain	2:1	Deionized water	0.320 (0.195)	0.323 (0.198)	Gray et al. (2016)
	6:1			0.224 (0.119)	
	10:1			0.169 (0.079)	
White medium grain	2:1	Deionized water	0.204 (0.105)	0.208 (0.106)	
	6:1			0.179 (0.082)	
	10:1			0.145 (0.054)	
Parboiled	2:1	Deionized water	0.216 (0.143)	0.229 (0.143)	
	6:1			0.112 (0.070)	
	10:1			0.073 (0.044)	
Short bold, medium slender, long slender	(3–6):1	–	0.575 (0.346)	0.348 (0.194)	Halder et al. (2014)
			0.125 (0.091)	0.116 (0.067)	
			0.297 (0.223)	0.227 (0.110)	
Polished basmati	2.5:1	Deionized water	0.162 (0.093)	0.141 (0.090)	Raab et al. (2009)
	6:1			0.103 (0.056)	
	Steamed			0.122 (0.061)	
Wholegrain basmati	2.5:1	Deionized water	0.131 (0.089)	0.119 (0.082)	
	6:1			0.072 (0.048)	
	Steamed			0.119 (0.076)	
Polished long grain	2.5:1	Deionized water	0.229 (0.138)	0.238 (0.144)	
	6:1			0.165 (0.070)	
	Steamed			0.177 (0.107)	
Wholegrain long grain	2.5:1	Deionized water	0.314 (0.183)	0.324 (0.165)	
	6:1			0.219 (0.102)	
	Steamed			0.280 (0.156)	
Italian parboiled	2.5:1	Deionized water	0.211 (0.157)	0.211 (0.157)	
Long grain parboiled	2.5:1	Deionized water	0.186 (0.115)	0.163 (0.086)	
Thai white	3:1	Ultrapure water	0.241 (0.173)	0.138 (0.091)	Jitaru et al. (2016)
	6:1			0.125 (0.064)	
	steamed			0.153 (0.097)	
White risotto	3:1	Ultrapure water	0.280 (0.237)	0.149 (0.127)	
	6:1			0.091 (0.044)	
	steamed			0.197 (0.156)	
Organic rice duo	3:1	Ultrapure water	0.535 (0.471)	0.306 (0.286)	
	6:1			0.162 (0.120)	
	steamed			0.316 (0.292)	
White Basmati	3:1	Ultrapure water	0.129 (0.115)	0.064 (0.064)	
	6:1			0.050 (0.035)	
	steamed			0.090 (0.073)	
Steamed black wholegrain	3:1	Ultrapure water	0.234 (0.214)	0.211 (0.199)	
	6:1			0.113 (0.095)	
	steamed			0.212 (0.192)	
Gontra Selection-3	6:1	0.152	0.627	0.445	Basu et al. (2015)
		0.023		0.383	
		0.152	0.495	0.346	
		0.023		0.302	
		0.152	0.572	0.418	
		0.023		0.350	
		0.152	0.549	0.368	
		0.023		0.327	
Zhenshan 97	6:1	Milli-Q water	0.171	0.103	Mihucz et al. (2007)
Risabell	6:1		0.116	0.070	
Koorostaj	6:1		0.139	0.058	
–	2:1	0.040	–	0.365	Signes et al. (2008)
	6:1			0.258	
BRRI dhan28	5:1	0.130	0.570	0.390	Rahman et al. (2006)
BRRI hybrid dhan1			0.690	0.440	
–	2:1 (pressure cooker)	Deionized water	0.085	0.082	Liao et al. (2018)
	4:1 (pressure cooker)			0.081	
	6:1 (pressure cooker)			0.080	
–	2:1 (stainless steel pot)			0.082	
	4:1 (stainless steel pot)			0.080	
	6:1 (stainless steel pot)			0.078	
Xinfeng 2	1.8:1 (steamer)	Deionized water	0.058	0.050	Liu et al. (2018)
	1.8:1 (pressure cooker)			0.050	
	1.8:1 (microwave oven)			0.049	
T-You 15	1.8:1 (steamer)	Deionized water	0.122	0.104	
	1.8:1 (pressure cooker)			0.107	
	1.8:1 (microwave oven)			0.104	

whether water soluble and thermosensitive compounds in cooked rice are affected as a function of a continual stream of percolating water. Moreover, designing and technological improvements are required to devise systems which facilitate the use of a continual stream of percolating water at household levels.

4.2. Introduction of rainwater harvesting systems

One of the major problems for elevated As levels in cooked rice in As endemic areas, as discussed in Section 2, is the use of As-contaminated water for cooking rice. Therefore, As free water sources can be adapted for washing and cooking rice. Rainwater is one of the best alternatives to be used for the preparation of rice in As endemic areas around the world. Rainwater harvesting units set up at the household level can collect rainwater during the rainy season and store it for long-term use. The first flush mode of the rainwater harvesting system may prevents debris (i.e. dead plant materials and bird feces) entering into the storage tank. Filters with different mesh sizes, placed in the water intake line, may also prevent debris entering into the storage tank. However, water quality assessment should be performed to assess whether water quality parameters meet guidelines for safe drinking purposes. If needed, water purification steps (i.e. chlorination and filtration) can also be introduced before using collected rainwater. The water quality of rainwater is dependent on the proximity of contaminant sources, meteorological conditions in a particular area, and roofing materials (Gwenzi et al. 2015; Kahinda et al. 2007). Potential risks (i.e. development of pathogens) associated with rainwater could be minimized by boiling the water for a certain time (~5–20 min) (WHO 2008). Engineering/technological interventions, proper housekeeping practices, and public education also lead to minimization of rainwater contamination and subsequently safeguard public health.

So far, only one study has reported the effects of rainwater on As content in cooked rice. O'Neill et al. (2013) demonstrated that in Prey Veng, Cambodia, As concentration in rice cooked in rainwater was significantly lower than that of raw rice. In that study, total As concentration in cooked rice was below the limit of detection (LOD). Studies revealed that total As content in cooked rice tends to be less than that of raw rice if the cooking water meets the WHO limit of $< 10\ \mu g\ L^{-1}$. The rain water used by O'Neill et al. (2013) contained a zero level of As. Therefore, governments and respective policy makers could initiate the introduction of rainwater harvesting systems, in particular for As endemic areas. The collected rainwater could eventually be used as safe drinking water and cooking water for communities. Rainwater harvesting is also a sustainable and economically feasible option for the global As dilemma. However, regular water quality monitoring for collected rainwater will also be required for the protection of public health.

5. Concluding remarks

Studies related to post-harvesting techniques and modification of cooking methods have been considered to evaluate the decrease of As content in cooked rice. Polishing of raw rice grains leads to a reduction in As content in cooked rice. Sequential washing of raw rice, cooking rice in As free excess water or in percolating cooking water have all remarkably decreased As content in cooked rice. Access to As free water sources such as rainwater harvesting systems is one of the most practical approaches to reduce the As content in cooked rice in As endemic areas. Most of the mitigation measures (i.e. sequential washing of raw rice and cooking rice in excess water) can be implemented at almost zero additional cost. However, use of the stream of percolating cooking water for cooking rice is still being investigated at the laboratory scale. Initial setup cost and low maintain cost will be involved for rainwater harvesting systems.

The key knowledge gaps regarding As removal from cooked rice remain unanswered and should be the focus of future studies. It will be worth studying the effect of long-term storage conditions for rice grains (i.e. storage of rice grains at varying temperature and humidity conditions) to examine whether long-term storage conditions affect As content in raw rice grains. There is a lack of information regarding the changes in As speciation in cooked rice during the different stages of cooking. It is important to investigate how As speciation changes over time, with respect to the mechanisms involved, during the cooking of rice. Optimum parameters (i.e. degree of polishing, number of washing steps, and rice: water ratio for cooking) need to be set for different rice varieties to decrease As content and to maintain required nutrient levels in cooked rice. Carefully designed cooking vessels may be able to effectively decrease As content in cooked rice. The effect of rainwater on reduced As concentration in cooked rice at different rainwater: rice cooking ratios also needs to be investigated in detail. Development of sequential analytical steps of the digestive process (i.e. to simulate the mouth, stomach, and intestines) would provide detailed information regarding the bioaccessibility of As species. Future research assessing As exposure in humans should also assess the As intake from gruel, if any, for a realistic representation of the As exposure scenario in As endemic areas around the world.

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6.3.3 Concluding remarks

The key causes associated with high As concentrations in cooked rice are the use of As-contaminated water for cooking rice and the use of raw rice which exceeds the recommended level for inorganic As (200 µg kg^{-1}). Cost-effective in vitro digestive methods (using artificial gastrointestinal digestion) can be used to evaluate the bioaccessibility of As in humans through rice consumption. In addition, in vivo bioassays can be done to assess the bioavailability of As through rice consumption by using animals, including mice and swine. Polishing of rice grains, washing the raw rice (1 - 4 washing steps), cooking rice in excess water (2-12: 1 of water: rice ratio) can remove As levels in cooked rice by 13% - 84%. However, the major drawback associated with these methods is the loss of nutrients from cooked rice. However, use of a continual stream of percolating water for cooking rice has been shown to significantly decrease inorganic As (~ 96%) in rice bran and, most importantly, this method had minimum effect on nutrient contents in cooked rice. Nevertheless, there is no conclusive evidence regarding the effects of storage conditions such as storage temperature and storage time on As concentrations in rice grains. Since As-contaminated water is used for cooking purposes in As endemic areas, introduction of rainwater harvesting systems would be a sustainable way to decrease As concentrations in cooked rice. In future research, it is important to assess the changes in As speciation in raw rice over time and during the cooking of rice with respect to the mechanisms.

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Appendix A

Article VIII

Herath, I., Kumarathilaka, P., Bundschuh, J., Marchuk, A. and Rinklebe, J., (2019). A fast analytical protocol for simultaneous speciation of arsenic by Ultra-High Performance Liquid Chromatography (UHPLC) hyphenated to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as a modern advancement in liquid chromatography approaches. Talanta, 208, 120457. (Published).

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A fast analytical protocol for simultaneous speciation of arsenic by Ultra-High Performance Liquid Chromatography (UHPLC) hyphenated to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as a modern advancement in liquid chromatography approaches

Indika Herath[a], Prasanna Kumarathilaka[a], Jochen Bundschuh[a,b,*], Alla Marchuk[c], Jörg Rinklebe[d,e]

[a] *Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, 4350, Queensland, Australia*
[b] *UNESCO Chair on Groundwater As Within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, 4350, Queensland, Australia*
[c] *University of Southern Queensland, Centre for Sustainable Agricultural Systems, Toowoomba, 4350, Queensland, Australia*
[d] *University of Wuppertal, School of Architecture and Civil Engineering, Institute of Foundation Engineering, Water- and Waste-Management, Laboratory of Soil- and Groundwater-Management, Pauluskirchstraße 7, 42285, Wuppertal, Germany*
[e] *Department of Environment, Energy and Geoinformatics, Sejong University, Seoul, 05006, Republic of Korea*

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Rice

ABSTRACT

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) hyphenated to High Performance Liquid Chromatography (HPLC) and Ion Chromatography (IC) are widely used for simultaneous speciation of arsenic (As). Longer retention time resulting in a slow separation is the major drawback of these existing approaches. Besides, fast separations achieved from HPLC based methods have always resulted in poor resolution and baseline separation between peaks. For the first time, the present study aimed to improve the existing HPLC related methods in order to develop a fast analytical protocol based on Ultra-High Performance Liquid Chromatography (UHPLC) hyphenated to ICP-MS detection for simultaneous separation and quantification of arsenite (As(III)), arsenate (As(V)), dimethylarsonate (DMA(V)) and monomethylarsenate (MMA(V)). Two types of ammonium-based mobile phases (i.e. $NH_4H_2PO_4$ and NH_4NO_3) were examined at different eluent concentrations and pH to choose the most effective eluent system. Results demonstrated that the mixed mobile phase containing 8.5 mM of $NH_4H_2PO_4$ and NH_4NO_3 (1:1) at pH 6.0 is the most effective eluent achieving the separation of As species with improved resolutions within 5 min which is almost a double saving in analysis time per sample compared to the existing methods (9–15 min). Faster separation is analytically cost effective in terms of ICP-MS running cost and energy consumption. Unlike HPLC, UHPLC did not generate a higher column back pressure with increasing flow rate up to 2.5 mL/min resulting in a faster separation with excellent resolution of peaks. Limits of detection for As species were in the range of 0.3–0.5 μg/L. The proposed method was applied to quantify As species present in commercially available rice varieties in Australia and Sri Lanka. Results of speciation analysis indicated that As(III) is the dominant species, ranging from 53 to 100% in the rice grains. The proposed analytical protocol based on UHPLC-ICP-MS provided an accurate and reliable identification and quantification of As species with the advantages of rapid separation, excellent resolution, and low detection limits. Such a recent trend in fundamental research could be a turning point for future environmental and biological research to further improve this strategy for the speciation of other toxic metal(loid)s in food, water and biological samples.

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[*] Corresponding author. Faculty of Health, Engineering and Sciences & UNESCO Chair on Groundwater As within the 2030 Agenda for Sustainable Development, University of Southern Queensland, West Street, Toowoomba, 4350, Queensland, Australia.
E-mail address: Jochen.Bundschuh@usq.edu.au (J. Bundschuh).

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1. Introduction

Arsenic (As) is an ubiquitous metalloid in environmental and biological systems which exits in various redox states as inorganic As and organoarsenicals [1]. The toxicity of As is utterly dependent on its chemical speciation [2]. Inorganic As species particularly arsenite (As(III)) are more toxic than the organic As species and hence, the inorganic As species are categorized as group 1 carcinogens by the International Agency for Research on Cancer (IARC) [3]. The advancement of analytical instrumentation in the determination of total As concentrations has enabled the understanding of its adverse consequences in the environment. However, the determination of total As concentrations is not always adequate under various environmental scenarios as the toxicological nature of As depends on its chemical speciation. Therefore, the development of a fast and cost-effective analytical tool for the identification and quantification of various chemical forms of As is of critical importance in order to access their toxicity, bioavailability, biogeochemistry and metabolism in different environmental suits.

During the last two decades, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) have been widely used for the determination of total As concentrations with the detection limits at μg/L(ppb) - ng/L (ppt) levels. However, the analysis of different chemical species of As rather than the total As is attributed to an understanding of its biotransformation and human health risks in a given environmental system. Therefore, identification and quantification of As species have been successfully performed using several chromatographic techniques in combination with ICP-MS detection such as ion chromatography (IC-ICP-MS) and high performance liquid chromatography (HPLC-ICP-MS). An IC-ICP-MS system has been successfully used to determine inorganic and organic As species, such as As(III), arsenate (As(V)), dimethylarsonate (DMA(V)) and monomethylarsenate MMA(V) in soil and biological samples [4–6]. However, the sensitivity, specificity and reliability of LC technology coupled with ICP-MS have advanced intensely to determine As species in various environmental and biological samples. Hence, HPLC coupled with ICP-MS is the widely applied technology to date for the analysis of inorganic and organic As species due to its high versatility, capable of being applied for a variety of sample matrixes which comprises even volatile and non-polar compounds [2,7–10].

Anion-exchange chromatography is predominately utilized for the separation of anionic inorganic and organic As compounds which mostly include As(III), As(V), DMA(V) and MMA(V) [11]. In anion exchange chromatography, negatively charged As ions are retained on the positively charged stationary phase of the column due to strong electrostatic interactions and are then eluted by competitive anions present in the mobile phase via ion exchange mechanisms [12]. It is clear that the optimum separation depends on both the type of column materials and the composition of the mobile phase. Chen et al. [5] have developed an analytical method based on IC-ICP-MS for the separation and quantification of As(III), As(V), DMA(V)) and MMA(V) in soil and plant tissues. In this study, the complete separation of the targeted As species occurred within 9 min using a PRP-X100 anion exchange column with 30 mM of $NH_4H_2PO_4$ at pH 4.0 as the eluent. The analytical approach developed in this study based on ammonium related mobile phases (i.e. $NH_4H_2PO_4$, NH_4HPO_4, NH_4NO_3, NH_4Ac, and NH_4HCO_3) has been successfully applied in HPLC-ICP-MS technology with appropriate modifications until now for the identification and quantification of As species in food, water, plant materials and biological samples [8,13–18]. In a very recent study, these four As species present in shellfish were simultaneously separated within < 5 min by a HPLC-ICP-MS system with a reverse-phase C18 column (150 mm × 4.6 mm, 5 μm) [19]. However, this method resulted in a poor resolution between DMA(V) and MMA(V) which is not reasonable enough for a sensitive quantification of As species in real samples. A rapid separation (< 3 min) of As(III), MMA(V), DMA(V) and As(V) was achieved from an analytical method developed based on HPLC-ICPMS using a Capcell Pak C18 MG column (250 mm × 4.6 mm, 5 μm) [20]. Nevertheless, this method produced a weak baseline separation between MMA(V) and As(III) resolving very close peaks for both species which may lead to a less accurate calibration of As standards. Baseline separation and resolution between peaks need to be achieved perfectly in order to establish an accurate and precise analytical protocol for real sample applications. Therefore, a faster separation with excellent resolution of As species using the existing analytical techniques has still remained as a challenging task.

After analysing the performances of the analytical instrumentations developed so far for the separation and analysis of As species, it is noteworthy to mention that most of the analytical methods developed in previous studies required quite a long separation time (9–15 min) to achieve a resendable resolution between peaks. This is likely to be due to the inability of changing operational conditions in conventional LC systems to achieve a fast separation of species because of the generation of high back pressure which may cause a critical damage to the column as well as the instrumentation. When dealing with a large number of samples, the total elution time per sample needs to be as short as possible because the ICP-MS running cost is expensive. Moreover, longer analysis time may cause a change in the stability of As species in diluted samples. Therefore, given these limitations associated with conventional HPLC coupled with ICP-MS systems and also to meet future demands, this study aims for the very first time to develop an analytical protocol based on a modern system of ultra-high pressure liquid chromatography (UHPLC) hyphenated to ICP-MS (UHPLC-ICP-MS) for the speciation of As.

The traditional HPLC technology has been reengineered, producing UHPLC to reach pressures of up to 18,000 psi which are capable of withstanding elevated backpressure generated through the system [21]. The UHPLC can accommodate 100–150 mm analytical columns packed with materials with 1.8 μm particles achieving an increased analysis speed, sensitivity, selectivity and specificity compared to the conventional HPLC [22]. The UHPLC technology is recognised as an ideal fast-separation technology, so that it is preferably used in commercial applications for the analysis of complex organic compounds including pharmaceuticals, peptides, metabolites, pesticides, and toxins in both isocratic and gradient modes [21,23]. With the rapid development of UHPLC related technologies, mass spectrometry (MS) is used as the detector and the combination of UHPLC with MS detection (UHPLC-MS) has advantageous features in terms of selectivity, sensitivity and capability of analysing complex organic compounds, such as oxidative stress biomarkers, methylparaben, triclocarban, and triclosan [22,24,25]. An UHPLC coupled to the ICP-MS system has been used to quantify glutathione (GSH) in human liver microsomes [26].

To our knowledge, no study exists related to the application of UHPLC-ICP-MS technology for the speciation of trace metal(loid)s, including As in environmental and biological samples to date. This could be due to the lack of fundamental applications of modern UHPLC techniques at a research scale to develop UHPLC related analytical columns for the separation of As species so that UHPLC columns are still not commercially available for the speciation of As and other metal (loid)s in the current market. Therefore, selecting an analytical column with a suitable stationary phase that can be used in UHPLC is the major challenge faced in developing analytical methods for As speciation. For the first time, our study aimed to improve the existing HPLC related approaches in order to develop a fast analytical protocol for simultaneous separation and quantification of As species, including As(III), As (V), MMA(V) and DMA(V), using an UHPLC hyphenated to ICP-MS detection as a modern advancement for the analysis of environmental, and biological samples. Eventually, the proposed method based on UHPLC-ICP-MS was applied for the identification and quantification of As species present in rice grains which are commercially available in Sri Lanka and Australia.

2. Experimental

2.1. Chemical reagents and solutions

All chemical reagents and solutions were analytical grade and purchased from Sigma Aldrich (New South Wales, Australia) and Choice Analytical (New South Wales, Australia). A 1000 mg/L of As standard solution (Choice Analytical, New South Wales, Australia) was used to prepare the calibration standards for the analysis of total As. For the analysis of As speciation, As(III) and As(V) calibration standards were prepared from 1000 mg/L of As(III) and As(V) standard solutions (Choice Analytical, New South Wales, Australia), respectively. Two 1000 mg/L of stock solutions for DMA(V) and MMA(V) were prepared from sodium cacodylate hydrate ($C_2H_8AsNaO_3$) and sodium methylarsenate (CH_4AsNaO_3) (Sigma Aldrich, New South Wales, Australia), respectively. Both As salt compounds were dissolved in distilled deionized water to prepare corresponding stock solutions. All the As stock solutions were kept at 4 °C in the dark. Calibration working standards were freshly prepared by diluting stock As solutions every time just before daily analysis.

2.2. Preparation of mobile phase

Two types of eluents based on different compositions of ammonium salts containing $NH_4H_2PO_4$ and NH_4NO_3 were systematically examined for the speciation of As within a reasonable retention and resolution. The first mobile phase (MP1) was prepared with $NH_4H_2PO_4$ and the second mobile phase (MP2) was prepared with a mixture of $NH_4H_2PO_4$ and NH_4NO_3 (1:1). The concentration and pH of both eluents were optimized in the range of 8.5–30.0 mM and pH 5–6.6, respectively through isocratic elution to select the best mobile phase to achieve a good separation of the targeted As species. The pH of desired eluents was adjusted with aqueous NH_3 and the eluents were filtered through a 5 μm glass fibre filter (Advantec, Japan) followed by the degassing in an ultrasonic bath (Unisonics, Australia). Fig. 1 illustrates the entire optimization process which was followed to develop the analytical method using UHPLC-ICP-MS system for the speciation of As.

2.3. Instrumentation of UHPLC coupled with ICP-MS

Modern UHPLC systems provide an excellent resolution, higher sensitivity and faster analysis compared to the conventional HPLC technology. For the first time, a modern UHPLC system (Flexar, PerkinElmer, USA) was coupled with ICP-MS detection for the separation and quantification of the targeted As species with an anion exchange column; PRP-X100, 250 × 4.1 mm, 10 μm (Hamilton, USA). This column was chosen based on the performance in existing HPLC-ICP-MS methods that are used for As speciation [27]. To quantify As species, the outlet of the analytical column was connected to the ICP-MS (PerkinElmer NexION™ 300X) through PEEK tubing (PerkinElmer, outer diameter - 1/16″, inner Diameter - 0.007″). The ICP-MS is equipped with a unique hyper skimmer cone (triple cone interface) and quadrupole ion deflector. Samples are introduced into the system through a concentric glass nebuliser and cyclonic spray chamber. This ICP-MS consists of a simultaneous dual mode detector which provides 10 times the dynamic range and can measure both high- and low-level analytes in a given sample. The elemental As is detected by ICP-MS at m/z 75. Table 1 summarizes the operating conditions of ICP-MS and UHPLC which are optimized for the quantification of As species. Data processing in UHPLC was performed using Chromera software.

2.4. Applicability of the proposed method in real sample analysis

The proposed method, based on the optimized conditions of mobile phase and UHPLC-ICP-MS, was applied for the identification and quantification of As species in 16 different rice varieties which are commercially available in Sri Lanka and Australia. Rice varieties obtained from Australian markets in this analysis belong not only to Australia but also to Sri Lanka, India, and Pakistan. However, our study does not intend to report data either in country/region-wise or brand names because the main aim of this investigation is to apply the proposed analytical approach for the speciation of As in real samples.

2.4.1. Speciation of As

Arsenic species were extracted from rice grains by following the method described elsewhere [28]. The rice grains were ground and sieved (0.5 mm pore size) to obtain in powdered form. Approximately 0.5 g of rice powder was treated with 7.0 mL of 0.25 mol/L HNO_3 and digested at 95 °C for 90 min using a microwave digestion system (Anton Paar, Multiwave 3000, Austria). The extractions were transferred to a volumetric flask and diluted to 20 mL with Milli-Q water (Elix® 35, Merck Millipore, New Zealand) followed by the centrifuge at 3000 rpm for 20 min (SPINTRON GT-20, Australia). The supernatants were filtered through a glass fibre syringe membrane filter (5 μm) and the samples were 2-times diluted with the same mobile phase just before loading onto the UHPLC autosampler. The targeted As species were then freshly analysed and quantified by UHPLC-ICP-MS following the six point calibration (1–120 μg/L) to cover the entire concentration range of interest. The speciation analysis was carried out in triplicate (n = 3) per each rice variety.

2.4.2. Determination of total As

To determine the total concentration of As in rice grains, approximately 0.5 g of powdered rice samples was treated with 6.0 mL of 70% nitric acid (HNO_3) and 1.0 mL of 30% hydrogen peroxide (H_2O_2) and digested at 180 °C for 45 min on the Anton Paar 3000 digestive system. After sample digestion, the solutions were transferred to a volumetric flask and diluted to 20 mL with Milli-Q water and filtered through a glass fibre filter. The total concentration of As in rice grains was determined by ICP-MS.

2.4.3. Analytical method validation tests

Analytical performance of the proposed method was examined by some of important validation tests, including accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). Accuracy and precision of the proposed analytical method were evaluated by recovery and repeatability tests. The closeness of agreement between the measured value and the certified reference value is known as the accuracy of an analytical result [29]. The accuracy of the analysed amounts of As species by UHPLC-ICP-MS was validated by the certified reference rice material (CRM-rice; ERM® - BC211). The certified amounts of As species present in the CRM-rice (as provided by the institute for reference materials and measurements of the European commission's joint research centre) and corresponding measured amounts along with statistical results are presented in Table 2. Reproducibility of the proposed method was tested by repeating a 10 μg/L of mixed As standard in 6 times, thereby determining the relative standard deviation (RSD) among 6 repeated measurements. The recovery of the analysed amounts of As species was examined based on the sum of As species analysed by UHPLC-ICP-MS was compared with the total concentration of As measured by ICP-MS. A mixed As standard at concentration of 10 μg/L was subjected for the determination of percentage recoveries of As species analysed by the proposed method. Table 3 summarizes the results obtained from the tests of analytical method validation.

The linearity is the ability of producing analytical results that are directly proportional to the concentration of the analyte [29]. The degree of the linear correlation between the concentrations and peak intensities was assessed based on the coefficient of determination (R^2) obtained from the corresponding calibration curves of As species (Table 3). The LOD refers to the smallest concentration of an analyte which can be reliably distinguished from zero according to the

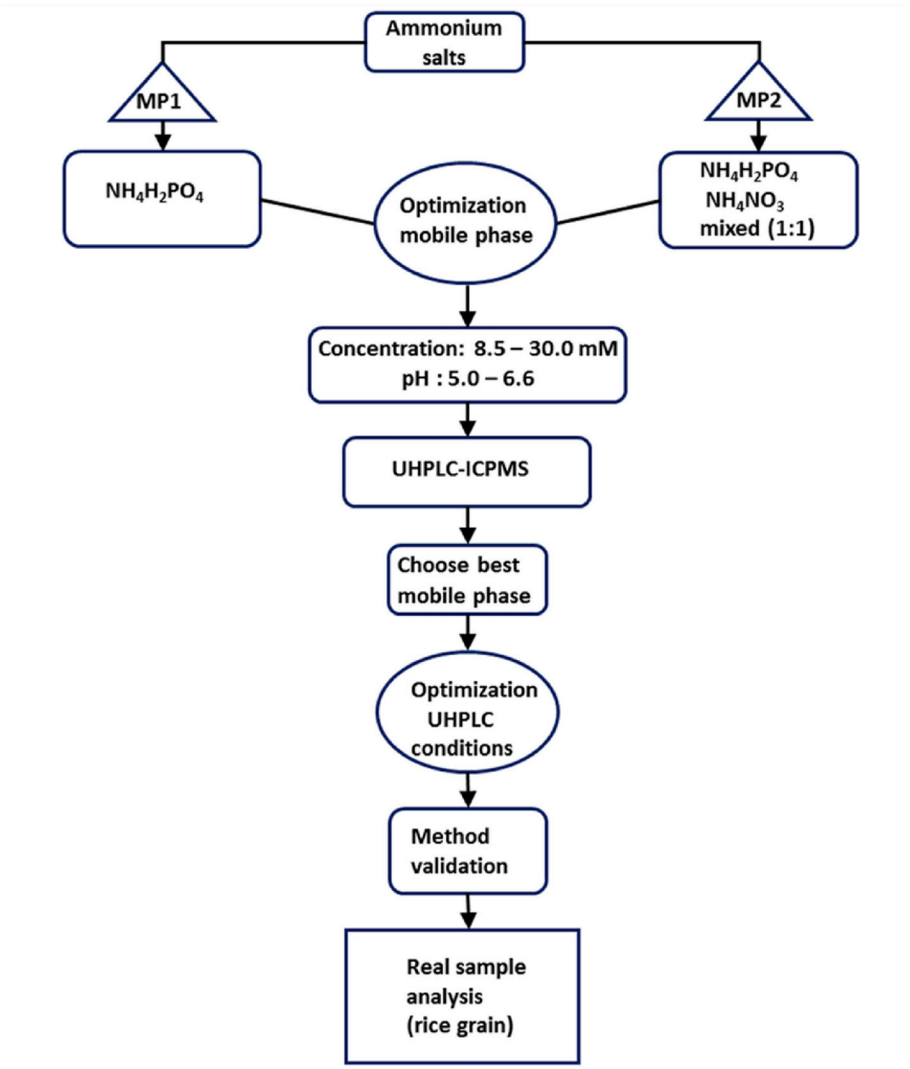


Fig. 1. Flowchart showing the entire optimization process for the development of an analytical protocol based on UHPLC-ICP-MS for the speciation of As (mobile phase 1 - MP1 and mobile phase 2 - MP2).

Table 1
Operational conditions of UHPLC-ICP-MS that are optimized for the speciation of As.

Parameters	Operational conditions
ICP-MS	Perkin-Elmer NexION™ 300X
RF power	1450 W
Plasma gas flow rate	15 L/min
Auxiliary gas flow rate	1.2 L/min
Nebulizer gas flow rate	0.82 L/min
Monitoring mass	*m/z* 74.9216
Integration time	1 s
Dwell time	250.0 ms
Sampling depth	7.0 mm
UHPLC	Flexar, Perkin-Elmer
Column	Hamilton PRP-X100 (250 × 4.1 mm, 10 µm)
Maximum pump pressure (FX-20 pump)	3200 psi
Composition of mobile phase	8.5 mM, $NH_4H_2PO_4$ and NH_4NO_3 at 6.0 pH
Flow rate	2.5 mL/min
Injection volume	50 µL
Column temperature	30 °C

Note: Inductively Coupled Plasma Mass Spectrometry - ICP-MS, Ultra-High Performance Liquid Chromatography - UHPLC.

guidelines provided by the International Union of Pure and Applied Chemists (IUPAC), whereas the LOQ is defined as the lowest concentration of analyte which can be quantitatively determined with suitable precision and accuracy (International Conference on Harmonisation (ICH) and United States Pharmacopoeia (USP)) [30]. In this study, the analysis of blanks does not involve any background noise due to the absence of analyte species in the blank solutions, so that the LOD and LOQ were determined according to the IUPAC criteria as 3 x S_a/m and 10 x S_a/m respectively, whereas S_a is the standard deviation of the response and b is the slope of the calibration curve (Table 3). The S_a can be estimated by the standard deviation of regression lines [30].

3. Results and discussion

3.1. Optimization of mobile phase

The most prevalent and precise method for the separation of negatively charged species is anion exchange chromatography in which negatively charged molecules are retained on positively charged materials in the stationary phase of the column [6]. Thus, the retention of an analyte on the stationary phase mainly depends on the concentration and charge of the competing ions as well as eluent pH. The use of ammonium salts as a mobile phase is particularly important because of their ability to be decomposed into volatile substances under plasma

Table 2
The analysed amounts of As species present in the CRM-rice and corresponding certified amounts along with statistical results for comparing the analytical results with the certified values.

As species	Certified value (μg/kg)	Measured value (μg/kg)	Recovery (%)	Reproducibility (n = 6) (%)	u_{CRM} (μg/kg)	u_m (μg/kg)	Δ_m (μg/kg)	U_Δ (μg/kg)
As(III)	124.0	123.9	99.9	1.9	11.0	2.9	0.06	22.7
DMA(V)	119.0	125.5	105.5	1.8	13.0	1.4	6.5	26.1
Total As	260.0	239.6	92.1	2.6	13.0	0.9	–	–

Note: Certified uncertainty (u_{CRM}), Measurement uncertainty (u_m), Absolute difference between measured and certified values (Δ_m), Expanded uncertainty (U_Δ), If $\Delta_m < U_\Delta$, there is no significance difference between the measured value and the certified value at a confidence level of 95% (The calculation procedure of U_Δ is described in supplementary materials (S1)). The total concentration of As was measured by ICP-MS.

conditions without depositing any salt residues which prevents the damage to sampler and skimmer cones of ICP-MS [5]. Therefore, two different types of mobile phases containing single $NH_4H_2PO_4$ salt (MP1) and a mixture (1:1) of $NH_4H_2PO_4$ and NH_4NO_3 (MP2) were tested based on existing LC approaches for the speciation of As.

3.1.1. Effects of eluent pH on retention time and resolution of peaks

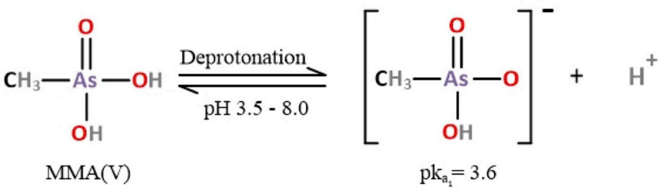
Fig. 2 and Fig. 3 depict the variation of retention time in the separation of the targeted As species with changing the pH of MP1 (30 mM) and MP2 (8.5 mM). The concentrations which were chosen for MP1 and MP2 are based on the results obtained from preliminary screening of chromatograms at different concentrations. Various pHs were tested for both MP1 and MP2 in the range of 5.0–6.6 as a good balance between the pKa values of corresponding As species.

Generally, the change in eluent pH leads to an alteration in the charges of As species and competing anions present in the mobile phase which can trigger anion exchange mechanisms to separate As species through the column. The stationary phase of the PRP-X100 column consists of a resin comprising a 55% cross-linked polystyrene-divinylbenzene copolymer functionalized with a positively charged quaternary ammonium anion-exchanger group ($–C_6H_4–CH_2–H_3N^+$) which is stable over a wide pH range (1–13 pH). This positively charged stationary phase possesses a high affinity for negatively charged As species due to electrostatic interactions. Depending on the ionic strength and pH of the mobile phase, bound As species are exchanged and eluted through the column, acquiring different retention times by competing anions present in the eluent.

As shown in Figs. 2 and 3, the pH of the mobile phase significantly impacts on the retention time of each species and the total elution time required for the separation of all the species increased with increasing eluent pH. Fig. 2 (b) shows the chromatograms obtained for MP1 containing 30 mM of $NH_4H_2PO_4$ at pH ranging from 5.1 to 6.6. At pH 5.1 and 6.6, mono-charged $H_2PO_4^-$ and di-charged $H_2PO_4^{2-}$ are the major ionization products of phosphate ion in the eluent, respectively (reaction(1)). Therefore, with changing the pH of MP1 from 5.1 to 6.6, the charge of the competing ion is increased from 1 ($H_2PO_4^-$) to 2 ($H_2PO_4^{2-}$) which leads to the creation of a strong eluting power [5].

$$H_2PO^-_{4(aq)} \Leftrightarrow HPO^{2-}_{4(aq)} + H^+_{(aq)} pk_a = 7.21 \tag{1}$$

Neutral H_3AsO_3 is the dominant species of As(III) (pKa - 9.2) in solutions at pH < 9, so that As(III) is less retained on the positively charged stationary phase and eluted first, resulting in a clear resolution of As(III) with all the pH values tested for both MP1 and MP2. Under acidic conditions (pH < 6.0), DMA(V) (pKa - 6.27) predominantly exits as a neutral species in the form of $(CH_3)_2AsO(OH)$ and the retention on the positively charged stationary phase is not influenced by the electrostatic interactions [31]. Thus, MP1 at pH 5.1 produced a poor retention for DMA(V) similar to neutral As(III) on the stationary phase which caused DMA(V) to be interfered with As(III) and eluted together with As(III), thereby leading to a significant overlap between the separation of As(III) and DMA(V) with MP1 at pH 5.1. Generally, in aqueous solutions at pH 3.5–8.0, MMA(V) (pKa_1 = 6.6 and pKa_2 = 8.2) exits as a weak acid in the form of mono-negatively charged $CH_3AsO_2(OH)^-$ due to its deportation (reaction 2) and hence, $CH_3AsO_2(OH)^-$ is the major species of MMA(V) at pH 5.1–6.6 in MP1. In the meantime, DMA(V) exists as neutral $(CH_3)_2AsO(OH)$ at pH < 6.0 in the eluent system. Because of this difference in charge between DMA(V) and MMA(V), both ions acquired distinct retention affinities on the stationary phase resulting in a clear separation between DMA(V) and MMA(V) with a raise in the pH of MP1 from 5.1 to 6.1. However, when further increasing pH from 6.1 to 6.6, separation of MMA(V) noticeably overlapped with DMA(V) that is likely to be due to strong eluting power exerted by $H_2PO_4^{2-}$ on single charged $CH_3AsO_2(OH)^-$ and thereby eluting together with DMA(V) at pH 6.6. On the other hand, DMA(V) is capable of becoming deprotonated and existing as mono-negatively charged $(CH_3)_2AsO(O)^-$ (reaction 3) at 6.6 pH in MP1 which is identical in charge to mono-negatively charged $CH_3AsO_2(OH)^-$, so that both anions may receive a similar displacement force by $H_2PO_4^{2-}$. This may cause an interference in the separation of both ions, thereby leading to a poor resolution between DMA(V) and MMA(V) with MP1 at 6.6. With regard to the chromatograms obtained for MP1 (Fig. 2 (b)), it is clear that the separation and resolution of the targeted As species with all the pH values tested for MP1 are not at satisfactory levels.



(2)

Table 3
Method validation results for testing the analytical performance of the proposed method based on UHPLC-ICP-MS.

Description	Species	Regression line	Coefficient of determination (R^2)	Limit of detection (LOD) (μg/L)	Limit of quantification (LOQ) (μg/L)	Reproducibility (n = 6) (%)	Recovery (%)
Calibration standards	As(III)	$Y = 6.59 \times 10^3x + 6.33 \times 10^2$	0.9996	0.47	1.59	1.7	98.3
	DMA(V)	$Y = 6.05 \times 10^3x + 2.93 \times 10^3$	1.0000	0.33	1.10	2.3	100.4
	MMA(V)	$Y = 5.90 \times 10^3x + 1.79 \times 10^3$	1.0000	0.47	1.57	2.3	106.7
	As(V)	$Y = 6.31 \times 10^3x + 2.21 \times 10^3$	1.0000	0.29	0.98	0.5	100.9

Note: Working linear range: 1–120 μg/L. Reproducibility and recovery tests were performed for the mixed As standard at concentration of 10 μg/L.

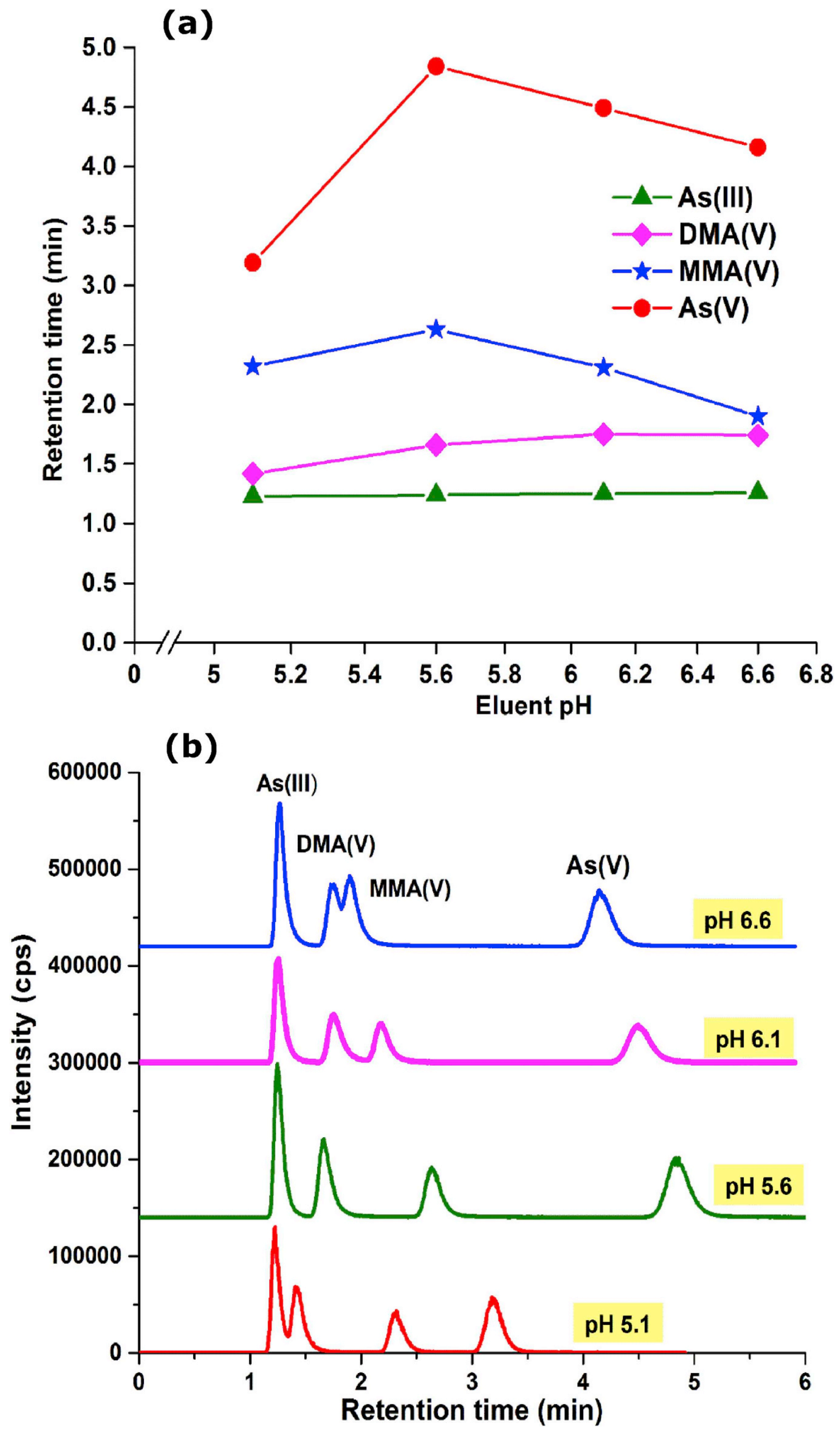
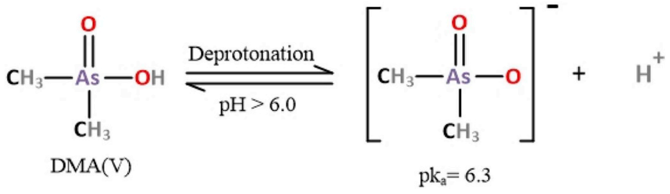


Fig. 2. Effects of eluent pH on the (a) variation of retention time (standard error $<$ 0.008 min) and (b) resolution of As species through isocratic elution of 30 mM, $NH_4H_2PO_4$ (MP1) (Concentration of tested As mixture - 120 µg/L, flow rate - 2.0 mL/min, injection volume - 50 µL and other UHPLC and ICP-MS parameters are given in Table 1).



(3)

Fig. 3 depicts the effects of the pH of MP2 in the retention and resolution of the targeted As species. The MP2 is a mixed eluent system containing two major competing anions, namely $H_2PO_4^-$ and NO_3^- which leads to an increased eluting power compared to MP1. In MP2 at pH 5, DMA(V) overlapped with the peak of As(III) due to similar behaviour in acidic pH as observed for MP1 (Fig. 3 (b)). However, with an increase in

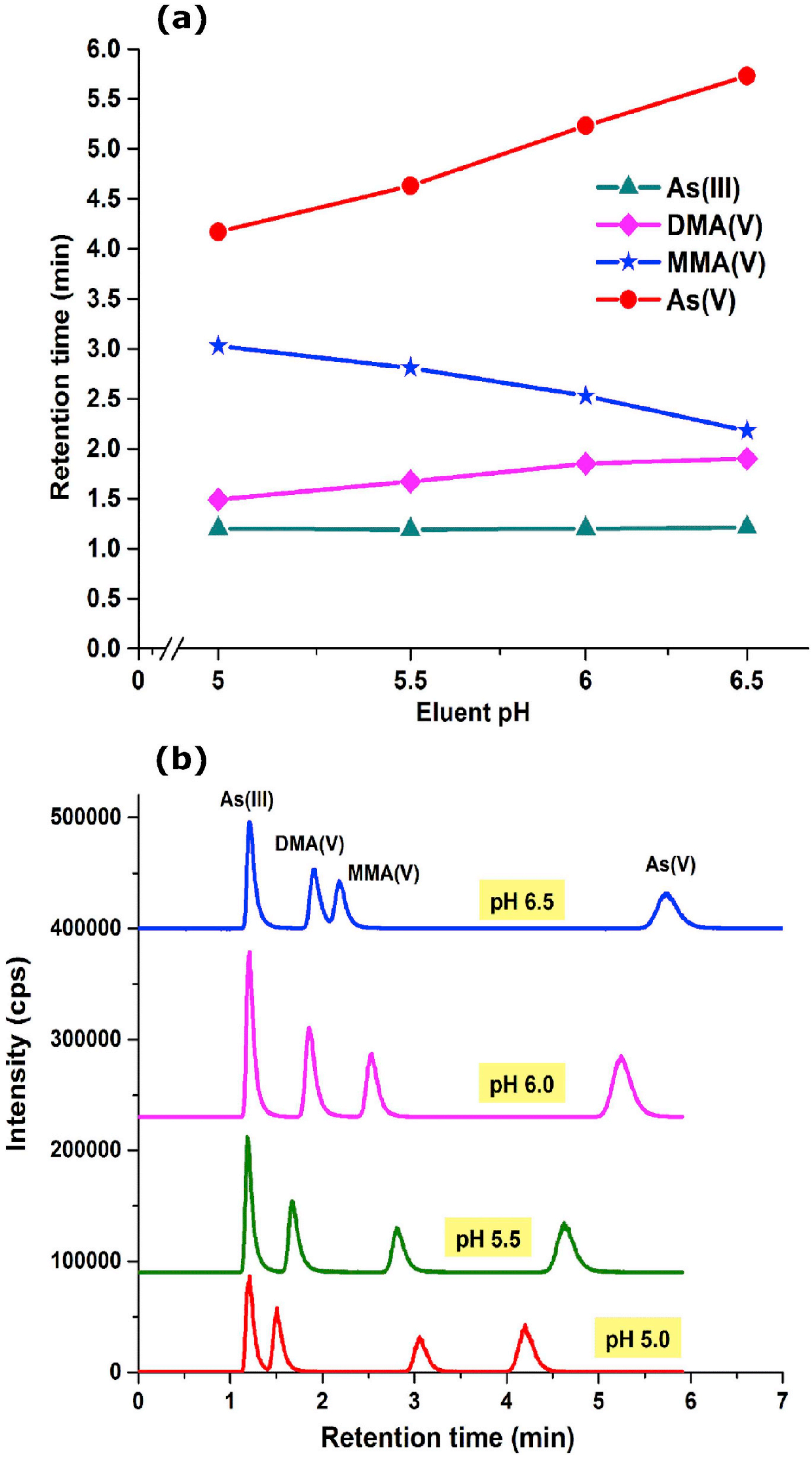


Fig. 3. Effects of eluent pH on the (a) variation of retention time (standard error < 0.008 min) and (b) resolution of As species through isocratic elution of 8.5 mM, $NH_4H_2PO_4$ and NH_4NO_3 (MP2) (Concentration of tested As mixture - 120 µg/L, flow rate - 2.0 mL/min, injection volume - 50 µL and other UHPLC and ICP-MS parameters are given in Table 1).

the pH of the eluent from 5.0 to 6.5, the resolution between As(III) and DMA(V) was improved to a considerable extent. The longest retention of As(V) appeared to be in MP2 at pH 6.5 and hence, the total time required for the separation of all the As species has significantly increased with an increase in eluent pH. Generally, As(V) (pKa_1 - 2.3, pKa_2 - 6.7, pK_{a3} - 11.6) exists predominantly as di-anionic $HAsO_4^{2-}$ at pH > 6.0 which can be strongly bound on the positively charged stationary phase. Because of this strong affinity of As(V) towards the stationary phase, the selected ionic strength of the eluent (8.5 mM) at pH 6–6.5 may not strong enough for anionic competing ions present in the eluent (particularly $H_2PO_4^{2-}$) to quickly displace and elute As(V) through the column. Furthermore, the raising of pH from 5.0 to 6.5 in MP2 causes a significant overlap of the separation between DMA(V) and MMA(V) resulting in a weak resolution of peaks. This is also due to the change in binding affinity of MMA(V) with the stationary phase at higher pHs following the similar ion exchange mechanisms as explained for MP1 at pH > 6.0. The MP2 at pH 6.0 achieved the best resolution for the separation of all the As species within 6 min. It is noteworthy to mention that even a slight change in pH of the MP2 can improve both resolution and retention time of As species to a significant extent. Thus, the performance of MP2 prepared at pH 6.0 was the best compared to MP1 for the separation of targeted As species achieving a shorter retention time for the resolution of all of the As species. Therefore, pH 6.0 was selected as the optimum pH for MP2 for the separation of targeted As species considering excellent resolution and faster elution.

3.1.2. Effects of eluent concentration on retention time and resolution of peaks

Results obtained from pH experiments revealed that MP1 at pH 5.6 provides a better resolution of As species compared to other pHs tested for this eluent, whereas the best peak resolution for all As species is achieved by MP2 at pH 6.0 (Figs. 2 and 3). The ionic strength or concentration of competing anions present in the mobile phase is the other critical parameter which can have a large impact on the resolution as well as the retention time of desired species. Therefore, several concentrations in the range of 8.5–30 mM were tested for both MP1 and MP2 at the respective optimized pH of 5.6 and 6.0 to choose the best type of mobile phase that could be applied for the simultaneous separation of As species.

Fig. 4 and Fig. 5 illustrate the effects of eluent concentration on changing the retention time and peak resolution of As species with MP1 and MP2 at 5.6 and pH 6.0, respectively. With an increase in the concentration of both MP1 and MP2 from 8.5 to 30 mM, the retention time of all of the species significantly decreased, resulting in a poor resolution between each As species at higher ionic strengths (> 17 mM). This could be due to strong electrostatic interaction between concentrated competing anions in the eluents and the stationary phase. As a result, anionic As species are unable to fully displace strongly bound eluent anions on the stationary phase which may lead to faster elution of As species resulting in a poor resolution between each species. Thus, the total separation time for all of the species decreased significantly with an increase in the concentration of the eluents while inadequately resolving the peaks between As(III), DMA(V) and MMA(V) at higher ionic strengths of both mobile phases tested. However, previous studies have demonstrated that 30 mM of $NH_4H_2PO_4$ buffer at pH 6.5 can attain a better separation of these four As species, although corresponding chromatograms of As species showed quite a weak resolution between As(III) and DMA(III) [4,5]. When such methods are applied in the analysis of As species in real environmental samples, there may be more overlap between As(III) and DMA(V) since the retention times of these species present in real samples are quite different from those of standard As mixtures prepared under laboratory conditions. Therefore, the resolution of species obtained from such methods cannot be accepted as an adequate separation for real environmental samples.

The 10 mM of MP1 at pH 5.6 provided a clear separation of all the species within 13 min which is too long to be considered as a cost effective analytical method (Fig. 4). Interestingly, chromatograms obtained from MP2 at pH 6.0 demonstrated that 8.5 and 10.0 mM of MP2 at 6.0 pH are capable of achieving an excellent separation of As species in less than 6 min (Fig. 5 (b)). However, the 10 mM of MP2 at pH 6.0 cannot be recommended as a suitable ionic strength to simultaneously separate these As species present in environmental samples because of poor resolution between DMA(V) and MMA(V). The decrease of eluent concentration from 10 mM to 8.5 mM at pH 6.0 resulted in an excellent resolution between DMA(V) and MMA(V) achieving enhanced separation of targeted As species within 6 min that is indeed analytically cost effective due to shorter analysis time per sample. Generally, eluents with mild ionic strength (< 10 mM) comprise weak competing ions which can produce only a feebler eluting power, thereby increasing the retention of desired analytes in the column. Nevertheless, the MP2 is a mixture of two strong competing anions, including $H_2PO_4^{2-}$ and NO_3^- at pH 6.0 which can act as strong pushers producing an increased eluting power, thereby rapidly displacing the targeted As species from the stationary phase. Therefore, 8.5 mM of MP2 at pH 6.0 was selected as an optimum mobile phase to separate the targeted As species with regard to high peak intensity, excellent peak resolution and rapid analysis time.

3.2. Optimization of UHPLC conditions

The 8.5 mM of MP2 at pH 6.0 was used to optimize the conditions of UHPLC, particularly the sample injection volume and flow rate in order to achieve improved resolution and retention time of the targeted As species. The injection volume was changed in different magnitudes ranging from 25 to 75 μL however the changes of injection volume did not significantly affect the resolution and retention time of As species. Thus, 50 μL was selected to run the samples as a good balance between the intensity of peaks and retention time.

The flow rate played a significant role in controlling the resolution of each As species and the retention time after being optimized by all other parameters. When the flow rate was increased from 1.0 mL/min to 2.0 mL/min, the retention time of As(V) significantly decreased to 5.5 min with an enhanced resolution of all the targeted species (Fig. 5 (b)), an excellent performance of the UHPLC compared to existing HPLC systems which have been used for the speciation of As. When the samples were run with the optimized eluent (8.5 mM MP2 at pH 6.0) at 1.0 mL/min, the system back pressure was recorded as ~1200 psi resulting in a long retention time (15 min) for As(V). Thus, the flow rate was increased up to 2.0 mL/min to decrease the retention time of As(V) without affecting the resolution of the other three As species. However, when increasing the flow rate, the amount of column back pressure generated by the UHPLC system must be taken into consideration because higher back pressure can damage the stationary phase of the column as well as the hyphenated UHPLC-ICP-MS system. The increase of flow rate up to 2.0 mL/min caused a rise of column pressure up to ~2800 psi which was not harmful for the column, as the back pressure of PRP-X100 column is recommended to be a maximum of 6000 psi. Therefore, the flow rate was maintained at 2 mL/min for mobile phase optimization experiments as a good balance between the resolution and retention time of the species (Figs. 2–5). Because of such low back pressure generation ability of the UHPL system at 2.0 mL/min, the flow rate was further increased to 2.5 mL/min in order to achieve a faster separation of the targeted As species. Interestingly, all of the As species were then separated within 5 min, reducing the retention time of As(V) up to 4.5 min without affecting the resolution between As(III), DMA(V) and MMA(V) as shown in Fig. 6. When the flow rate was increased from 2.0 to 2.5 mL/min, the column back pressure was raised, ranging from 3100 to 3300 psi, which was still not harmful for the column and UHPLC system. In existing HPLC technology, by increasing the flow rate above 2.0 mL/min, optimization of resolution and retention time of species is not possible because of the generation of a high back pressure which may damage the whole hyphenated system. Hence, this UHPLC system provided such an excellent ultra-performance due to the presence of dual-reciprocating piston 18,000 psi pump (FX-20 pump) that can generate and withstand the system pressure maximum of 15,000–18,000 psi. The major limitation of using HPLC columns in UHPLC is that the existing HPLC columns are not capable of tolerating such a high pressure as is developed in UHPLC. Thus, the development of UHPLC based analytical columns are an urgent necessity to achieve the ultra-performance ability of UHPLC systems for the separation of analyte species more quickly. Therefore, the analytical approach developed using a HPLC column in this study will pave the way to develop

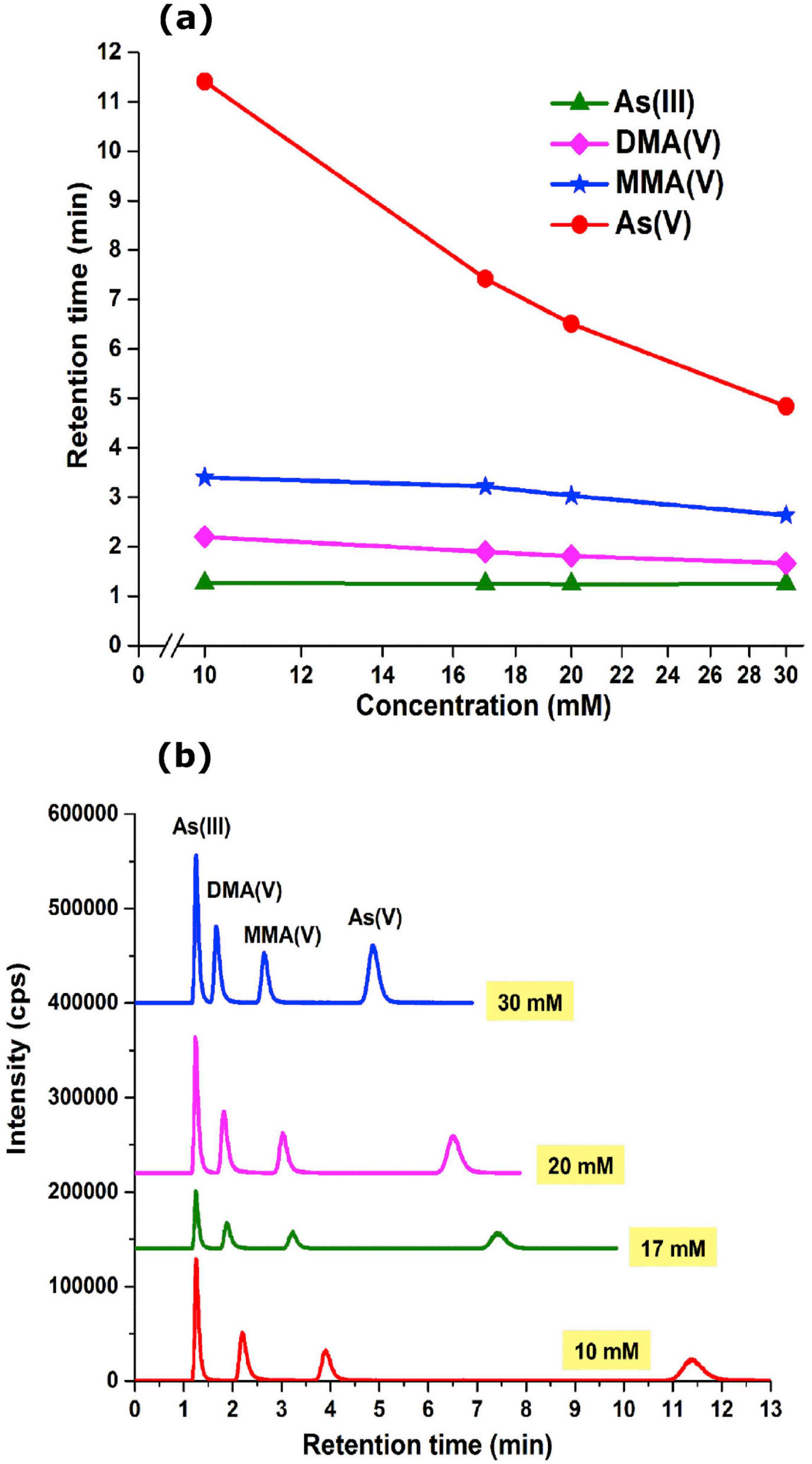


Fig. 4. Influence of the concentration of competing ions present in the mobile phase (MP1) containing $NH_4H_2PO_4$ at pH 5.6 on (a) change in retention time (standard error < 0.008 min) and (b) resolution of As species (Concentration of tested As mixture - 120 µg/L, flow rate - 2.0 mL/min, injection volume - 50 µL and other UHPLC and ICP-MS parameters are given in Table 1).

suitable UHPLC based analytical columns for the speciation of As species in the near future.

Table 4 summarizes the performance of the proposed method based on UHPLC in this study compared to the existing methods that are commonly used for the speciation of As. It is noteworthy to mention that the existing HPLC-ICP-MS systems have required 10–15 min for the complete separation of these four As species with reasonable resolutions using similar types of anion exchange columns (Table 4). Hence,

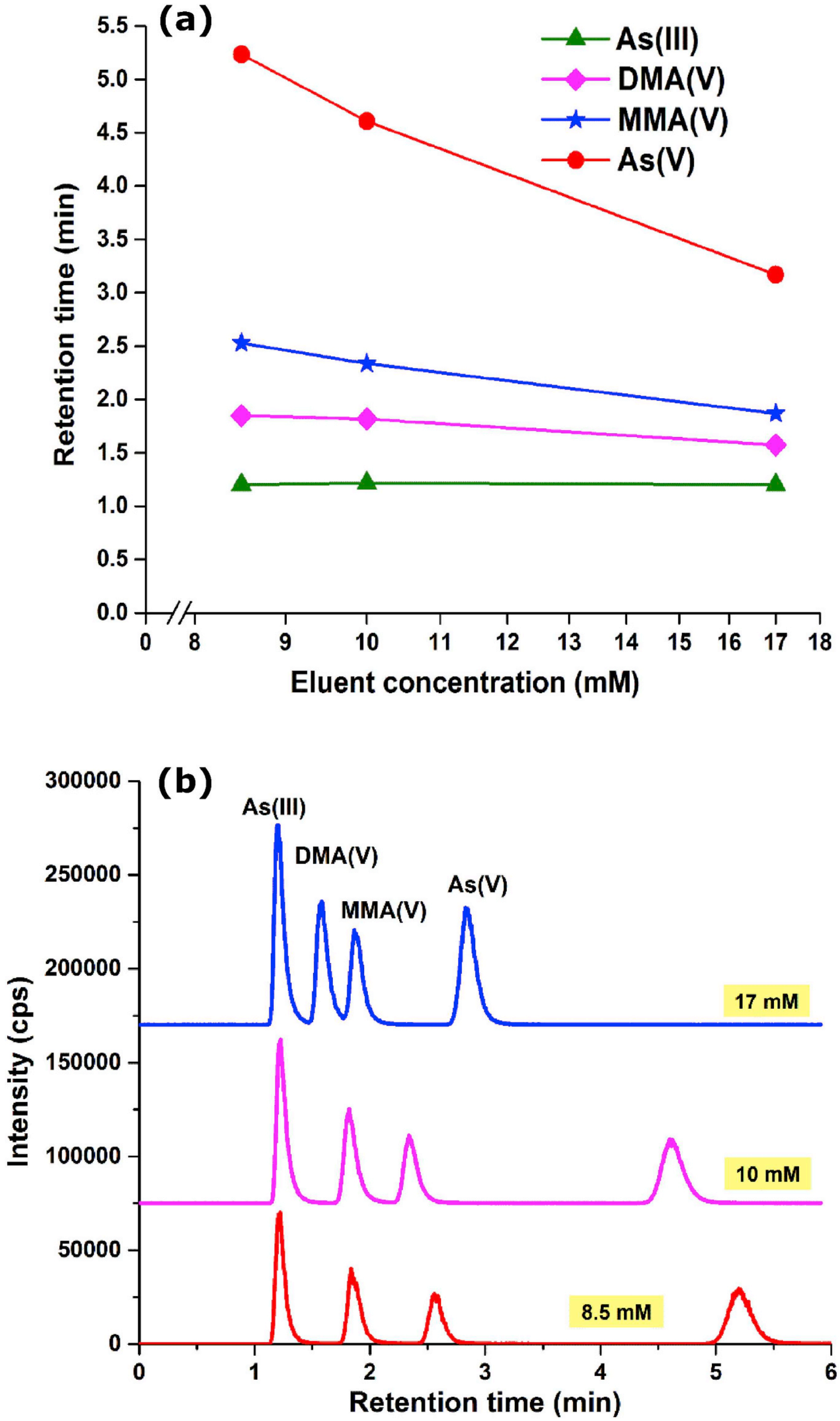


Fig. 5. Influence of the concentration of competing ions present in the mixed mobile phase (MP2) containing 8.5 mM of $NH_4H_2PO_4$ and NH_4NO_3 at pH 6.0 on (a) change in retention time (standard error < 0.008 min) and (b) resolution of As species (Concentration of tested As mixture - 120 µg/L, flow rate - 2.0 mL/min, injection volume - 50 µL and other UHPLC and ICP-MS parameters are given in Table 1).

achieving a fast separation time per sample with improved resolution and retention time is analytically cost effective for the analysis of a large number of samples due to the higher cost required for the consumables of ICP-MS. Furthermore, this could provide a significant saving in operational time for the operator as well. Therefore, such a modern development in analytical instrumentation will be beneficial for future biological and water research to analyse an extensive number of samples in a short time.

3.3. Analytical performance and validation of the proposed method

Separation of the targeted As species was achieved within 5 min under optimized conditions of UHPLC and the elution order was as, As (III) < DMA(V) < MMA(V) < As(V). As shown in Fig. 5, the resolution of species is far better even at low concentrations (< 5 µg/L). The column back pressure was maintained below 3500 psi, keeping the flow rate at 2.5 mL/min considering the life time of the column,

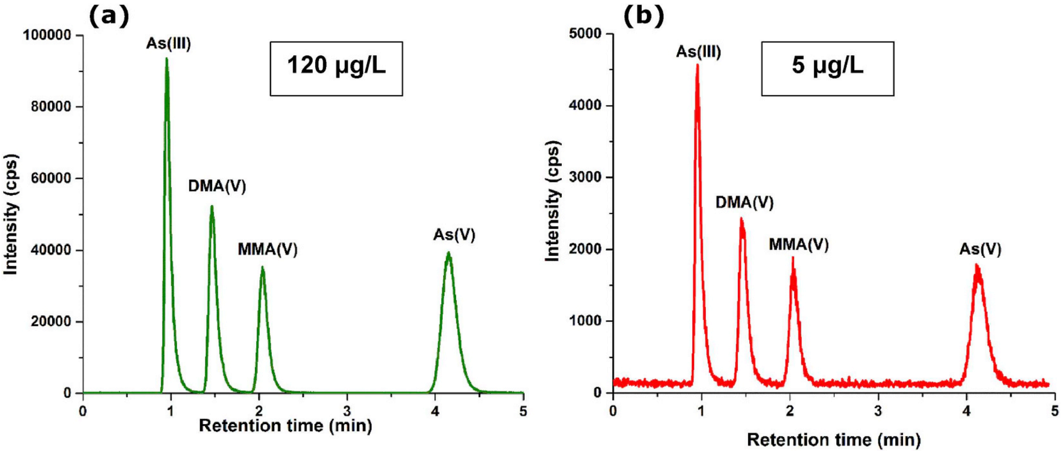


Fig. 6. Chromatogram of a mixture of As species at concentrations of (a) 120 µg/L (b) 5 µg/L obtained with the optimized UHPLC conditions and mobile phase containing 8.5 mM, $NH_4H_2PO_4$ and NH_4NO_3 at pH 6.0 (Flow rate - 2.5 mL/min, injection volume - 50 µL and other UHPLC-ICP-MS conditions are given in Table 1).

consumption of the mobile phase and waste generation. However, the potential existed to further reduce the retention time of As(V) by increasing the flow rate more than 2.5 mL/min, because at every increment of the flow rate, the retention of As(V) is reduced significantly without affecting the resolution between other species. This is an exciting promising characteristic feature resulting from this proposed method, based on UHPLC-ICP-MS because maintaining a considerable resolution between As(III), DMA(V) and MMA(V) is a challenging task while retaining the total elution time < 5 min.

Table 3 summarizes the method validation results obtained from this fast analytical protocol for the quantification of As species present in mixed As standards. Results obtained from all the validation tests corroborated the high accuracy and precision of the proposed analytical method. Calibration curves for each species were obtained by plotting the peak area against the concentration of the targeted As species. All calibration curves produced a linear pattern over a concentration range of 1–120 µg/L with the coefficient of determination (R^2) > 0.999 with 50 µL injection volume of mixed As standards. The LOD of each As species achieved by the proposed method ranged from 0.3 to 0.5 µg/L that is low enough to analyse As species at trace levels (µg/L) in real samples. Values of LOQ lied in the range of 1.0–1.6 µg/L which imply that these four As species can be reliably quantitated at and above this concentration range under the proposed analytical conditions. Reproducibility of measured concentrations of As species was determined from the relative standard deviation (RSD) among 6 repeated measurement of a sample (Table 3). Lower RSD values ranging from 0.5 to 2.3% indicate high precision of the proposed method for the quantification of these As species. Moreover, percentage recovery values obtained for all the As species were in the range of 100 ± 7 which confirms the excellent separation of the targeted As species under optimized conditions.

The accuracy and reliability of the proposed method was further validated by testing with the CRM-rice which contains predominately As(III) and DMA(V) as As species (Fig. 7). Retention times of As(III) and DMA(V) (1 and 1.5 min, respectively), obtained for mixed As standards were in well-agreement with that of CRM-rice, confirming the excellent resolution of targeted As species by this method (Fig. 7). Further, excellent recovery and repeatability in the concentrations of As(III) and DMA(V) attained for CRM-rice confirmed the column performance, the resolution of peaks and the precision of quantitative data (Table 2). The absolute difference between the measured concentration and certified value for As(III) and DMA(V) present in the CRM-rice is 0.061 and 6.5 µg/kg, respectively which is only a deviation of 0.05 and 5.50% from the certified values for As(III) and DMA(V), respectively. Moreover, these absolute difference values of As(III) and DMA(V) are significantly smaller than the expanded uncertainty of corresponding two species (22.7 and 26.1 µg/kg, respectively) at a confidence level of 95%, which confirms that there is no significant difference between the measured concentrations and the certified values of As species present in the CRM-rice (Table 2). Therefore, the proposed method based on UHPLC-ICP-MS can be recommended as an accurate and reliable analytical protocol for a quantification of As species in various types of environmental and biological samples. Therefore, the proposed method was applied to identify and quantify the desired As species present in rice grains which are commercially available in Australia and Sri Lanka.

Table 4
A comparison between the retention times achieved from the proposed method in this study with existing other methods for the speciation of As.

Study	Instrumentation	Column	Mobile phase	Retention time/min				Total run time/min
				As(III)	DMA(V)	MMA(V)	As(V)	
This study	UHPLC-ICPMS	anion exchange- PRP-X 100	8.5 mM $NH_4H_2PO_4$ and NH_4NO_3 at 6.0 pH	1.0	1.5	2.2	4.3	5.0
[27]	HPLC-ICPMS	anion exchange- PRPX100	30 mmol/L $(NH_4)_2CO_3$ at pH 8.50	2.8	3.3	6.5	12.0	15.0
[39]	HPLC-ICPMS	anion exchange- PRP-X100	Gradient elution with (A) - 1.25 mmol/L Na_2HPO_4 and 11.0 mmol/L KH_2PO_4 (B) - 2.5 mmol/L Na_2HPO_4 and 22.0 mmol/L KH_2PO_4	3.6	5.5	7.0	11.3	13.0
[17]	HPLC-ICPMS	Diamonsil C18 column	4 mM Tetrabutylammonium hydroxide (TBAH) at pH 6.0	0.4	2.1	3.3	8.7	10.0
[40]	HPLC-ICPMS	anion exchange- IonPac®AS7 column	A gradient of 0.04 mM HNO_3 and 50 mM HNO_3	2.0	4.0	8.0	8.8	10.0
[15]	HPLC-ICPMS	IonPac AS16	A gradient of NaOH ranging 1–100 mM	7.5	6.0	13.8	19.5	23.0
[5]	IC-ICPMS	anion exchange- PRP-X 100	30 mM $NH_4H_2PO_4$ at pH 5.6	2.5	3.1	5.1	8.1	10.0
[6]	IC-ICPMS	PRP-X100	30 mM $NH_4H_2PO_4$ at pH 5.6	2.4	3.1	4.8	7.7	10.0

Note: High Performance Liquid Chromatography coupled to Inductively Coupled Plasma Mass Spectrometry - HPLC-ICPMS, Ultra-High Performance Liquid Chromatography coupled to Inductively Coupled Plasma Mass Spectrometry - UHPLC-ICPMS.

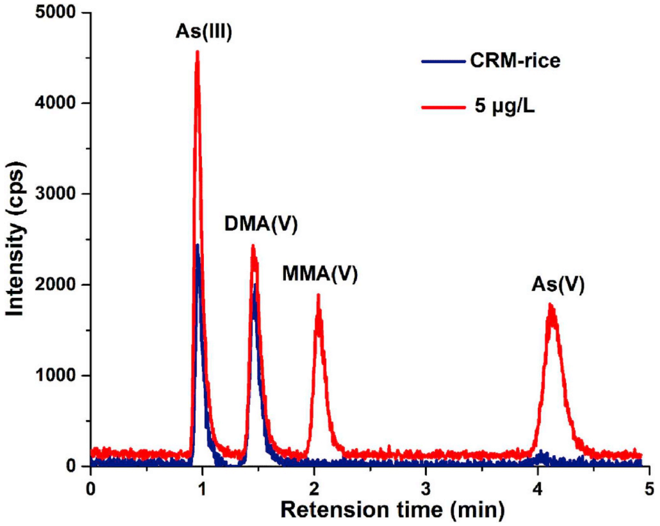


Fig. 7. Chromatograms showing the validation of retention time for As(III) and DMA(V) obtained from the certified reference rice material (CRM-rice) with a mixed As standard at concentration of 5 µg/L.

3.4. Quantification of As species in rice grains

Rice (*Oryza sativa* L.) is the staple food which feeds over 50% of the world's population [32,33]. Arsenic enrichment of rice at elevated levels has become a critical life-threatening issue due to its toxicological effects on human health. Rice grown in several parts of Bangladesh, India, China and Japan contains elevated amounts of arsenic because of high geogenic arsenic levels in ground- and surface-water sources that are used for irrigation as well as paddy soil contamination through mining activities [13,32,34–36]. It is hypothesised that arsenic exposure at toxic levels though food and water might be a reason for the chronic kidney disease of unknown etiology of people in the dry zone in Sri Lanka [37]. Australia is one of main rice exporters in the world. However, it also imports rice in considerable quantities from Sri Lanka, India, China and Bangladesh because of increased interest and demand from local multi-ethnic populations. Therefore, it is very important to identify and quantify different toxic arsenic species in rice produced from countries where the contamination of arsenic in ground-surface- and irrigated-water has been a critical issue.

Amounts of As species extracted from different rice varieties which were quantified by the proposed method using the modern UHPLC-ICP-MS system are given in Table 5. The sum of species concentrations correlated closely with the total acid digested As concentrations, achieving an excellent extraction efficiency ranging from 75 to 99% (except for two rice varieties) which confirmed that the extraction method based on microwave-assisted digestion with 0.25 M of HNO_3 can perform well for the detection and analysis of targeted As species by the proposed method. With regard to the results obtained from species analysis, it is obvious that As(III) was the prevailing species in all of the rice samples ranging from 53 to 100%, whereas DMA(V) was detected at lower quantities in only six rice varieties, ranging from 0 to 47% of the sum of the four As species. The concentration of As(III) increased with the total As concentration in the grains, while DMA(V) remained in quite a narrow range from 20.3 to 71.5 µg/kg. Inorganic As(III) is generally more toxic than DMA(V) and hence, potential health risks can also be increased with prolonged consumption of these rice varieties in large quantities. Interestingly, amounts of total As in all the rice varieties lay below the maximum permissible limit for inorganic As in rice for adults (200 µg/kg) recommended by the World Health Organization (WHO) [38]. Only one type of rice variety contained a higher amount of As (192.2 µg/kg) which is close to the limit of safe guideline value. This variety was identified as a red long grain basmati rice comprising a thick bran layer (unpolished) which was prominent compared to other varieties. This thick bran layer may enable As to be localized at the surface to a considerable extent. Therefore, the findings of this study would encourage future research to explore the accumulation of more toxic As species and their localization over different parts of the rice grain using modern synchrotron-based X-ray techniques, including synchrotron X-ray fluorescence (S-XRF) and X-ray absorption near edge structure (µ-XANES).

4. Conclusions

The present study aimed to develop a fast analytical protocol based on an UHPLC hyphenated to ICP-MS detection for the speciation of As which can be used to analyse environmental and biological samples. Results demonstrated that the mixed mobile phase containing 8.5 mM of $NH_4H_2PO_4$ and NH_4NO_3 at pH 6.0 is the most appropriate eluent for the separation of targeted As species by an PRP-X100 anion exchange column coupled to ICP-MS as the detector. Optimized chromatographic conditions in UHPLC achieved the separation of targeted As species with an excellent resolution within 5 min which is a double saving in analysis time per sample compared to the existing HPLC-ICP-MS approaches (9–15 min). Interestingly, unlike HPLC technology, the optimized UHPLC system did not generate a high column back pressure

Table 5
Amounts of As species in commercially available rice varieties quantified by the proposed method based on UHPLC-ICPMS system.

Rice variety	Amount of As species (µg/kg)				Sum of species (µg/kg)	Total As (µg/kg)	Extraction efficiency (%)
	As(III)	DMA(V)	MMA(V)	As(V)			
VRT-1	74.5 ± 5.3	n.d	n.d	n.d	74.5 ± 5.3	87.3 ± 1.0	85.3
VRT-2	80.3 ± 1.8	71.5 ± 2.2	n.d	n.d	151.8 ± 4.0	155.6 ± 1.3	97.5
VRT-3	59.2 ± 6.4	n.d	n.d	n.d	59.2 ± 6.4	64.9 ± 1.9	91.2
VRT-4	58.1 ± 4.7	n.d	n.d	n.d	58.1 ± 4.7	71.9 ± 7.2	80.8
VRT-5	36.5 ± 2.9	n.d	n.d	n.d	36.5 ± 2.9	46.1 ± 2.0	79.2
VRT-6	99.4 ± 1.1	30.6 ± 2.4	n.d	n.d	130.0 ± 3.4	130.9 ± 2.5	99.3
VRT-7	162.3 ± 15.6	20.4 ± 1.1	n.d	n.d	182.7 ± 16.7	192.7 ± 0.8	94.8
VRT-8	43.0 ± 1.2	n.d	n.d	n.d	43.0 ± 1.2	70.9 ± 2.4	60.7
VRT-9	75.2 ± 0.1	20.3 ± 0.7	n.d	n.d	95.5 ± 0.7	100.4 ± 10.0	95.1
VRT-10	48.6 ± 0.9	35.9 ± 0.9	n.d	n.d	84.5 ± 0.5	89.9 ± 1.8	93.9
VRT-11	67.6 ± 2.0	n.d	n.d	n.d	67.6 ± 2.0	70.6 ± 9.6	95.8
VRT-12	65.8 ± 1.6	n.d	n.d	n.d	65.8 ± 1.6	94.3 ± 3.8	69.8
VRT-13	88.2 ± 4.5	23.6 ± 2.0	n.d	n.d	111.8 ± 6.5	113.8 ± 2.5	98.2
VRT-14	29.6 ± 0.1	n.d	n.d	n.d	29.6 ± 0.1	30.3 ± 0.1	97.6
VRT-15	67.6 ± 0.9	n.d	n.d	n.d	67.6 ± 0.9	68.4 ± 3.6	98.8
VRT-16	92.5 ± 3.5	n.d	n.d	n.d	92.5 ± 3.5	108.5 ± 3.4	85.2

Note: Rice variety - VRT, n.d - not detected, Sum of species – Sum of As(III), DMA(V), MMA(V) and As(V).

with an increased flow rate even up to 2.5 mL/min which resulted in a faster separation with an excellent resolution of the targeted As species. The absolute percentage difference between mean measured concentration and the certified value for As(III) and DMA(V) in certified reference rice material was only 0.05 and 5.50% of the certified values for As(III) and DMA(V) respectively, which further confirmed the accuracy and reliability of the proposed method for As speciation. The speciation of As in rice samples indicated that As(III) is the dominant species ranging from 53 to 100% in the rice grains. Amounts of total As in all of the rice varieties ranged from 30.3 to 192.7 μg/kg which lie below the limit for inorganic As in rice for adults (200 μg/kg) recommended by the WHO. Overall, the proposed analytical protocol based on UHPLC coupled to ICP-MS provided accurate and reliable identification and quantification of the targeted As species, along with the advantages of lower detection limits, excellent resolution and faster analysis. The approach of UHPLC-ICP-MS as a modern analytical advancement in liquid chromatography would pave the way to encourage future research to develop UHPLC based analytical columns for the analysis of more complexed As species, including thio-As complexes in environmental, biological and geological systems.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.120457.

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Appendix B

Article IX

Vithanage, M., Kumarathilaka, P., Oze, C., Karunatilake, S., Seneviratne, M., Hseu, Z-Y, Gunarathne, V., Dassanayake, M., Ok, Y.S., Rinklebe, J., (2019). Occurrence and cycling of trace elements in ultramafic soils and their impacts on human health: a critical review. Environment International, 131, 104974. (Published).

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Review article

Occurrence and cycling of trace elements in ultramafic soils and their impacts on human health: A critical review

Meththika Vithanage[a,b,*], Prasanna Kumarathilaka[b], Christopher Oze[c], Suniti Karunatilake[d], Mihiri Seneviratne[e], Zeng-Yei Hseu[f], Viraj Gunarathne[a], Maheshi Dassanayake[g], Yong Sik Ok[h,**], Jörg Rinklebe[i,j,***]

[a] *Ecosphere Resilience Research Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda 10250, Sri Lanka*
[b] *School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia*
[c] *Department of Geology, Occidental College, Los Angeles, CA 90041, USA*
[d] *Planetary Science Lab, Geology & Geophysics, Louisiana State University, USA*
[e] *Department of Botany, The Open University of Sri Lanka, Nawala, Sri Lanka*
[f] *Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan*
[g] *Department of Biological Sciences, Louisiana State University, USA*
[h] *Korea Biochar Research Center, O-Jeong Eco-Resilience Institute, Division of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Republic of Korea*
[i] *University of Wuppertal, School of Architecture and Civil Engineering, Institute of Foundation Engineering, Water- and Waste-Management, Laboratory of Soil- and Groundwater-Management, Pauluskirchstraße 7, 42285 Wuppertal, Germany*
[j] *University of Sejong, Department of Environment and Energy, Seoul 05006, Republic of Korea*

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ABSTRACT

The transformation of trace metals (TMs) in natural environmental systems has created significant concerns in recent decades. Ultramafic environments lead to potential risks to the agricultural products and, subsequently, to human health. This unique review presents geochemistry of ultramafic soils, TM fractionation (i.e. sequential and single extraction techniques), TM uptake and accumulation mechanisms of ultramafic flora, and ultramafic-associated health risks to human and agricultural crops. Ultramafic soils contain high levels of TMs (i.e. Cr, Ni, Mn, and Co) and have a low Ca:Mg ratio together with deficiencies in essential macronutrients required for the growth of crops. Even though a higher portion of TMs bind with the residual fraction of ultramafic soils, environmental changes (i.e. natural or anthropogenic) may increase the levels of TMs in the bioavailable or extractable fractions of ultramafic soils. Extremophile plants that have evolved to thrive in ultramafic soils present clear examples of evolutionary adaptations to TM resistance. The release of TMs into water sources and accumulation in food crops in and around ultramafic localities increases health risks for humans. Therefore, more focused investigations need to be implemented to understand the mechanisms related to the mobility and bioavailability of TMs in different ultramafic environments. Research gaps and directions for future studies are also discussed in this review. Lastly, we consider the importance of characterizing terrestrial ultramafic soil and its effect on crop plants in the context of multi-decadal plans by NASA and other space agencies to establish human colonies on Mars.

1. Introduction

A thorough understanding of the importance of the abiotic environment and its interaction with living organisms is a challenge in soil and environmental research. Trace metals (TMs) are common environmental contaminants whose toxicity is an increasing concern from

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* Correspondence to: M. Vithanage, Ecosphere Resilience Research Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda 10250, Sri Lanka.

** Corresponding author.

*** Correspondence to: J. Rinklebe, University of Wuppertal, School of Architecture and Civil Engineering, Institute of Foundation Engineering, Water- and Waste-Management, Laboratory of Soil- and Groundwater-Management, Pauluskirchstraße 7, 42285 Wuppertal, Germany.

E-mail addresses: meththika@sjp.ac.lk (M. Vithanage), yongsikok@korea.ac.kr (Y.S. Ok), rinklebe@uni-wuppertal.de (J. Rinklebe).

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an ecological and nutritional viewpoint (Kazakou et al., 2008; Sungur et al., 2015). The term "TM" has been adopted to describe any metallic element with a relatively high density that can be toxic to living organisms at low concentrations (Nagajyoti et al., 2010). However, it is not the density but the chemical characteristics of TMs that are most influential in determining their toxicity. Nieboer and Richardson (1980) classified heavy metals as class B metals, which are non-essential and highly toxic elements such as Hg, Ag, and Pb. TMs can be bioaccumulative by not being degraded in an ecosystem or easily metabolized. For this reason, uptake of TMs by plants may lead to the accumulation at different levels in the food chain (Antoniadis et al., 2017).

Both anthropogenic activities and natural processes lead to the release of TMs into the environment. Industrial inputs of TMs to the biosphere are of great environmental concern (Wijesekara et al., 2016; Young et al., 2005). The process of weathering rocks and minerals can also serve as inputs for TMs into the environment (Cooper, 2002; Robles-Camacho and Armienta, 2000). The deleterious effects on the environment of TMs of natural origins cannot simply be neglected, as these effects can be extreme. An example of an environment which is extremely affected from TMs is ultramafic soils, which contain elevated concentrations of TMs such as chromium (Cr), nickel (Ni), cobalt (Co), manganese (Mn), lead (Pb), zinc (Zn), copper (Cu), and vanadium (V) coupled the with low availability of calcium (Ca) (Lee et al., 2001; Ndjigui et al., 2008). The exceedingly high concentrations of TMs in ultramafic soils may cause challenges to agricultural crops, due to the potential risk of bioaccumulation (Bandara et al., 2017a; Bandara et al., 2017b; Herath et al., 2015). For instance, Kanellopoulos et al. (2015) found that agricultural soils near an ultramafic source contained high levels of Cr, Ni, and Co. Acidic conditions in the ultramafic soil matrix may promote the release of TMs (i.e., Ni and Mn) from ultramafic soils into surrounding environments (Kumarathilaka et al., 2016b; Rajapaksha et al., 2012; Vithanage et al., 2014).

A limited number of extremophile plants (extremophytes) have evolved to grow in hostile ultramafic environments. Ultramafic rocks have dramatic effects on the vegetation that grows on them. Most plants cannot grow on ultramafic soils, leaving distinctive suites of extremophytes to occupy ultramafic habitats. The floristic diversity associated with serpentine soils formed above ultramafic rocks is surprising, considering that these soils are toxic to most plants. The species richness observed in ultramafic environments in some instances has been reported as a result of divergent selection leading to speciation where ultramafic adapted species are sister taxa of species growing in non-ultramafic soils (Anacker and Strauss, 2014; Baldwin, 2005). Ultramafic barrens of California often look like moonscapes, however, different plant species of low biomass, rarely found in other parts of the world, are present there (Richard, 2009). Ultramafic flora exhibits unique adaptations to its extreme micro-environment (Brooks, 1987; Roberts and Proctor, 2012; Teptina et al., 2018; Van der Ent et al., 2015). The rocky, granular textured soils, lack of organic material, low soil water-holding capacity, low Ca/Mg ratio, low nutrient content, and TM toxicity in ultramafic soils create an unfavorable environment for plant growth and development (Brady et al., 2005; Rajakaruna et al., 2003; Seneviratne et al., 2015). Adaptations to ultramafic soils have independently evolved multiple times in terrestrial plants and within finer geographic scales (Brady et al., 2005; Kay et al., 2018; Krämer, 2018). These endemic plants have developed molecular to macro-level adaptations to withstand the unfavorable conditions created by the ultramafic edaphic factors (Reeves et al., 2015). Phytoremediation approaches seek to use the capacity of such plant species to be sequestrate heavy metal(loid)s (Seneviratne et al., 2016; Wójcik et al., 2017). A comprehensive understanding of cellular and biochemical processes is essential to this work. However, a few studies have focused on comprehensive reviews of plant life on ultramafic soils (Richard, 2009).

Understanding TM behavior provides useful information on the mobility, bioavailability and potential toxicity of TMs in the ultramafic environment. A handful of studies have been reported related to the geochemistry (Dissanayake, 1982; Dublet et al., 2017; Dublet et al., 2012; Fan and Gerson, 2011; Noël et al., 2017; Noël et al., 2015; Rajakaruna et al., 2002; Rajapaksha et al., 2012) and plant ecology of ultramafic environments (Hidalgo-Triana et al., 2018; Kay et al., 2018; Teptina et al., 2018; Weerasinghe and Iqbal, 2011). Fewer studies have been conducted to explore important issues such as the mobility, bioavailability, and release rates of TMs; the mechanisms of cellular uptake and conduction of TMs within plants; and the influence of TMs released from ultramafic environments on the health of the ecosystem (Tashakor et al., 2017; Tashakor et al., 2018). Reviews in the context of physicochemical properties of ultramafic soils, TM fractionation in ultramafic soils (i.e. sequential and single extraction techniques) are limited and TM uptake and accumulation mechanisms of ultramafic flora, and ultramafic-associated risks to human and agricultural crops are non-existent (Vithanage et al., 2014). Hence, given that ultramafic substrata constitute a stressful environment for plant growth, the objectives of this review are i) to assess recent findings concerning TM distribution, speciation, and plant availability in ultramafic soil profiles, and ii) to highlight TM uptake, translocation, and accumulation mechanisms in plant species. We also briefly discuss recent insights into the ecosystem health impacts of TMs in ultramafic soils and the importance of characterizing terrestrial ultramafic soil sites as references for martian regolith that future human colonists may need to use as a growth substrate for crop plants. Hence, this becomes a unique review for ultramafic soils, which gathers a variety of data on geological origin, trace metal release and fractionations, environmental risk and uptake, and risk to human health.

2. Geological setting of serpentine bodies

The term ultramafic refers to igneous or metamorphic rocks containing < 45 wt% silica (SiO_2) (Susaya et al., 2010). Serpentinite is a metamorphic rock formed as a result of low temperature (300–600 °C) hydrothermal alteration of ultramafic rocks (Page et al., 1999). The general formula for all members of the serpentine group is $(Mg,Mn,Fe,Co,Ni)_{3-x}SiO_2O_5(OH)_4$ (Bayliss, 1981). Serpentinites contain one or more serpentine group minerals such as lizardite ($Mg_3Si_2O_5(OH)_4$), chrysotile ($Mg_3Si_2O_5(OH)_4$) and antigorite ($(Mg,Fe^{2+})_3Si_2O_5(OH)_4$). More precisely, those serpentine group minerals are formed through the metamorphic transformation of ultramafic rocks such as peridotite and pyroxenite, which consist of iron (Fe) and Mg-rich silicate minerals such as olivine ($(Mg,Fe^{2+})_2[SiO_4]$) and pyroxene ($XY(Si,Al)_2O_6$) from the Earth's mantle. Magnetite ($Fe^{2+}Fe_2{}^{3+}O_4$), Cr-rich magnetite ($Fe^{2+}(Fe^{3+},Cr)_2O_4$) and chromite ($FeCr_2O_4$) can largely be associated with serpentinites. Similarly, mixed-composition minerals including talc ($Mg_3Si_4O_{10}(OH)_2$), chlorite ($(Mg,Fe)_5Al[(OH)_8AlSi_3O_{10}]$), tremolite ($[Ca_2][Mg_5][(OH)_2Si_8O_{22}]$), and brucite ($Mg(OH)_2$) are associated with serpentinites (Morrison et al., 2009; Oze et al., 2004b). Recent synchrotron-based Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy revealed that Ni resided in layered-phyllosilicate and chain-inosilicate minerals and was associated with Fe oxides and primary serpentine minerals such as lizardite (Siebecker et al., 2017).

Soils derived from peridotite and serpentinite are also referred to as serpentine soils due to the fact that similar plant species exist on peridotite soils as on serpentine soils (Oze et al., 2004b). Peridotite and serpentinite possess similar chemical compositions. For example, they both contain a high level of magnesia (> 35 wt% of MgO) and low amount of silica (< 45 wt% of SiO_2). However, serpentinite has relatively higher water content (up to 13%) than peridotite (Coleman, 1971). In addition, the two rock types are characterized by different mineral compositions. Thus, soils derived from these two rock types possess different properties as well as different morphologies (Alexander, 2004). For instance, soils formed on peridotites are typically redder than soils derived from serpentines. Additionally,

Table 1
Total metal concentration with respect to depth in different ultramafic environments in the world.

Location	Depth (cm)	Concentration (mg kg^{-1})									Reference
		Ni	Cr	Mn	Co	Cd	Cu	Pb	Zn	V	
World average in soils	–	2–750	5–1500	1–4000	1–40	0.01–2.0	2–250	2–300	1–900	90–150	(Alfaro et al., 2015; Cappuyns and Slabbinck, 2012; Grygo-Szymanko et al., 2016; Nagajyoti et al., 2010)
Rudnik mountain, Serbia	0–20	550	–	–	–	–	–	–	–	–	(Antić-Mladenović et al., 2011)
Galicia, NW Spain	20	76–373	1499–4309	–	1472–7132	–	–	–	–	21–140	(Arenas-Lago et al., 2016)
Marivan, Iran		1600	–	–	–	–	–	–	–	–	(Ghasemi and Ghaderian, 2009)
Ranau Valley, Malaysia	0–20	6322	4873	43,063	1005	–	239	–	597	–	(Aziz et al., 2015)
Borneo, Sabah, Malaysia	5–20	1080–5450	420–10,980	1427–7330	170–690	–	–	–	–	–	(van der Ent et al., 2017)
Central Greece	0–25	334	234	823	31	–	36	13	66	57	(Kanellopoulos et al., 2015)
Serra de Nogueira, Portugal	10–30	102–2342	200–6822	1007–1853	56–175	–	31–225	19–41	63–242	–	(Freitas et al., 2004)
Niquelandia, Brazil	0–20	4577	5533	6667	–	–	–	–	–	–	(Garnier et al., 2006)
	20–60	3660	7015	1045	–	–	–	–	–	–	
Szklary Massif, SW Poland	4–25	1329	2671	–	119	–	25	40	108	–	(Kierczak et al., 2008)
Tras-os-Montes, NE Portugal	0–15	1574	4384	2451	180	–	150	21	88	–	(Díez Lázaro et al., 2006)
Mt. Prinzera, Italy	0–20	2466	2231	–	106	–	17	10	56	62	(Lombini et al., 1998)
Melide, NW Spain	0–15	6–940	10–1162	–	–	–	2–80	–	–	–	(Miranda et al., 2009)
Barazón, Spain	0–20	937	–	1297	73	–	–	–	–	–	(Álvarez-López et al., 2016)
Nove Dvory, Czech Republic	Bed rock	3054	2682	–	103	–	–	–	–	–	(Quantin et al., 2008)
Mohelno, Czech Republic	Bed rock	2198	3206	–	97	–	–	–	–	–	
Holubov, Czech Republic	Bed rock	2824	1802	–	112	–	–	–	–	–	
Bitincka, Albania	15	1737	513	–	184	4	6	80	52	–	(Shallari et al., 1998)
Gjegjan, Albania	15	1104	574	–	289	4	27	87	49	–	
Guri I Kuq, Albania	15	819	365	–	130	2	44	85	51	–	
Pogradec, Albania	15	2442	635	–	259	5	8	98	63	–	
Prrenjas, Albania	15	3579	3865	–	476	14	36	172	93	–	
Redlschlag, Eastern Australia	–	2580	1910	–	172	4	52	16	61	–	(Wenzel et al., 2003)
Haut Limousin, France	60–80	4142	7466	5886	724	–	–	–	–	–	(Caillaud et al., 2009)
	80–100	5320	6756	4569	750	–	–	–	–	–	
	100–120	4625	8675	1936	248	–	–	–	–	–	
	120–140	4018	5812	465	117	–	–	–	–	–	
	Rock (> 140)	1587	2262	620	86	–	–	–	–	–	
Ussangoda, Sri Lanka	15	6776	10,707	1117	157	–	30	–	173	–	(Vithanage et al., 2014)
Yudhaganawa, Sri Lanka	15	6567	14,880	2609	555	–	14	–	307	–	
Ginigalpelessa, Sri Lanka	15	5945	9948	2543	219	–	15	–	182	–	
Indikolapelessa, Sri Lanka	15	4705	6737	1349	241	–	24	–	108	–	
Niquelandia, Brazil	0–15	3606	5185	–	980	–	–	–	–	–	(Garnier et al., 2009)
	15–30	25,767	8066	–	115	–	–	–	–	–	
	30–70	73,786	5960	–	295	–	–	–	–	–	
	70+	32,216	9400	–	189	–	–	–	–	–	
Shih-Tao, Taiwan	0–15	3222	3192	–	–	–	–	–	–	–	(Cheng et al., 2011)
	15–50	2352	2548	–	–	–	–	–	–	–	
	50–80	3458	3146	–	–	–	–	–	–	–	
	80–110	3991	3214	–	–	–	–	–	–	–	
Wan-Ron Hill, Taiwan	0–10	950	650	735	–	–	–	–	–	–	(Ho et al., 2013a)
Soroako, Indonesia	0–15	7051	17,216	1076	57	–	–	–	–	–	(van der Ent et al., 2013a)
Klamath mountains, USA	0–12	2718	1247	1750	173	–	24	–	72	–	(Alexander, 2014)
Morais Massif, Portugal	0–15	2800	2800	2400	–	–	29	–	73	–	(Alves et al., 2011b)
Samar island, Philippines	0–20	1840	–	–	–	–	–	–	–	–	(Susaya et al., 2010)
California, USA	10–20	4060	11,670	–	–	–	–	–	–	–	(Morrison et al., 2009)
Trazy-Guerioum, Koniambo massif, Kone, New Caledonia	NA	3300	–	–	–	–	–	–	–	–	(Jourand et al., 2010)
Pindjen Water Fall Road, Kone, New Caledonia	NA	3500	–	–	–	–	–	–	–	–	
Mont-Dore, Plum Road, New Caledonia	NA	2800	–	–	–	–	–	–	–	–	
Euboea, Greece	20	1200-8900	540–3800	600–2200	60–460	0.1–1	15–44	3–370	16–900	25–70	(Megremi, 2010)
Hudenisht, Albania	0–20	3180	677	1760	182	–	15.7	–	3,18	–	(Bani et al., 2014)
Velika (Great) Morava River valley, Serbia	0–20	138	–	–	–	–	–	–	–	–	(Rinklebe et al., 2016)

(*continued on next page*)

Table 1 (*continued*)

Location	Depth (cm)	Concentration (mg kg^{-1})									Reference
		Ni	Cr	Mn	Co	Cd	Cu	Pb	Zn	V	
Western Anatolia and the Eastern Thrace, Turkey	0–15	25.7–2680	631	1570	166	–	15.9	47.7	164	29	(Ünver et al., 2013)
Malbog, Philippines	0–20	1840	–	–	–	–	–	–	–	–	(Susaya et al., 2010)
Beni Bouchra, Morocco	0–10	1821	311	–	88	–	–	–	–	–	(Ater et al., 2000)
Andaman Islands	10–15	4316	4437	–	533	–	–	–	–	–	(Pal et al., 2005)

magnetite, which is resistant to weathering, is the main source of Fe in serpentines, whereas, olivine is easily weathered and is the major source of Fe in peridotites (Alexander, 2004). Thus, peridotites produce more (oxy)hydroxides than serpentines, leading to the red color of the peridotite-derived soils. Due to serpentinization, related deformation, and diapiric migration, serpentinites are more fractured than peridotites. As a result, steeper slopes tend to develop on peridotites while serpentinites yield shallower slopes (Alexander and DuShey, 2011).

3. Global distribution of ultramafic soils

Ultramafic rocks, which cover approximately 1% of the surface of the Earth, are commonly found in ophiolite belts along tectonic plate margins (Garnier et al., 2009; Lee et al., 2001). The weathering processes of ultramafic rock and the resulting soils differ between locations due to climate, the nature of the parent material, and other environmental factors such as topography, biota, and time (Garnier et al., 2009). Ultramafic outcrops are well documented on every continent except Antarctica (Table 1) (Morrison et al., 2009; Mun, 1988; Ndjigui et al., 2008; Oze et al., 2004b; Rajapaksha et al., 2012; Wenzel et al., 2003; Westerbergh and Saura, 1992). Fig. 1 shows a typical ultramafic environment found in tropical countries. Ultramafic soils were reported on islands (such as Shetland) over 40 years ago, but have not been recently investigated (Shewry and Peterson, 1975). Ultramafic environments are also present in cold environments such as in Greenland (Pons et al., 2011). The uniqueness of flora inhabiting on ultramafic environments has long been explored (Brooks, 1987; Roberts and Proctor, 2012; Van der Ent et al., 2015). The most extensively studied ultramafic flora is those of tropical and Mediterranean climatic regions. The plant communities in tropical and Mediterranean regions are found to decreased floristic diversity and lower productivity in comparison to those on non-ultramafic environments. Ultramafic environments in tropical and Mediterranean regions are home to unique flora, significantly rich in endemic species and ecotypes (Galey et al., 2017). The number of endemics in Northern Hemisphere is remarkably lower than that of the tropical and Mediterranean regions (Teptina et al., 2018).

Ultramafic outcrops occur in highly populated areas. For instance, a wide distribution of ultramafics has been identified in populous areas within the Circum-Pacific margin and the Mediterranean (Oze et al., 2004b). An ultramafic body in Shih-Tao Mountain in the coastal range of eastern Taiwan, which is 2 km away from Taitung city, contains peridotite, pyroxenites, and serpentinite (Cheng et al., 2011). The ultramafic soil in the Eastern part of the Rudnik Mountain, near Salasi Village, Serbia, is extensively used for crop cultivation (Antić-

Fig. 1. An ultramafic environment at Indikolapelessa, Sri Lanka.

Mladenović et al., 2011). Extensive ultramafic coverage is found in Atalanti, central Greece, where ultramafics cover 64 km^2 (Kanellopoulos et al., 2015). A few ultramafic localities are also found in the villages of Malbog, Napataan and Palanas in Eastern Samar, Philippines (Susaya et al., 2010). Ultramafic outcrops located in and around populated areas may increase the risks of TM accumulation in agricultural soils and vegetation and the groundwater contamination (discussed in detail in Section 5.7).

4. Physicochemical characteristics of serpentine soils

Serpentine soils are red, green, blue, or black, depending on their chemical compositions (Kazakou et al., 2008; Kierczak et al., 2016). The variations in the mineralogy of serpentine outcrops have been investigated in detail (Caillaud et al., 2009; Kelepertzis et al., 2013; Kierczak et al., 2007). The characteristic chemical properties of serpentine soils are as follows:

(1) Low Ca: magnesium (Mg) ratio
Plant growth is significantly affected by the proportions of exchangeable cations in soil. The high abundance of Mg released from ultramafic weathering makes it the dominant cation present on soil exchange sites, resulting in the characteristic low exchangeable Ca: Mg ratios of serpentine soils (Burt et al., 2003). Serpentine soils are not necessarily Ca-limited where the low Ca:Mg ratios are commonly the result of an overabundance of Mg (Oze et al., 2008). Serpentine soils, thus, impose strong limitations on the fertility of plants. In temperate climates, Mg is only partially leached during soil formation due to incomplete hydrolysis. In comparison, in tropical climates, complete hydrolysis occurs and, consequently, almost all available Mg is leached (Kierczak et al., 2007).

(2) Deficiencies in essential plant nutrients
Low levels of plant-available macronutrients are a prevailing issue in serpentine soils. Low organic matter in the soils inevitably leads to low macronutrient concentrations. Additionally, the lack of P and K in the parent materials results in low concentrations of P and K in serpentine soils (Burt et al., 2001). For this reason, only a few plant species can survive in serpentine substrata. However, the identity of the limiting nutrient varies from one serpentine soil location to another. For example, serpentine soils in California, USA, have low N concentrations, whereas European serpentine soils contain low levels of P (Chiarucci et al., 1999).

(3) High levels of toxic TMs
Serpentinite parent materials and the resulting soils contain high levels of toxic metals such as Cr, Ni, Co, Mn, Zn, and V. Therefore, serpentinites act as a non-anthropogenic source of toxic elements by releasing them into the environment during natural weathering processes. The toxicity of serpentine soils is termed "serpentine syndrome" and leads to endemism and low primary productivity (Oze et al., 2008).

4.1. Total TM content

Ultramafic environments display a high geochemical background of TMs due to the weathering of minerals (Amir and Pineau, 2003; Quantin et al., 2002). The distribution of TMs along with the soil profile primarily depends on the weathering susceptibility of their initial host minerals, although climatic conditions (i.e., humidity, rainfall, snowfall, and temperature) influence the soil forming processes (Caillaud et al., 2009). Geochemical studies have shown that TM concentrations in ultramafic soils are remarkably higher than that of non-ultramafic soils, summarized in Table 1.

Studies related to TMs distribution at the mineral scale demonstrate how TMs may be released into the environment. Ultramafic soil profiles in the Czech Republic have been studied using a combination of geochemical and mineralogical techniques and results show that Cr is mainly mobilized from pyroxenes, amphiboles, and Cr-spinels in bedrock, whereas, Ni is released from olivines and small Fe-Ni sulfide inclusions (Quantin et al., 2008). The distribution of TMs along the ultramafic soil profile in South Limoges in France has shown that silicates (i.e. serpentine and clinochlore) retain Cr, Ni, and Mn while oxides retain Co and Cr (Caillaud et al., 2009). During the initial weathering stage, Ni and Cr can be concentrated in secondary clays such as Fe-montmorillonite and trioctahedral vermiculite whereas Mn and Co are mainly retained in secondary oxides. At the top of the ultramafic profile, Ni, Mn, and Co have been found to retain in secondary oxy-hydroxides, possibly due to the Fe segregation. An assessment of the ultramafic massif in south west Poland has revealed that Ni content in Cr-magnetite, forsterite, iddingsite and serpentine are up to 0.96, 0.55, 0.99 and 0.55 wt% of NiO, respectively. Additionally, the levels of Cr in Cr-magnetites and clinochlores were up to 21.77 and 1.68 wt% of Cr_2O_3, respectively (Kierczak et al., 2007).

Chemical and mineralogical investigations have further demonstrated that Cr and Ni in Shih-Tao Mountain in Taiwan are dominantly concentrated in chromites and silicates, respectively (Cheng et al., 2011). Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) analysis has revealed that chemical modification of the chromites was more common near the soil surface and the available Cr concentration from the shoulder to the footslope also increased (Cheng et al., 2011). Kelepertzis et al. (2013) investigated two weathering profiles of Thiva Valley in central Greece and found that Ni is primarily associated with serpentine (0.5 wt% of NiO) and secondary weathering products such as smectites and goethite (0.5 and 1.1 wt% of NiO, respectively). In contrast, Cr was predominantly bound to chromite (54.7 wt% of Cr_2O_3) and a lesser extent to Cr-magnetite and enstatite (12.2 and 0.8 wt% of Cr_2O_3) (Kelepertzis et al., 2013). Geochemical and mineralogical analyses were also performed in the agricultural region of Mouriki-Thiva in central Greece (Antibachi et al., 2012). The geology of that area is characterized by ultrabasic rocks and the total concentrations of Ni and Cr ranged from 621 to 2639 and 134 to 856 mg kg^{-1}, respectively. In addition, Cr was mainly incorporated into chromite, whereas Ni was mainly associate with in olivine and serpentine (Antibachi et al., 2012).

Regional scale geochemical studies demonstrate that geochemistry and mineralogy of ultramafics are strongly influenced by the transport of weathered parent materials. Weathering processes lead to the concentration of TMs in the secondary phases (Caillaud et al., 2009). However, their distribution does not show a consistent pattern at most of the investigated ultramafic weathering profiles (Shallari et al., 1998). This could be attributed to variations in weathering rates of different TM-bearing minerals as well as variations in the TM mobility in the soil profile (Table 1).

Total TM concentrations are useful as references for assessing contamination levels and the associated environmental risks (Kierczak et al., 2008). Nickel concentrations have been found to range from 1510 to 5970 mg kg^{-1} in ultramafic soils in Oregon and Maryland, whereas, Cr, Mn, and Co concentrations were 580–2910, 1380–3360 and 87–311 mg kg^{-1}, respectively (Siebecker et al., 2017). However, total TM concentrations do not provide sufficient information regarding the mobility and availability of TMs. Assessing the chemical fractionation of TMs in ultramafic soils is therefore important to determine the mobility and bioavailability of TMs (Díez Lázaro et al., 2006; Garnier et al., 2006).

4.2. TM fractionation

The distribution of TMs within their various geochemical binding forms, which is referred as TM fractionations, is an important factor to determine the mobility and bioavailability of TMs in the environment (Kierczak et al., 2008). Sequential extraction is a commonly used method to evaluate the speciation of TMs in ultramafic environments (Amir et al., 2005; Dère et al., 2007). The extraction efficiency of TMs bound to different fractions is found to depend on the strength of the extractant, a number of steps involved, and the geochemical properties of the ultramafic soil.

Tessier et al. (1979) defined five metal bound fractions (i.e. (I) exchangeable, (II) carbonate, (III) Fe and Mn oxides, (IV) organic and (V) residual) in his sequential extraction method. Using the method described by Tessier et al. (1979), Antić-Mladenović et al. (2011) determined that Ni in Rudnik Mountain ultramafic soils was mainly remained in the residual fraction (91.3%). The remaining Ni was retained in different fractions in the following order: Fe and Mn oxides bound > organic matter bound > carbonate bound > exchangeable (Antić-Mladenović et al., 2011). Similarly, metal bound fractions in four different locations in Sri Lanka (Ussangoda, Yudhaganawa, Indikolapelessa and Ginigalpelessa) were evaluated following the method described by Tessier et al. (1979) and the portion of Mn, Ni, and Cr retained in different fractions from greatest to least were as follows: (1) Mn: Fe and Mn oxides bound > residual > organic matter bound > exchangeable > carbonate-bound, (2) Ni: residual > Fe and Mn oxides bound > organic matter bound > exchangeable > carbonate-bound, and (3) Cr: residual > organic matter bound > Fe and Mn oxides bound > exchangeable > carbonate bound (Vithanage et al., 2014).

A seven-step sequential extraction method (i.e. (I) water soluble, (II) exchangeable, (III) bound to Mn oxides, (IV) bound to amorphous or poorly crystallized Fe oxides, (V) bound to well crystallized Fe oxides, (VI) oxidizable fraction and (VII) residual) has been used to evaluate the solid-phase fractionation of Cr in ultramafic soil localities in Niquelandia, Brazil (Garnier et al., 2006). The results showed that Cr was mainly bound to well crystallized Fe oxides (50 to 85 wt% of Cr). The same sequential extraction procedure was used to investigate Cr fractionation in paddy soils derived from serpentine in Wan-Ron Hill in Eastern Taiwan (Hseu and Iizuka, 2013). The results showed that Cr is primarily associated with recalcitrant minerals in the serpentine derived paddy soil matrix. Similarly, ultramafic soils in Galicia (NW Spain) contain high portions of Co, Cr, Ni, and V in the residual fraction (Arenas-Lago et al., 2016). Rinklebe et al. (2016) also found that Ni is mainly retained in the residual fraction in ultramafic soils in the Morava River Valley, Serbia.

A six-step sequential extraction method (i.e. (I) exchangeable, (II) acid-soluble, (III) easily reducible, (IV) moderately reducible, (V) sulfide/organic and (VI) residual) has been used to evaluate the TM fractionation in an ultramafic massif in Szklary, SW Poland. The Cr in ultramafic soils in Szklary is found to be mainly retained in the residual fraction (> 76%), and mineralogical analysis further confirmed that Cr is incorporated into highly resistant spinels (Cr-magnetite) (Kierczak et al., 2008). In addition, Co was largely retained in sulfide/organic and residual fractions. Díez Lázaro et al. (2006) also followed a six-step sequential extraction method (i.e. (I) exchangeable, (II) Mn oxide bound, (III) organic matter bound, (IV) amorphous Fe oxide bound, (V) crystalline Fe oxide bound, and (VI) residual and demonstrated that Cr in ultramafic soils is largely retained in the residual fraction, whereas, Ni, Co, and Cu are predominantly bound to Fe oxides, Mn oxides, and organic matter fractions, respectively. Moreover, a four-step sequential extraction method (i.e. (I) reducible, (II) oxidizable, (III) acid-extractable and (IV) residual) has been used by Hseu (2006) and the results have demonstrated that Cr and Ni are predominantly associated with the residual fraction.

In summary, direct comparisons of the results of various sequential extraction studies are hampered due to different extraction methods using various extractants and involving an assorted number of extraction steps. In addition, the limited selectivity of extractants and the redistribution of metals among newly formed phases may confounded sequential extraction results. For instance, an exchangeable fraction of a particular TM may is easily leached by a natural salt. The carbonate-bound fraction is sensitive to changes in pH of the environment, as it is soluble under acidic conditions (Tokalioglu et al., 2000). Metals bound to the Fe-Mn oxides fraction can mobilized due to the changes of redox conditions in the environment (Hursthouse et al., 2003). Natural organic matter (i.e. humic and fulvic acids) has a high capacity to complex with metal ions (Tessier et al., 1979). The residual fraction can retain metals in recalcitrant crystalline structures. Strong oxidizing acids such as hydrofluoric, nitric and perchloric acids are required to decompose the residual fraction. Therefore, the metals associated with the residual fraction can only be mobilized due to the process of natural weathering, which is a slow and long-term process (Tessier et al., 1979). Climatic conditions also affect the weathering patterns of minerals (i.e., Fe/Mn oxides precipitate in humid tropical climates but are formed in cold and temperate climates) (Antić-Mladenović et al., 2011). To obtain a more precise and accurate data about TM fractionation in ultramafic soils, a combined method of sequential extraction and non-destructive analytical methods (i.e. XRD, SEM-EDX, and EMPA) is therefore suggested.

5. Risks to plants in ultramafic soils from TM mobilization

5.1. Bioavailability of metals

For a comprehensive ecotoxicological risk assessment, it is essential to determine the bioavailability of TMs in ultramafic soils (Díez Lázaro et al., 2006). As reported from the various definitions of bioavailability existing in the literature, the term "bioavailability" used in this manuscript refers to the availability of TMs in the soil-water systems for the uptake by plant species. Bioavailability of metals in soil systems is dependent on a multitude of factors such as climatic conditions (i.e. temperature and water regime), the origin of the metal, distribution among the soil constituents, edaphic properties (i.e. pH, redox potential, distribution coefficients of TMs (K_d), texture, cationic exchange capacity, carbonate, organic matter, amount of oxides (i.e. Mn and Fe oxides) (Antić-Mladenović et al., 2017; Kumarathilaka et al., 2018a; Shaheen et al., 2013), microbial activity of the rhizosphere microflora, and the chemical composition of root exudates (Alves et al. 2011c; Antoniadis et al., 2017; Echevarria et al., 2006; Kumarathilaka et al., 2018c; Oze et al., 2004a). At present, no single method can fully determine TM bioavailability in ultramafic soil systems, and thus a variety of chemical extraction techniques are used to assess TM bioavailability

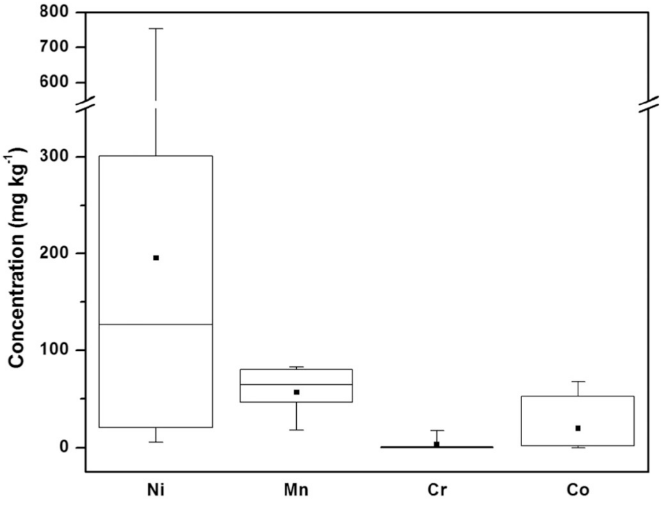

Fig. 2. Box and whisker plot for bioavailability of TMs in selected ultramafic locations. Data from Álvarez-López et al. (2016), Ater et al. (2000), Aziz et al. (2015), Díez Lázaro et al. (2006), Ho et al. (2013b), Jourand et al. (2010), Pal et al. (2005), Ünver et al. (2013), van der Ent et al. (2013a), van der Ent et al. (2017), Vithanage et al. (2014), and Wenzel et al. (2003). Different extractants have been used to obtain these levels of TMs. The black dots in the figure show the mean of the data set.

in ultramafic soil systems (Quantin et al., 2008).

Fig. 2 shows bioavailable concentrations of TMs in various ultramafic locations around the world. In comparison, the bioavailability of Ni is high (6–754 mg kg^{-1}) followed by Mn (18–83 mg kg^{-1}) and Co (1–68 mg kg^{-1}). Cr shows the least bioavailability (0.02–17.80 mg kg^{-1}). Different extractants have been used to evaluate the bioavailability of TMs in various ultramafic locations. In one study, diethylene triamine pentaacetic acid (DTPA) has extracted 13–90 mg kg^{-1} of Ni (Quantin et al., 2008). In contrast, Cr was not found to be bioavailable, suggesting that Cr is tightly bound to well-crystallized Fe-oxides and primary rock-derived phases. Similarly, DTPA and ammonium acetate (NH_4OAc)-extractable Cr concentrations have been examined in the ultramafic toposequence in eastern Taiwan, and extractable Cr concentrations were found to be below 5 mg kg^{-1} (Hseu, 2006). The bioavailability of Ni, Mn, and Cr in Sri Lankan ultramafic soils have also been assessed and neither calcium chloride ($CaCl_2$) nor DTPA extractants were found to release Cr, which was consistently < 0.5 mg kg^{-1}, suggesting that Cr in ultramafic soils may tightly bound to the mineral phases (Vithanage et al., 2014). High amounts of Ni and Mn can be released by $CaCl_2$ or DTPA extractants although the concentration of TMs (i.e. Ni, Mn) extractable by $CaCl_2$ was approximately 50% lower than that extractable by DTPA. Use of ethylene diamine tetraacetic acid (EDTA) as an extractant has shown the highest concentrations of bioavailable metals in the most acidic locations (Díez Lázaro et al., 2006). The majority of the Ni and Co has been released from a combination of the exchangeable and Mn oxide bound fractions. Six different single extraction procedures (i.e. distilled water, 0.11 M acetic acid, 1 M NH_4OAc; pH 7.0, 0.01 M $CaCl_2$, DTPA and 0.1 M hydrochloric acid (HCl)) for Co extractability of ultramafic soils in eastern Taiwan have shown that water extractable Co concentrations ranged between 0.15 and 0.93 mg kg^{-1}, whereas, HCl-extracted Co concentration is 22.1 mg kg^{-1} (Hsiao et al., 2009).

The mobility of Ni, Cr, and Co, using the EDTA extraction method has been investigated at six ultramafic sites in Poland (Kierczak et al., 2016). The results have shown that Ni possessed the highest EDTA extractable fraction, whereas Cr has the lowest mobility in all studied soils. The lowest concentrations of EDTA-extractable Ni and Co (up to 7 and 4% of total concentrations, respectively) have been found in serpentinites devoid of primary minerals (i.e. olivine and pyroxene) that had non-pseudomorphic textures. In contrast, the highest concentrations of EDTA-extractable Ni and Co (up to 18 and 16% of total concentration, respectively) have been observed in partially serpentinized peridotite (i.e. hornblende peridotite with a pseudomorphic texture), suggesting that the type and origin of the ultrabasic parent rock governs the mobility of Ni and Co (Kierczak et al., 2016). Amir and Pineau (2003) analyzed the relationship between the activity of microorganisms and extractable concentrations of TM in ultramafic soils in New Caledonia and found that extractable concentrations of Ni and Co in New Caledonian ultramafic soils are directly proportional to the microbial activity.

To date, most studies have focused on Cr and Ni mobility in ultramafic-related systems. Soil concentrations of Ni are generally reported to be between 2 and 750 mg kg^{-1}; however, they occasionally exceed 10,000 mg kg^{-1} (Alves et al. 2011c). Typically, Ni substitutes for Mg^{2+} in olivine and pyroxenes (Becquer et al., 2006). Consequently, during ultramafic soil development, Ni released by weathering of primary minerals is in a position to substitute for Mg in clay minerals (i.e. smectites and vermiculite) (Lee et al., 2004). Ni is adsorbed on Fe and Mn oxide surfaces at pH ≥ 5, although it is retained only weakly on clay and Fe and Mn oxide surfaces (Lee et al., 2004). Under near-surface conditions, Ni is also released from ultramafic silicates. As a result, Ni is readily adsorbed by exchangeable sites in soil systems as hydroxyl-oxide precipitates. Thus, Ni is more extractable than Cr in ultramafic soils (Lee et al., 2001; Oze et al., 2004b).

Average concentrations of Cr in soils from around the world are about 5–1500 mg kg^{-1} (Hseu and Iizuka, 2013). In ultramafic rocks, oxides (i.e. chromite and magnetite) and to a lesser extent silicate phase (i.e. serpentine and pyroxene) are the main Cr-bearing minerals (Oze et al., 2004b; Quantin et al., 2008). During serpentinization, magnetite and serpentine minerals are incorporated different amounts of Cr into their structures (Oze et al., 2004b). Chromite undergoes incongruent dissolution during metamorphism, and, consequently, forms ferrichromite and magnetite rims (Garnier et al., 2008; Oze et al., 2004b). In general, the availability of Cr is found to be relatively low by various chemical extractions, since Cr-bearing minerals tend to be nearly insoluble as well as slowly weathered (Garnier et al., 2008). Garnier et al. (2008) demonstrated that the total amounts of potentially labile Cr increased from the shoulder to the footslope, suggesting that increased availability of Cr is due to the weathering of chromite (Garnier et al., 2008). Cr released from chromite remains in the soil system in both Cr (III) and Cr(VI) states (Cheng et al., 2011). High-valent Mn oxides may stimulate the oxidation of Cr(III) to Cr(VI) in serpentine soil solutions (Oze et al., 2004b).

From previous studies, it is clear that weathering of ultramafic minerals leads to the mobilization of TMs, particularly Cr into the surface and groundwater. Cr(VI) is of particular concern because of its high mobility and toxicity (Gil et al., 2006). The speciation of Cr in the environment is primarily controlled by redox potential (E_H) and pH. In general, Cr(VI) is the dominant form under oxidizing conditions, while Cr(III) predominates under more reducing conditions. Despite being highly resistant to weathering, it appears that chromite and more soluble Cr-containing silicates are possible sources of mobilized Cr in ultramafic soils, where Cr oxidation is induced by the presence of Fe, Mn, and dissolved organic carbon under specific environmental conditions (Rajapaksha et al., 2013). A study reported that rapid dissolution of chromite and subsequent oxidation of Cr(III) to Cr(VI) might occur in the presence of birnessite (Oze et al., 2007). The sources of Cr in groundwater in Leon Guanajuato Valley, Central Mexico were also examined by Robles-Camacho and Armienta (2000) who found that Cr is released into the groundwater from the Guanajuato ultramafic outcrops. Similarly, groundwater contamination with Cr(VI) has been assessed in La Spezia province, Italy by Fantoni et al. (2002). The Cr(VI) concentrations in groundwater samples in La Spezia province, Italy, ranged from 5 to 73 μg L^{-1}, exceeding the permissible level for drinking water (5 μg L^{-1}). The authors suggested that Cr-rich ultramafics are the major source of the Cr contamination in the groundwater.

TM oxidation and mobilization may be influenced by the presence of hydrogen peroxide (H_2O_2) produced by serpentinization, biological weathering, and photochemical processes in a wide variety of terrestrial environments (Cooper and Zika, 1983; Cooper et al., 1987; Willey et al., 1996; Oze et al., 2016). For example, Cr(III) from chromite ($FeCr_2O_4$) has been shown to be rapidly oxidized with low concentrations of H_2O_2, especially at neutral to basic conditions (Oze et al., 2016). As H_2O_2 can be presented to serpentine soils by a variety of means, even prior to pedogenesis, this is a relatively unknown factor related to TM fractionation and release into the soil solution.

The mobilization of TMs from ultramafics may increase the risk of TMs accumulation in animals and humans. However, an understanding of the fate and transport mechanisms of TMs in groundwater systems remains limited. It is clear that the availability of TMs in ultramafic soils is predominantly influenced by the identity of the TM bearing phases, which are in turn affected by the climatic conditions in the region as well as the weathering history of the soil. For instance, Ni availability is much higher when Ni is bound to phyllosilicates than when it is bound to well-crystallized Fe oxides (Massoura et al., 2006). Similarly, soils with large amounts of amorphous Fe oxides and small amounts of goethite possess a high Ni availability (Massoura et al., 2006). Accordingly, moderately weathered ultramafic soils have higher Ni availabilities than very intensively weathered goethite-rich soils. While these conditions are important, they are sometimes insufficient to explain the availability of TMs in ultramafic environments, since other factors also affect TM release.

5.2. Uptake of ultramafic TMs into plants

Many researchers have assessed TMs in plants growing in ultramafic environments with the goal of identifying hyperaccumulators. Nickel has been particularly studied in plants to assess the potential for phytomining via hyperaccumulation (Chaney, 1983; Reeves, 2003; Reeves et al., 2007; van der Ent et al., 2013a). In Brazil alone, hyperaccumulation ($> 1000\,\mathrm{mg\,kg^{-1}}$ in dry plant matter) of Ni has been found in > 30 different species (Reeves et al., 2007). In the Mediterranean and California, a clear distinction between the accumulator and non-accumulator plants was observed; however, in Brazil, the distribution is more continuous, and median Ni concentrations are much higher (Reeves et al., 2007). Globally, around 400 Ni hyperaccumulators have been identified to date (van der Ent et al., 2013b) although hyperaccumulation is generally considered a relatively rare phenomenon, as only 0.5–1% of native ultramafic plant species exhibit Ni hyperaccumulation (van der Ent et al., 2013b). Nickel hyperaccumulators have been listed by country, where Cuba has 140, Southern Europe and Minor Asia have 80–90, Brazil has about 40, and New Caledonia has 65 Ni hyperaccumulators (Jaffré et al., 2013; Reeves et al., 2007; Reeves et al., 1996). However, due to a lack of research efforts, very few Ni hyperaccumulators have been recorded in Malaysia, Indonesia, and Sri Lanka, despite these countries being biodiversity hot spots. Additionally, ecological studies have revealed that the number of hyperaccumulator species in serpentinite environments is less in comparison with the non-ultramafic environments (Baker and Brooks, 1989; Roberts and Proctor, 2012; Teptina et al., 2018; Teptina and Paukov, 2015). However, recent research investigating ultramafic occurrences in South and South East Asia has documented many different plant species with unusually high concentrations of TMs in their foliage (Galey et al., 2017).

5.3. Translocation factor

Iron, Cu, Mn, Ni, and Zn are essential to plant health and growth. However, Cr and Co are non-essential elements and are toxic to plant species even at low concentrations. They can also be transported to humans via the food chain (Aziz et al., 2015). The translocation factor (TF) describes the translocation of metals from one part to another within a particular plant species. For instance,

$$TF = \frac{Concentration\,(shoot)}{Concentration\,(root)}$$

If TF > 1, translocation of metals from root to shoot in a particular plant species takes place in an efficient manner (Fayiga and Ma, 2006). Table 2 summarizes TM levels in roots, stem, and leaves of different plant species in and around ultramafic localities. Most of the plant species successfully stimulate translocation of TMs including Cr, Cu, Mn, Ni, Zn and Co, indicating that high levels of metals are found in plant shoots and leaves compared to the roots (Table 2). In contrast, certain plant species such as *Oryza sativa*, *Cerastium arvence*, and *Biscutella laevigata* lack the capability for translocation of TMs and consequently, less TMs are accumulated in the shoots and leaves of those plants compared to their roots (Table 2).

The accumulation of metals in the different plant tissues (i.e. leaf, stem, and root) are significantly affected by the identity of the metal and the plant species under consideration (Díez Lázaro et al., 2006). Plant species such as *Cytisus multiflorus*, *Daphne gnidium*, and *Genista hystrix* has been reported to contain the lower metal concentrations in their roots, stems and leaves, while species including *Cistus ladanifer*, *Lavandula stoechas*, *Phlox subulata*, and *Thymus mastichina* have shown high metal concentrations distributed throughout the plant (Díez Lázaro et al., 2006). There are several studies on the mechanisms of metal translocation and the role of different types of ligands in metal transportation in plants. The xylem sap of a Ni hyperaccumulator (*Alyssum serpyllifolium* ssp. *lusitanicum*) endemic to the ultramafic soil in northeast Portugal was studied to determine Ni translocation mechanisms (Alves et al., 2011a). The results demonstrated that 70% of Ni was transported as a free, hydrated cation in the xylem, whereas, 18% of Ni was transported by complexation of Ni with carboxylic acids, mainly citric acid. Altogether, oxalic acid, malic acid, malonic acid, and aspartic acid complexed < 13% of total Ni. Furthermore, < 1% of Ni complexes are transported via amino acids (i.e. glutamic acid and glutamine).

Interestingly, a recent study done by van der Ent et al. (2017) showed that Ni was concentrated in the phloem in roots and stems. A maximum of 16.9% Ni in the phloem sap of *Phyllanthus balgooyi* was reported; however, in some species (i.e. *Phyllanthus securinegioides* and *Rinorea bengalensis*), the maximum concentrations were reported in the epidermis and in the spongy mesophyll (van der Ent et al., 2017). The above-mentioned study showed that shoot translocation of TMs is a major feature of ultramafic vegetation. In hyperaccumulating plants in the *Euphorbiaceae* family, higher levels of Ni have been found to accumulate in the epidermis of leaves and the laticifer tubes of the stems (Berazaín et al., 2007). A study by Robinson et al. (2003) has confirmed that a Ni hyperaccumulating plant, *Berkheya coddii*, has significantly higher Ni levels in the upper epidermis in leaves than the rest of the leaf. Similarly, *Streptanthus polygaloides* retain high levels of Ni in the leaf epidermis (Sánchez-Mata et al., 2014). Despite these recent advances, further research is needed to understand the role of metal chelation in long-distance transport, the role transporters play in crossing the plasma membrane, and the involvement of organic acids in TM transport through the xylem.

5.4. The bioaccumulation factor

Metal uptake by plant species is dependent on the availability of metals, which in turn depends on the supply of metals from less plant-available forms in the soil system (Lu et al., 2016; Moon et al., 2016). Plant uptake mechanisms for TMs including As, Cd, and Ni at the molecular level have also been investigated in detail. The bioaccumulation factor (BAF) is a measure of the transfer of metals from one trophic level to another. For instance,

$$BAF = \frac{Concentration\,(shoot)}{Concentration\,(soil)}$$

Based on their BAF values, plant species are divided into hyperaccumulators ($BAF > 1\,\mathrm{mg\,kg^{-1}}$) and excluders ($BAF < 1\,\mathrm{mg\,kg^{-1}}$) (Baker, 1981). Metal hyperaccumulator plants can accumulate TMs in their shoots at levels (in $\mathrm{mg\,kg^{-1}}$) of > 10,000 (Mn and Zn), > 1000 (Cu, Co, Cr, Ni and Pb) and > 50 (Cd) (Wenzel and Jockwer, 1999; Wenzel et al., 2003). For instance, the Ni concentration in plants growing in non-ultramafic soils ranges from 0.1 to $5\,\mathrm{mg\,kg^{-1}}$ dry weight, while plants growing on ultramafic soils may contain up to $100\,\mathrm{mg\,kg^{-1}}$ dry weight of Ni. In addition, Ni hyperaccumulator plant species can contain over $1000\,\mathrm{mg\,kg^{-1}}$ in dry leaf tissue (Brooks et al., 1977; van der Ent et al., 2013b). The study conducted by Shallari et al. (1998) in eight ultramafic and industrial sites of Albania found $9\,\mathrm{mg\,kg^{-1}}$ of maximum Cd concentration in stems and leaves of *Dittrichia graveolens* and BAF was approximately one. In comparison, BAF of the *Herniaria hirsute* reached $2\,\mathrm{mg\,kg^{-1}}$ for Cd. Co concentrations in plant tissues ranged from 1 to $86\,\mathrm{mg\,kg^{-1}}$, and Cr concentrations were between 3 and $275\,\mathrm{mg\,kg^{-1}}$. A wide range of Ni concentration was detected in the investigated plant species (a minimum of $4\,\mathrm{mg\,kg^{-1}}$ for *Epilobium dodonaei* and a maximum of $12{,}625\,\mathrm{mg\,kg^{-1}}$ for *Alyssum markgrafii*). None of the plant samples investigated was found to be a metal hyperaccumulator, as the BAF of all the investigated samples was < 1 (0.02, 0.10, 0.01 and 0.03 for Mn, Zn, Co, and Cu, respectively) (Aziz et al., 2015). All plant species investigated in Salcedo watershed in eastern Samar Island, Philippines contained low Ni concentrations with the mean of $14.7\,\mathrm{mg\,kg^{-1}}$ (Susaya et al., 2010). The authors, therefore, suggested that food plants (i.e. Gabi, watermelon,

Table 2
Metal concentrations in the different plant parts of the various study species.

Plant species	Plant part	Concentration (mg kg^{-1})						Reference
		Cr	Cu	Mn	Ni	Zn	Co	
Alyssum serpyllifolium	Root	3.3–19	2.5–9	10–80	90–2340	15–70	–	(Díez Lázaro et al., 2006)
	Stem	2.5–34	2.5–12	20–85	150–4500	13–140	–	
	Leaves	5–27	3–18	50–180	670–31,200	10–255	–	
Cistus ladanifer	Root	1.4–17	2.5–15	25–350	1.6–75	5.5–140	–	
	Stem	2.5–26	3–11	30–467	2–50	15.5–500	–	
	Leaves	1.8–128	2.5–15	20–2000	3–50	30–300	–	
Lavandula stoechas	Root	1.8–20	3.5–16	12.5–340	0.8–80	8–44.5	–	
	Stem	1.2–17	4.5–11.2	15.5–325	2.5–40	15–165	–	
	Leaves	1.6–51	3–27.5	40–2200	2.5–95	30–170	–	
Santolina semidentata	Root	3–26	6–15.5	20–70	25–85	10–20	–	
	Stem	4.5–5.5	8.5–10.5	15–30	14.5–20	15–25	–	
	Leaves	4–21	21.5–33	55–135	39.5–55	40–70	–	
Thymus mastichina	Root	2.5–30	5.5–25	14.5–235	2.5–100	4–240	–	
	Stem	2.5–19	4.5–10.5	14.5–145	2.5–35	8–65	–	
	Leaves	2.4–55.5	5.5–19	21.5–485	4–180	17.5–145	–	
Oryza sativa L.	Root	585	12	477	430	18	48	(Aziz et al., 2015)
	Shoot	15	0.75	602	8	37	3	
Silene armeria	Root	55	69	–	51	189	57	(Lombini et al., 1998)
	Shoot	14	9	–	21	66	11	
Cerastium arvence	Root	77	69	–	83	172	16	
	Shoot	29	9	–	45	40	5	
Minuartia laricifolia	Root	24	13	–	124	87	8	
	Shoot	32	9	–	54	68	11	
Dianthus gr. sylvestris	Root	130	8	–	144	65	12	
	Shoot	175	6	–	148	52	13	
Biscutella gr. laevigata	Root	65	8	–	91	102	11	
	Shoot	15	6	–	27	61	11	
Alyssum bertolonii	Root	26	4	–	91	49	11	
	Shoot	52	8	–	27	108	24	
Herniaria hirsuta	Whole plant	275	22	–	808	29	63	(Shallari et al., 1998)
Inula germanica	Whole plant	89	20	–	211	24	31	
Filago eriocephala	Whole plant	35	14	–	460	46	14	
Alyssum murale	Whole plant	13	7	–	5057	49	24	
Alyssum markgrafii	Whole plant	19	56	–	12,625	291	23	
Verbascum blattaria	Whole plant	23	23	–	60	26	8	
Consolida orientalis	Whole plant	12	13	–	49	54	3	
Lotus ornithopodioides	Whole plant	63	14	–	232	75	21	
Teucrium polium	Whole plant	50	9	–	167	12	19	
Colocasia esculenta	Leaves	–	–	–	14.2	–	–	(Susaya et al., 2010)
Phyllanthus amarus	Leaves	–	–	–	53.7	–	–	
Artocarpus heterophylla	Leaves	–	–	–	19.2	–	–	
Moringa oleifera	Leaves	–	–	–	10.5	–	–	
Lycopersian esculentum	Leaves	–	–	–	11.1	–	–	
Solanum melongena	Leaves	–	–	–	6.22	–	–	
Alyssum chalcidicum	Leaves	–	–	–	2910	–	–	(Kanellopoulos et al., 2015)
Allium cepa	Bulb	0.7	6	22	2.9	39	2.3	(Economou-Eliopoulos et al., 2011)
Allium cepa	Shoot	1.8	6	60	2	24	3.8	
Allium sativum	Bulb	0.7	8	15	3.1	46	6.1	
Allium sativum	Shoot	1.4	4	34	1.8	28	2	
Allium porrum	Root	8	8	23	11	19	1.9	
Allium porrum	Shoot	0.7	5	16	1.6	17	2	
Beta vulgaris	Root	0.6	8	23	0.5	12	2.7	
Beta vulgaris	Shoot	0.8	7	115	1.3	11	2.4	
Triticum spp.	Root	100	14	140	150	12	20	
Triticum spp.	Shoot	1.7	2.5	25	1.2	12	16	
Triticum spp.	Seeds	1.3	4.6	30	3	26	6.5	
Solanum nigrum	Leaves	120	–	94	140	42	–	(Megremi, 2010)
Cichorium intybus	Leaves	48	–	89	88	78	–	
Sonchus oleraceus	Leaves	54	–	100	93	37	–	
Allium sativum	Leaves	6	–	32	17	15	–	
Sinapis arvensis	Leaves	79	–	82	72	92	–	
Daucus carota	Leaves	3.5	–	52	17	9	–	
Verbascum spp.	Leaves	82	–	73	210	86	–	
Alyssum murale	Leaves	–	–	30	20,100	310	90	(Bani et al., 2014)

(*continued on next page*)

Table 2 (*continued*)

Plant species	Plant part	Concentration (mg kg^{-1})						Reference
		Cr	Cu	Mn	Ni	Zn	Co	
Crotalaria micans	Root	13	–	33.2	19.5	–	–	(Ho et al., 2013a)
	Shoot	6.08		10.4	48.3			
Miscanthus floridulus	Root	28.2	–	53	45	–	–	
	Shoot	8.99		11.6	11.1			
Leucaena leucocephala	Root	20.4	–	28.9	15.7	–	–	
	Shoot	7.03		15.2	22.9			
Bidens pilosa	Root	36.9	–	69.4	32.7	–	–	
	Shoot	12.5		27.4	45.9			
Pueraria lobata	Root	18.7	–	46	30.1	–	–	
	Shoot	12.1		17.3	51.4			
Melilotus indicus	Root	15.8	–	30.2	22.4	–	–	
	Shoot	7.18		21.8	17.2			
Conyza canadensis	Root	13	–	44.6	24.7	–	–	
	Shoot	13.5		20.6	42.7			
Phyllanthus balgooyi	Root	–	–	120	4780	120	7	(van der Ent et al., 2017)
	Bark	–	–	37	310	8.8	4	
	Wood	–	–	20	560	11	5.8	
	Branches	–	–	89	5270	64	26	
	Leaves	–	–	240	4680	88	31	
Phyllanthus securinegioides	Root	–	–	26	1270	24	16	
	Bark	–	–	68	5860	78	8.8	
	Wood	–	–	11	860	10	5	
	Branches	–	–	94	7630	74	20	
	Leaves	–	–	190	16,530	92	30	
Rinorea bengalensis	Root	–	–	–	–	–	–	
	Bark	–	–	36	3970	240	5.1	
	Wood	–	–	13	880	35	4.8	
	Branches	–	–	57	5140	120	13	
	Leaves	–	–	370	5740	95	19	
Alyssum pintodasilvae (*Barazón*)	Shoot	–	–	–	5713	–	–	(Álvarez-López et al., 2016)
	Whole plant				148			
(*Samil*)	Shoot	–	–	–	6665	–	–	
	Whole plant				117			
Alyssum malacitanum (*S. Bermeja*)	Shoot	–	–	–	9527	–	–	
	Whole plant				279			
(*S. Aguas*)	Shoot	–	–	–	5178	–	–	
	Whole plant				152			
Alyssum bertolonii	Shoot	–	–	–	8727	–	–	
	Whole plant				299			
Noccaea goesingense	Shoot	–	–	–	6241	–	–	
	Whole plant				211			

jackfruit, horse-radish tree, guava, tomato, eggplant) grown in eastern Samar Island, Philippines, may not have adverse health effects on humans.

Crops and pasture grown on ultramafic soils are likely to accumulate high concentrations of TMs and thus may constitute a health hazard for animals such as cattle. For example, Miranda et al. (2009) demonstrated that Ni and Cu levels in the kidneys of cattle near the ultramafic locality in Galicia, NW Spain have been increased by 20% and 32%, respectively. In contrast, Cr levels in cattle tissue were within the normal range (Miranda et al., 2009). This study shows that ultramafic soils significantly influence Ni and Cu accumulation in animals, leading to the risk of toxicity. Additional direct threats to human consumption of crops grown in ultramafic soils have been reported for crops such as rice. For example, the Ni content from brown and polished rice grown in paddy fields inclusive of ultramafic soils in eastern Taiwan was shown to exceed the tolerable Ni intake for adults recommended by the World Health Organization (WHO) (Hseu and Lai, 2017). Hence, further studies are required to evaluate the risk that TMs pose through the soil-plant and animal cycle.

5.5. *TM uptake mechanisms in plants*

Ultramafic flora possesses a remarkable ability to tolerate TMs and develop under TM stress. They excel at both uptake and translocation of TMs throughout the plant body. The adapted plant species to ultramafic environments can be metal hyperaccumulators or excluders (Baker, 1981; Rascio and Navari-Izzo, 2011). The metal hyperaccumulator plant species can uptake and translocate metals 100 to 1000 times more efficiently than non-hyperaccumulating plant species (Krämer, 2010; van der Ent et al., 2013b). The translocation of metals from roots to aerial parts of the plant through the xylem transport is a characteristic feature of hyperaccumulator phenotypes (Brady et al., 2005; Clemens et al., 2002). The metal hyperaccumulator, *Arabidopsis halleri* has been used as a model plant to study TM uptake and translocation (Briskine et al., 2017; Chiang et al., 2006; Frérot et al., 2010; Hanikenne and Nouet, 2011). *A. halleri* can accumulate high Zn and Cd levels as high as 2.2% and 0.28% respectively in its leaf in dry biomass (Talke et al., 2006).

Transport of soil TMs across the plasma membrane is an important feature of hyperaccumulators. Membrane transport proteins and intracellular high-affinity binding sites play an essential role in metal transport across the plasma membrane. The six major transporter systems effectuating TM transport across the membrane are: the Zn-regulated transporter and Fe-regulated transporter protein (ZIP) family, the cation diffusion facilitator (CDF) family, the P_{1B}-type subfamily of P-type ATPases, the natural resistance-associated macrophage protein (NRAMP) family, the yellow-stripe 1-like (YSL) subfamily of the oligopeptide transporter (OPT) superfamily, and three subfamilies of ATP-binding cassette (ABC) transporters (Hall and Williams, 2003; Kumarathilaka et al., 2018b; McGrath and Zhao, 2003).

Interestingly, the genes coding the ZIP transporter family members were found to be expressed at very high levels in the roots and shoots of hyperaccumulators such as *Thlaspi caerulescens* (Assunção et al., 2001). The high constitutive expression of metal ion transporter genes in extremophytes even in the absence of high concentrations of the relevant ion in control soil treatments has been suggested as one of the hallmark genetic features of extremophytes adapted to extreme soils (Krämer, 2018; Oh et al., 2013). The extremophyte model, *Arabidopsis halleri* is naturally adapted to high Zn and Cd and this is thought to be partly a result of high gene copy number of the *HEAVY METAL ATPASE4* (*HMA4*) gene as well as its high constitutive expression leading to enhanced partitioning of Zn from roots into shoots (Briskine et al., 2017; Hanikenne et al., 2008; Talke et al., 2006). The genes highly expressed in *A. halleri*, compared to its close TM-sensitive Arabidopsis relatives, contribute to its hypertolerance to TMs include, Ca^{2+}/H^{+} antiporter, *CAX1*, and *METAL TOLERANCE PROTEIN1* (*MTP1*) (Baliardini et al., 2015; Talke et al., 2006). Furthermore, increased Zn influx in roots of the hyperaccumulating *Thlaspi* species was detected with respect to high Ni and Zn concentration of the roots (Kazakou et al., 2010; Pence et al., 2000). Moreover, the ultramafic and the non-metallicolous populations of *Thlaspi caerulescens* showed low affinity for Cd and Zn over Ni. This may be due to the Ni hyperaccumulation of the ultramafic population in its natural environment. Moreover, this suggests that a Zn transport system might also lead to the entrance of Ni into roots (Assunção et al., 2008).

Non-hyperaccumulator plants retain most of the TMs they uptake in their root cells, detoxifying them by chelation in the cytoplasm or accumulating them in vacuoles. In contrast, hyperaccumulators characteristically translocate TM to the shoot through the xylem in an efficient manner (Haydon and Cobbett, 2007). Enhanced chelation of Ni (II) by nicotianamine in the xylem has also been suggested as a Ni tolerance mechanism in the hyperaccumulator *Thlaspi caerulescens*. Ni–nicotianamine (Ni-NA) complexes have been detected in the roots of Ni-treated Zn-hyperaccumulator *Thlaspi caerulescens* (Vacchina et al., 2003). In *Arabidopsis halleri*, *NA synthase* is highly expressed in roots facilitating high Zn accumulation (Weber et al., 2004). Vacchina et al. (2003) demonstrated that the introduction of nicotianamine synthase gene expressed in *Arabidopsis thaliana* into transgenic tobacco plants led to an increase in Ni tolerance. Furthermore, transgenic tobacco plants which produce nicotianamine by overexpressing *HvNAS1*, grew with minimal toxicity symptoms in a Ni-enriched ultramafic soil (Kim et al., 2005).

Free histidine also acts as a Ni-binding ligand that facilitates the xylem transport of Ni. In the hyperaccumulator, *Alyssum lesbiacum*, free histidine production was found to be activated in the presence of high Ni concentrations, but not in the non-accumulator, *Alyssum montanum* (Charnock and JAC, 1996). Similarly, enhanced release of Ni into the xylem is coincident with the release of histidine from the free histidine pool in the roots of both *Alyssum lesbiacum* and *Brassica juncea* (Kerkeb and Krämer, 2003). In addition to histidine, YSL family members also help in xylem loading and unloading Ni-NA chelates (Colangelo and Guerinot, 2006). The upregulation and the localization of the expression of these *TcYSL* genes together with the transport activity of Ni-NA chelates around the vascular system was shown to be required for the long-distance transport of metal ions in *T. caerulescens*. The entry of Ni–NA into the symplastic transport in the root and the unloading of the Ni–NA complexes from the xylem in the leaves both depended on the capacity of *TcYSL3* to catalyze the influx of Ni–NA. That catalysis was vital for direct detoxification and storage of Ni, which is another mechanism of TM tolerance in plants (Freeman et al., 2006). Both processes are prominent in hyperaccumulators (Bidwell et al., 2004). Interestingly, leaves are considered to be the major storage organs of TMs as well as the major detoxification sites, while avoiding damage to the photosynthetic machinery (Freeman et al., 2006). The accumulation of Ni in the vacuoles of epidermal cells in the leaves of *Hybanthus floribundus* (a hyperaccumulator of Ni) was found to be concentrated on the outside of cell walls, indicating that apoplastic compartmentation facilitating Ni tolerance (Bidwell et al., 2004). Metal cations bound to smaller ligands of organic acids can be highly accumulated inside vacuoles. For example, citrate is the main Ni-bounded ligand in the leaves of *Thlaspigoesingense* (Bernal et al., 1994). Bioaccumulation of TMs may cause allelopathic effects on neighboring plants (Seneviratne et al., 2016); however, our knowledge is not sufficient to confirm this with ultramafic soils. Studies on TMs such as Cu and Co-accumulation in plants are even more limited (Lange et al., 2017) mainly due to the scarcity of the sites and the absence of model plants equipped with genetic and physiological resources. More studies are required to assess those physiological and biochemical damages and their mechanisms in plants in ultramafic ecosystems.

5.6. Tolerance to low Ca:Mg ratio

The Ca:Mg ratio is an important factor for ultramafic flora (Álvarez-López et al., 2016). High concentrations of Mg in ultramafic soils are antagonistic to plant Ca uptake. Ca shows a unidirectional transport from the root elongation zone to the shoot due to immobilization of Ca in the shoot. High concentrations of Mg in the ultramafic soil induces a Ca deficiency in the growing root tips, which leads to necrosis of the root tip (O'Dell et al., 2006). The plants grown in ultramafic environments show the tolerance towards high Mg and comparatively lower Ca levels in ultramafic soils (Ghasemi et al., 2015; Palm and Van Volkenburgh, 2014). For instance, *Mimulus guttatus* grown in ultramafic environments has shown an increased tolerance to low Ca:Mg than that of non-ultramafic populations (Selby, 2014). A single, major QTL (quantitative trait locus) of LG13 was found to control both the ability of plants to survive on ultramafic soils and their tolerance to low Ca:Mg ratios. Furthermore, native ultramafic plant species have exhibited more Ca absorption and less Mg absorption than plants in non-ultramafic soils (Ghasemi et al., 2015). Ultramafic plants have the ability to maintain a sufficient internal concentration of Ca under the lower Ca:Mg (< 1) found in ultramafic soils (Ghasemi et al., 2015). *Alyssum inflatum*, a Ni hyperaccumulator which is endemic to ultramafic habitats was found to demonstrate a high tolerance to a low Ca:Mg ratio and increase in plant Ni accumulation with increasing Ca:Mg ratio (Ghasemi and Ghaderian, 2009). However, an increase in soil Ca content was found to lower the Ni uptake of *A. serpyllifolium* (a non-hyperaccumulator), leading to a decrease in shoot Ni concentration (Brooks et al., 1981). Substantial work has been done on Ca^{2+} and Mg^{2+} transport systems and the homeostatic balance between the two nutrients (reviewed in Tang and Luan (2017)). However, limited studies (reviewed in Brady et al., 2005) have looked into molecular, genetic, and physiological mechanisms unique to ultramafic flora adapted to high Mg^{2+} and low Ca^{2+}soils.

5.7. Ecosystem and human health-related medical geology

Ultramafic localities are being used as croplands and residence areas in many different regions globally. The fractionation, release, and availability of TMs depend on both environmental factors and pedogenic activities (Fig. 3). Hence, ultramafic soil localities pose risks to human and ecosystem health. The ultramafic body in Shih-Tao Mountain in Taiwan is part of an urban watershed, directly influencing 100,000 residents (Cheng et al., 2011). The release of TMs can influence water quality. Subsurface waters in ultramafic terrains in Peninsular Malaysia and the State of Subah in Malaysia contained elevated concentrations of Cr and Ni (23–172 and 95–936 $\mu g\ L^{-1}$, respectively). However, surface waters contained safe levels of TMs for human consumption (Tashakor et al., 2018). Some ultramafic soils are under cultivation, including viticulture and rice. Wheat (*Triticum aestivum*) has been cultivated on ultramafic soils in the eastern part of the Rudnik Mountain, near Salasi Village in Serbia (Antić-Mladenović et al., 2011). Rice grown on ultramafic environments in Hualien and Taitung in

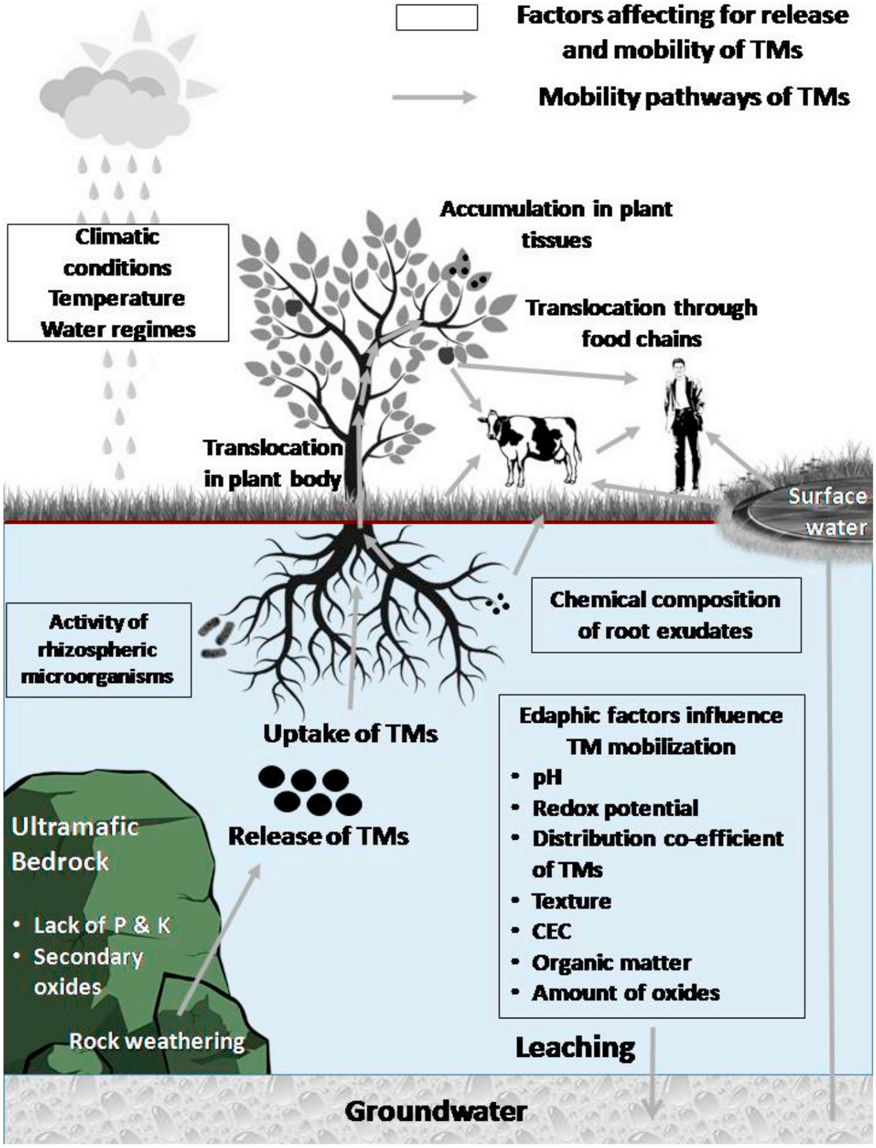

Fig. 3. Schematic diagram of the trace element release into the environment.

eastern Taiwan has been found to have high Ni levels (1.50–4.53 and 2.45–5.54 mg kg^{-1} in brown and polished rice, respectively) (Hseu and Lai, 2017). On the basis of the WHO's provisional tolerable Ni intake for adults, daily consumption of rice may lead to an excessive Ni intake by humans. Intensive agricultural activities and livestock farming are also taking place on ultramafic terrains (64 km^2) in Atalanti, central Greece (Kanellopoulos et al., 2015). The measured Ni level in the leaves of *Alyssum chalcidicum* in Atalanti was 2910 mg kg^{-1}. This characteristic example shows the relationship between soil geochemistry and metal uptake by plant species.

When food plants contain increased concentrations of TMs, this can create health problems. High Ni accumulations were noted in *Phyllanthus amarus* Schum. & Thonn (54 mg kg^{-1}) and *Stachytarpeta jamaicensis* (65 mg kg^{-1}), in the villages of Malbog, Napataan, and Palanas in Eastern Samar in the Philippines (Susaya et al., 2010). However, none of the investigated plants met the criterion for a hyperaccumulator plant (< 10,000 mg kg^{-1}), and non-food plants had higher maximum Ni concentrations than did food plants. The recommended daily intake of Ni is < 1 mg kg^{-1} (Susaya et al., 2010). The possible concentrations of Ni intake per food plant species at daily consumption levels of 1000, 250, and 125 g were calculated. For most food plant species consumed in great quantities (1000 g day^{-1}), the Ni intake level would be higher than the recommended daily intake. Additionally, even the consumption of 125 g day^{-1} of *Psidium guajava* L., *Artocarpusheterophylla* Lam., *Moringa oleifera* Lam., *Solanum melongena* L., and *Lycopersicon esculentum* Mill. would exceed the daily recommended threshold (Susaya et al., 2010). Another study examined the effect of high soil concentrations of Ni and Cr on perennial grapevines growing in ultramafic soil in Serbia (Licina et al., 2010). The levels of Ni and Cr in the grapevines showed the pattern root > leaves > shoots > grapes. The Ni and Cr concentrations in roots were 40.7–68.8 and 23.3–41.3 mg kg^{-1}, respectively, whereas in grapes the concentrations were 0.4–0.9 and 0.1–0.6 mg kg^{-1} (Licina et al., 2010).

Around 100,000 ha of floodplain soils in the Velika Morava valley, central Serbia, were found to have high Ni level (Jakovljevic et al., 1997). Later studies confirmed that the Ni in the floodplain soils originated from the weathering of ultramafic rocks which outcrop in the Velika Morara River system. The Velika Morava River valley is an important agricultural region which is exposed to frequent flooding. Rinklebe et al. (2016) investigated the release dynamics of Ni at controlled redox potentials (E_H) in floodplain soils in this valley. The authors found that Ni can be mobilized at low E_H, while an elevation in E_H from reducing to oxidizing conditions results in immobilization of Ni. The mobilization of Ni was attributed to the formation of Ni-dissolved organic carbon complexes at low E_H, and the immobilization of Ni was suggested to result from the formation of Fe/Mn (hydro)oxides and subsequent co-precipitation of Ni during oxidation (Rinklebe et al., 2016). The influence of ultramafic rocks on the contamination of agricultural plants by Cr due to natural processes was studied in the Assopos basin in Greece (Economou-Eliopoulos et al., 2011). Mineralogical studies revealed that Cr in the soil is mainly associated with chlorite, chromite, Fe-chromite, Cr-bearing goethite, and silicates. The Cr content in plants ranged from 1.8 to 2.3 mg L^{-1} in shoots, 1.3 to 100 mg L^{-1} in roots, and 1.5 mg L^{-1} in a bulb plant. It is interesting to note that some plants which possess small, fine fibrous roots located near the surface of the soil (i.e. wheat (*Triticum* spp.), onion (*Allium cepa*) and leek (*Allium porrum*)), contained higher Cr concentrations than plants which possess a thick main root extending deeper into the soil, such as beets (*Beta vulgaris*) and verbascum (*Verbascum* spp.). It is thus important to obtain a better ecological risk assessment of floodplain sites under agricultural use.

Both geogenic and anthropogenic sources have contributed to high levels of Cr present in groundwater in many countries. Leaching of geogenic Cr(VI) in Greece has led to an elevated level of total Cr (28.5 μg L^{-1}) and Cr(VI) (25.3 μg L^{-1}) in the groundwater (Dermatas et al., 2012). However, a few wells contained up to 190 μg L^{-1} total Cr, most of which occurred as Cr(VI) (up to 160 μg L^{-1}). Additionally, it was suggested that the presence of Cr(VI) in that area was due to a combination of geogenic (i.e. ophiolites) and anthropogenic sources. Similarly, up to 212 μg L^{-1} of Cr(VI) was detected in irrigated groundwaters from the wider area of Thiva Basin in central Greece (Tziritis et al., 2012). In Thiva town, the Cr(VI) concentration ranged from 13 to 212 μg L^{-1}, whereas, in Mouriki village, Cr(VI) values ranged from < 9 to 14 μg L^{-1}. Ultramafic formations are a possible source of elevated Cr(VI) in groundwater. Therefore, investigation of the geo-chemical properties is required to ensure the quality of agricultural products of that region. Additionally, tap water from 600 groundwater-supplied sites in Greece was assessed for potential Cr(VI) contamination (Kaprara et al., 2015). Notably, 25% of the samples contained Cr(VI) levels above 10 μg L^{-1}. The higher Cr(VI) concentrations in aquifers may be related to the underlying geology which is rich in ultramafics. Kaprara et al. (2015) suggested that high Cr(VI) levels in drinking water may occur due to slow MnO_2-catalyzed oxidation of Cr (III) into Cr(VI). Groundwater contamination due to ultramafics has also been identified in Leon Guanajuato Valley, Central-Mexico (Robles-Camacho and Armienta, 2000) and Sacramento Valley, California, USA (Mills et al., 2011). High Cr(VI) concentrations were found in the exchangeable soil fraction in Wan-Ron Hill in eastern Taiwan, ranging from 34.8 to 183 μg kg^{-1} (Ho et al., 2013b). In a California Coast Range ultramafic deposit, water-extractable Cr(VI) in soil has been found to increase with depth, with a total soil reservoir of 7.8 to 12 kg/km^2 Cr (VI) and soil concentrations ranging from 25 to 172 μg L^{-1} (McClain et al., 2017).

Open cast mining of ultramafic ore deposits has also been found to threaten the surrounding environment. The overburden and ore dumps in mining sites are commonly stored on the ground surface. Leaching of TMs from mining wastes, particularly during the rainy seasons, and dispersion of fine particles via wind lead to the contamination of groundwater, surface water bodies, and nearby lands (Losfeld et al., 2015). For instance, mine seepage and leachate migration from a chromite ore deposits (i.e. Sukinda mine, Orissa, India) have caused high Cr(VI) concentrations in nearby groundwater sources (0.032–0.452 mg L^{-1}) (Dhakate et al., 2008). Pattnaik and Equeenuddin (2016) also demonstrated that soils in the area near the Sukinda mine, Orissa, India, have been contaminated by Cr(VI) (1.45–16.7 mg kg^{-1}) due to the weathering of ultramafic rocks. Mining of laterite ore deposits has also been found to increase the risks to environmental health (Losfeld et al., 2015; Lweya et al., 2015). Lateritisation involves in situ neoformation of mineral phases which host TMs such as Cr, Ni, and Cu (Quesnel et al., 2017). Economou-Eliopoulos et al. (2016) demonstrated that Cr(VI) concentrations in leachates from laterite ore in Kastoria, Greece, reached levels as high as 1.3 mg L^{-1}. Groundwater sources near a laterite ore in central Evia, Greece, have also contained values ranging from < 2 to 360 μg L^{-1} (Economou-Eliopoulos et al., 2017). The groundwater samples near a laterite mine in North Western Zambia contained 0.12–0.15 mg L^{-1} of Cu, well below the WHO drinking water quality guideline of 2 mg L^{-1} (Lweya et al., 2015). At present, mining of Ni has shifted from high-grade Ni sulfides to low-grade ore Ni laterites, containing approximately half of the mined Ni ores. The spatial distribution of Cr in soils near Ni-laterite ores in C. Evia and Assopos-Thiva basins, Greece, has been investigated by Megremi et al. (2019) and the Cr levels in the respective locations ranged from 50 to 190 mg kg^{-1} and 130 to 310 mg kg^{-1}. Therefore, it is essential to restore or rehabilitate mining sites to minimize the potential for TM contamination of agricultural lands and water sources.

The release of TMs from ultramafic outcrops has garnered significant attention in recent years, with a particular focus on understanding the mechanisms (i.e. oxidation, reduction, adsorption, and desorption) which affect TM fates in water resources. More precisely, physicochemical properties (pH-Eh and natural organic matter) and adsorption-desorption reactions govern the mobility and bioavailability of TM in the environment (Kumarathilaka et al., 2016a; Kumarathilaka and Vithanage, 2017; Lilli et al., 2015). Mobility studies have demonstrated that TM (i.e. Cr) leaching phenomena from ultramafic soils are very fast; however, sorption capacities are also significant. For example, Cr complexation with ferric oxide surfaces limits Cr mobility and transport in the environment. Further, soils and sediments with calcareous components more efficiently adsorb Cr(VI) compared to siliceous clusters. The presence of Fe and Mn may increase Cr(VI) concentrations, as it can be released from the surface of Mn oxides, be oxidized, and subsequently re-adsorb onto Fe oxides (Ho et al., 2013b; Rajapaksha et al., 2013). Thus, it is necessary to investigate the leaching mechanisms of TMs in different lithologies to fully elucidate the processes which govern their fate and transport in the subsurface.

The far-reaching significance of ultramafic-rich soil and associated TM bioavailability is apparent from the role of such soils as an analog to regolith that humans may encounter on Mars. Pioneering work has highlighted that ultramafic-bearing soil may readily leach TM, especially in the presence of perchlorate (Kumarathilaka et al., 2016b) which has been detected at reactive abundance across several sites on Mars (Wilson et al., 2016). Combined with the expected higher abundance of serpentine-bearing regolith on Mars, any use Martian regolith materials to grow crop plants to support human habitats will need to address the health risks potentially associated with the higher bioavailability of TMs. Meanwhile, ongoing in situ and laboratory work on the martian halogen cycle (Karunatillake et al., 2013; Zhao et al., 2018) suggest that the regolith can serve as a source for reactive halogen phases on Mars via photochemical processes, which may, in turn, enhance the pervasiveness of salts like perchlorates and the associated TM bioavailability in serpentine-bearing soil. Collectively, such observations can be best understood only with additional work on soil analogs to complement current soil simulants (Cannon et al., 2019), particularly at poorly characterized settings on Earth, such as in Sri Lanka. Furthermore, given endemic extremophytes in and their genetic adaptations to serpentine soils (Oh et al., 2014; Turner et al., 2010), the serpentine-bearing soil along the suture zone in Sri Lanka likely harbors extremophyte species. Characterizing their physiological responses and underlying genetic adaptations will help identify ways to genetically modify crop plants for reduced TM hazards on extended human missions on Mars, wherein regolith will serve as a key growth substrate (Wamelink et al., 2014) as already anticipated in lunar settings (Liu et al., 2008).

6. Conclusions

Ultramafic outcrops have a higher concentration of TMs, particularly Ni, Cr, and Co, than non-ultramafic soils. For this reason, fauna and flora in and surrounding areas of ultramafic localities may be at potential risk of TM accumulation. Cr mainly binds with the residual fraction of ultramafic soils and is not readily bioavailable under natural environmental conditions. The mineralogy of ultramafic soil profiles is largely dependent on the location. Many extremophytes growing in ultramafic soils are effective at the translocation of TMs, but several species are not. Plant species grown in ultramafic substrata can accumulate high levels of TMs in their vegetative and reproductive tissues.

Limited information is currently available on TM fractionation, translocation, bioaccumulation, and their allelopathic effects on other plants in the ultramafic environment. Key knowledge gaps regarding the mobility and bioavailability of TMs in ultramafic environments remain. These include the following: (1) investigation of the relationship between physicochemical properties and the mobility and bioavailability of TMs in different ultramafic localities, (2) assessment of temporal and spatial variations of groundwater and surface water quality in and around ultramafic environments, (3) the long-term effect of natural and anthropogenic activities on the process of ultramafic weathering, and (4) risk assessment studies related to TM ingestion and bioaccessibility for humans in and around ultramafic localities. Such studies should seek to enhance current knowledge on TM release mechanisms in ultramafic soils and the metabolism of TMs in ultramafic flora at molecular levels. This knowledge could then be used to develop effective strategies to mitigate the mobilization of TMs in the ultramafic environment or develop methods to extract TMs through the process of phytoremediation -. Such work will also provide necessary insight on refining Mars soil simulants to advance crop-plant experiments for future human habitats.

Declaration of Competing Interest

Authors declare no conflict of interest.

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Appendix C

Book chapter I

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7 Characteristics of Particulate Plastics in Terrestrial Ecosystems

Kumuduni Niroshika Palansooriya, Hasintha Wijesekara, Lauren Bradney, Prasanna Kumarathilaka, Jochen Bundschuh, N. S. Bolan, Teresa Rocha-Santos, Cheng Gu, and Yong Sik Ok

CONTENTS

7.1 INTRODUCTION

Rapid development in industrial and agricultural activities and overconsumption by humans have sped up the manufacturing of plastic wastes and their consequent disposal into terrestrial and aquatic ecosystems. Recent statistics showed that worldwide production of plastics has increased at an unprecedented rate (from 1.5×10^6 tons in 1950 to 335×10^6 tons in 2016)

(Liu et al. 2018). Even though different methods (e.g., recycling, reuse, and reduction) have been introduced to minimize the disposal of plastics, most of the plastic debris is still released into the environment.

Contamination of terrestrial environments with particulate plastics (PPs) is continuously increasing and is considered to be one of the major threats to human and animal health (Horton et al. 2017). Depending upon the characteristics of the PPs in terrestrial environments, their physical and biogeochemical behavior and risks to humans and animals can show large variation (Bradney et al. 2019). For instance, PP size is an important physical characteristic that determines the occurrence and movement of PPs in soil. After being disposed of in the environment, PPs gradually break down into small fragments, due to the action of physical, chemical, and biological drivers. Liu et al. (2018) demonstrated that two different types of PPs, namely, microplastics (sizes of 20 µm–5 mm) and mesoplastics (5 mm–2 cm), occurred in farmland soils of Shanghai, China. Their study also found that the abundance of microplastics and mesoplastics were 78.0 and 6.75 items/kg in shallow soils, respectively, whereas the abundance of microplastics and mesoplastics were 62.5 and 3.3 items/kg in deep soils, respectively. The results show that the distribution of PPs varies in the soil profile due to their different sizes. These PPs can be subjected to migration and transformation through various means, such as soil erosion, mechanical abrasion, and photodegradation (Song et al. 2017; Hurley & Nizzetto 2018); however, the degree of migration and transformation depends on where the PPs are present in the soil profile.

The size, color, shape, and abundance of PPs in terrestrial environments are the key factors that determine their bioavailability to soil organisms. Due to distinguishable sizes, shapes, or colors, PPs can be readily ingested by terrestrial animals, especially birds (Zhao et al. 2016; Auta et al. 2017). For example, seabirds, such as little auks are found to be color selective on their prey. These little auks generally consume light-colored PPs rather than dark-colored PPs, thereby signifying active contamination of birds that mistake PPs for their natural prey (Amélineau et al. 2016). Furthermore, Huerta Lwanga et al. (2016) reported that earthworms (*Lumbricus terrestris*) preferentially take in small-sized (<50 µm) PPs, exhibiting a size selective ingestion. These types of feeding behaviors of terrestrial organisms must be studied to understand the fate and risk of PPs in terrestrial ecosystems.

The chemical properties of PPs also play an important role in terrestrial ecosystems. The chemical composition and the availability of various chemical additives in PPs may determine their degradability and associated risk to terrestrial ecosystems. Various additives, such as stabilizers, plasticizers, monomers, anti-oxidants, clarifiers, colorants, and flame retardants are used during manufacturing of plastics to improve their properties (de Souza Machado et al. 2018). Therefore, PP products have embedded within their morphological structure a complex chemical formation. In the soil, these additives can leach out and may affect soil fauna. In addition, soil PPs can also adsorb contaminants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and organochlorine pesticides because of their hydrophobic surface (Horton et al. 2017). Subsequently, these toxicants can be released into terrestrial environments depending upon environmental conditions. Previous studies observed that organic and inorganic contaminants released from PPs accumulate and adversely affect terrestrial organisms, such as soil microorganisms (Wijesekara et al. 2018), earthworms (Hodson et al. 2017), and seabirds (Fife et al. 2015).

AQ 1

With the increasing concern regarding the potential impacts of PPs in terrestrial environments, there is a growing need to develop techniques to extract, identify, characterize, and quantify these impacts. The methods for analysis of PPs in terrestrial environments follow the same ones used for sediments and water columns, since no standardized methods have been developed for extracting and quantifying PPs in terrestrial environments. Techniques, such as scanning electron microscopy-energy dispersive X-ray spectroscopy, environmental scanning microscopy-energy dispersive X-ray spectroscopy, Fourier transform infrared spectroscopy (FTIR), micro FTIR (µ-FTIR) and micro-Raman (µ-Raman) spectrometry, thermal desorption gas chromatography mass spectrometry (TED-GCMS), and pyrolysis gas chromatography mass spectrometry (Py-GCMS) are used for morphological and chemical characterizations of PPs.

In this chapter, the characteristics of PPs in terrestrial ecosystems are discussed, so as to thoroughly outline their behavioral impacts on terrestrial environments and potential risks. Summaries of physical, chemical, and advanced characteristics of PPs, and different extraction, identification, and quantification techniques, are also presented, and research gaps are highlighted.

7.2 CHARACTERISTICS OF PARTICULATE PLASTICS

Particulate plastics are broadly defined as synthetic polymers less than 5 mm in size, and they can be of a primary or secondary origin (Frias & Nash 2019). However, this broad definition means that PPs can encompass a diverse range of physical and chemical properties or characteristics. Polyethylene, polypropylene, polystyrene, polyester, polyamide, and polyacrylonitrile are a few examples of the many synthetic polymers used to manufacture PPs. Additionally, chemicals such as phthalates, bisphenol A (BPA), or trace metals are incorporated with the polymers as additives. Furthermore, the negative environmental impacts of PPs have resulted in renewed interest in biodegradable plastics and environmentally sustainable alternatives. AQ 2

The physical and chemical properties of PPs play an important role in terrestrial ecosystems. For instance, properties, such as surface roughness, hydrophilic/hydrophobic properties, chemical structure, and molecular weight have an impact on PP vulnerability to biodegradation. Table 7.1 shows the impact of PP properties on their susceptibility to biodegradation.

7.2.1 PHYSICAL PROPERTIES

7.2.1.1 Size

Particulate plastic is a broad term encompassing micro and nano-plastics. The literature, however, is often divided when it comes to defining their size, thereby making comparisons of results between papers difficult. Arthur et al. (2009) first defined microplastics as <5 mm. Although generally accepted (Ng et al. 2018; Ferreira et al. 2019; Schwaferts et al. 2019), researchers have argued that the upper size limit of microplastics should be more “intuitive,” i.e., <1 mm (Browne et al. 2011; Van Cauwenberghe et al. 2013).

TABLE 7.1
General Impact of Certain Particulate Plastic Properties on Susceptibility to Biodegradation

Property	Impact on Biodegradation	Sample Format
Molecular weight	Only low molecular weight compounds can be assimilated by microbial cells and enzymatically degraded. Carbon-chain backbones do not biodegrade until the molecular weight is <1000 g/mol	Molecular
Chemical structure and morphology	Certain functional groups provide sites for enzymatic cleavage (ester, ether, amide, urethane)	Molecular
	Branched structures are more difficult for microbes to assimilate	Molecular
	Amorphous materials biodegrade faster than crystalline ones	Macro
Surface hydrophobicity	Hydrophobic surfaces inhibit biofilm formation, hydrophilic surfaces (water contact angle 40°–70°) promote it	Surfaces of thin films
Water absorption	Bulk hydrophilicity and water absorption give microbes access throughout the bulk material	Macro
	Water absorption softens polymers, and softer materials biodegrade faster than harder ones	Macro
Surface roughness	Microbes adhere to rougher surfaces more easily than smooth ones	Surfaces of thin films

Source: Reproduced from Ng, E.-L. et al., *Sci. Total. Environ.*, 627, 1377–1388, 2018. With the permission from publisher.

The lower size limit of microplastics is far more varied, and, consequently, there is often an uncertainty between the upper limits of nano-plastics and the lower limits of microplastics. While nano-plastics typically begin at 100–1000 nm (i.e., 0.1–1 μm) (Gigault et al. 2018; Ng et al. 2018; Ferreira et al. 2019), a variety of values has been reported for the lower size limit of microplastics, e.g., 0.1 μm, 1 μm, 20 μm, or 333 μm (Arthur et al. 2009; Wagner et al. 2014; Ng et al. 2018; Schwaferts et al. 2019). Other papers also provided sub-categories to further classify plastics. For example, Wagner et al. (2014) specified "large" microplastics as being between 1 and 5 mm and "small" microplastics as 20 μm–1 mm. Additionally, a paper from Schwaferts et al. (2019) used the term "sub-μ-plastic" for plastics between 100 nm and 1 μm.

Alternatively, some papers do not use the term nano-plastics, instead choosing to leave the lower limits of microplastics undefined. However, Arthur et al. (2009) did acknowledge that sampling restrictions (i.e., net size) meant that recovering particulates smaller than 333 μm from the marine environments would be a challenge. Although the use of 333 μm mesh nets is standard sampling practice, mesh sizes can be smaller, e.g., 80 μm plankton nets (Dris et al. 2015). Therefore, sampling restrictions are influential in determining the size range of microplastics. However, these restrictions may differ between aquatic and terrestrial environments, thereby hindering comparisons between them.

This chapter uses the term PPs, which encompasses both micro and nano-plastics, and is therefore defined as ranging from 5 mm down to the nano-meter range.

7.2.1.2 Color

Particulate plastics are manufactured in a wide array of colors. Commonly reported colors include black (Gewert et al. 2017; Anderson et al. 2018; Nelms et al. 2018), clear (Nelms et al. 2018), red (Gewert et al. 2017; Anderson et al. 2018; Nelms et al. 2018), blue (Eriksson & Burton 2003; Gewert et al. 2017; Anderson et al. 2018), white (Eriksson & Burton 2003; Gewert et al. 2017), green (Eriksson & Burton 2003; Gewert et al. 2017), yellow (Eriksson & Burton 2003), and brown (Eriksson & Burton 2003). Furthermore, the color of the PP is often influenced by its shape. A substantial percentage of fibers were reported as red, blue, and black (Gewert et al. 2017; Anderson et al. 2018; Nelms et al. 2018), whereas pellets were often reported as white (pristine) or yellow and off-white (aged) (Karkanorachaki et al. 2018; Wang et al. 2018; Acosta-Coley et al. 2019).

The particulate plastic's colors also influence how easily they are observed, and therefore can impact their sampling likelihood. PPs that are brighter in color, e.g., red, are more likely to be observed and extracted than those that blend into the substrate (Nelms et al. 2018). PP color may also influence the concentrations that are ingested by organisms. An organism may mistakenly ingest PP if the plastic's color closely resembles its normal food. Although papers readily report color as a characteristic, it is subjective, and should not be relied on to accurately determine and differentiate PPs (Lusher et al. 2017).

7.2.1.3 Shape

Particulate plastics can be manufactured into a range of different shapes (Figure 7.1), e.g., fibers, beads, hexagonal patterns, fragments, foams (i.e., polystyrene), and pellets (i.e., nurdles) (Lusher et al. 2017). Fibers are one of the more common shapes and are used in the clothing industry to make synthetic textiles (Frias & Nash 2019). Unfortunately, clothes that are machine washed will shed fibers. This results in the release of PPs into the environment. One study found that about 100 fibers were released into washing machine effluent per polyester fabric, with one garment shedding about 1900 fibers (Browne et al. 2011). Furthermore, polyester fleece shed 180% more fibers when compared to polyester blankets and shirts (Browne et al. 2011). PPs can also be spherical (i.e., beads), and these are commonly referred to as microbeads. Microbeads have received considerable attention in recent years and are now banned from many commercial cosmetic products (USA House of Representatives 2015; Canadian Environmental Protection Act 2017). Fragments are also prevalent in the environment, but are not usually manufactured into a microbead shape. Instead, they occur as secondary PPs (i.e., the breakdown of larger plastic) (Tanaka & Takada 2016).

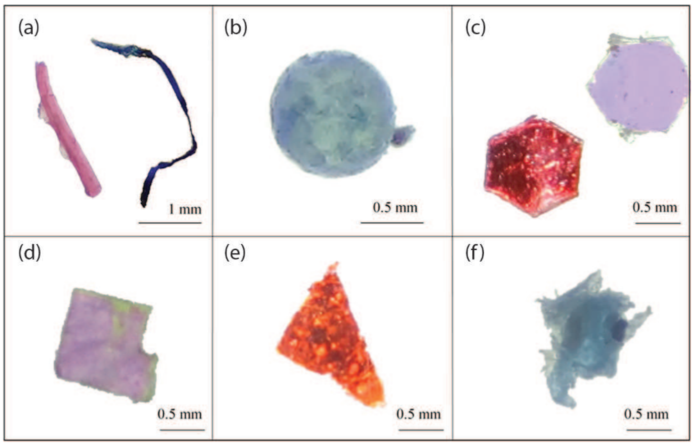

FIGURE 7.1 Selected examples of differently shaped particulate plastics found in Australian biosolids and compost under stereomicroscopy: (a) fibers, (b) spherical, (c) hexagonal, (d) square, (e) triangle, and (f) irregular. (Reproduced from Bradney, L. et al., *Environ. Int.*, 131, 104937, 2019. With the permission from publisher).

7.2.2 CHEMICAL PROPERTIES

7.2.2.1 Polymer Type

Particulate plastics are manufactured using a range of base polymers, e.g., polyethylene, polypropylene, and polystyrene. The choice in polymer is dependent on how a product will be used and its desired properties and characteristics. For example, low-density polyethylene is a flexible polymer AQ 3 and is used for plastic bags and film wrap, whereas polystyrene is an excellent insulator, making it ideal for roofing, refrigerators, and disposable food containers (Wijesekara et al. 2018).

Polyester, polyamide, and polyacrylonitrile are frequently used as synthetic fibers (Schwarz et al. 2019). However, one study found that a large proportion of fibers released into the environment are made from “natural” sources, e.g., wool and cotton, rather than PPs (Stanton et al. 2019). Another study found that two-thirds of sampled fibers were made from synthetic rubber (i.e., neoprene, nitrile rubber, styrene butadiene rubber, and ethylene propylene), with nearly half the fibers made from ethylene propylene (Nelms et al. 2018). The remaining third were identified as synthetic plastics (i.e., polyacrylamide (PAM), polyethylene terephthalate, poly butylene terephthalate, polyethylene, and polypropylene) (Nelms et al. 2018). Most of the fibers found in the environment are made from plastic-based polymers, although this is not always the case. Therefore, fibers must be properly identified before being classified as PPs.

Microbeads replaced natural products (e.g., ground almonds and apricot pits) to provide exfoliation from facial cleansers and other personal care products. Although many countries are now banning the use of microbeads, some products still contain them. A 2018 report by O’Farrell (2018) analyzed personal care and cosmetic products sold commercially in Australia. Polyethylene appeared on the ingredients list of 138 products. This was followed by nylon-12 contained within 92 products, polymethyl methacrylate within 33 products, and polyethylene terephthalate within 15 products. All other PPs were found in less than ten products and included polylactic acid (PLA), polypropylene, polytetrafluoroethylene, and several other nylon polymers.

7.2.2.2 Associated Chemical Bonds

Fourier-transform infrared spectroscopy is commonly used to identify PP polymers based on their chemical bonds. However, chemical bonds may be affected by factors such as weathering. A study conducted by Brandon et al. (2016) examined changes in the chemical bonds (i.e., hydroxyl, carbonyl groups, and carbon-oxygen) of polypropylene, high-density polyethylene, and low-density polyethylene pellets before and after three years of exposure to different experimental treatments. The four treatments were designed to simulate natural weathering conditions in the marine environments and included exposure to darkness and seawater (i.e., benthic environments), seawater and sunlight (i.e., marine surface), dry conditions and sunlight (i.e., coastline), and dry conditions and darkness (i.e., control). Overall, the chemical bonds showed time-dependent (i.e., non-linear) changes in their bond-indices. For example, high-density polyethylene showed decreased peak heights for all three chemical bond structures when exposed to the weathering treatment of darkness and seawater. Ioakeimidis et al. (2016) observed that native functional groups decreased and new functional groups (i.e., alkyne bonds at 620 cm^{-1} and 1435 cm^{-1}) were formed in polyethylene terephthalate bottles aged for <15 years. These changes in chemical bond structures hinder the identification of aged PPs in the environment, as many FTIR reference spectrums are designed for pristine polymers. Degradation by UV light can cause chemical bonds to break, and weathered PPs may therefore have slight variations in their chemical bond structures. Photodegradable plastics are specifically designed to undergo UV degradation, as they contain UV-sensitive additives that cause a weakening of bonds when exposed to sunlight (Fotopoulou & Karapanagioti 2019).

7.2.2.3 Chemical Additives

Particulate plastics not only consist of synthetic polymers, but also contain several chemical additives. These additives are used to help improve the physical and chemical properties of the plastics. For example, additives may be included to improve the mechanical strength of the plastics (e.g., fillers and reinforcements), increase resistance to degradation from heat and light (e.g., stabilizers), improve the flexibility of the plastic (e.g., plasticizers), or function as a flame retardant (Fries et al. 2013; Avio et al. 2017; Hahladakis et al. 2018; Godoy et al. 2019).

Many additives are included within the manufacturing of PPs, e.g., nonylphenol, phthalates, BPA, and polybrominated diphenyl ethers (Talsness et al. 2009; Koelmans et al. 2014). Phthalates are used as plasticizers and are commonly added to rigid PPs, such as polyvinyl chloride (Fries et al. 2013; Hahladakis et al. 2018). Furthermore, phthalates are susceptible to leaching, thereby adding another source of potential contamination caused by PPs; this contamination has been shown to affect marine organisms (Halden 2010; Fries et al. 2013). BPA is another well-known plastic additive. It is used as a flame retardant and stabilizer or anti-oxidant, where it is commonly used in polycarbonate plastics (Hahladakis et al. 2018). BPA has received considerable negative attention surrounding its use in food containers due to leaching and its subsequent implications on human AQ 4 health (e.g., endocrine disruptor). Other studies have also demonstrated that BPA can impact marine organisms (Anderson et al. 2016). Polybrominated diphenyl ethers is included in PPs as a flame retardant (Hahladakis et al. 2018). Unfortunately, polybrominated diphenyl ethers is a persistent organic pollutant, a known endocrine disruptor, and can cause neurotoxicity in certain organisms. Nonylphenol acts as an anti-oxidant or plasticizer and can be added to polyvinyl chloride and high-density polyethylene plastic bottles (Loyo-Rosales et al. 2004; Koelmans et al. 2014). These chemical additives are transported by the PPs and leach into the environment over time. Therefore, organisms not normally exposed to the chemicals may ingest them, thereby causing adverse effects.

7.2.3 ADVANCED CHARACTERISTICS

7.2.3.1 Trace Metals

Trace metals may be introduced to the PPs either as additives or as a by-product of the manufacturing process. A previous study suggested that a majority of trace elements found on PPs are derived AQ 5 from the manufacturing process (Wang et al. 2017). A study conducted by Godoy et al. (2019) identified trace metal additives (i.e., Al and Mg) within cosmetic PPs in Spain, and another study, conducted by Fries et al. (2013), found Al, Ti, Ba, and Zn. Furthermore, titanium dioxide nano-particles (added as white pigments or UV blockers) were also identified on the surface of PPs (Fries et al. 2013). Pb and Cd were often included in plastics as inorganic pigments. However, environmental laws and regulations have facilitated a reduction in the use of these additives (Hahladakis et al. 2018). However, Pb and Cd are still used as heat stabilizers in plastics, such as polyvinyl chloride (e.g., medical grade), along with Ba, Zn, and Sn (Sastri 2010). However, they are strongly bound to the polymer matrix and the likelihood of migration and leaching from the polymer is small.

Alternatively, trace metals can also sorb to PPs from the environment, and this process is influenced by factors, such as weathering and aging of the plastic's surface (Bradney et al. 2019). Particulate plastics can act as a vector for trace metal contamination. Furthermore, PPs and associated trace metals can be ingested by organisms, leading to bioaccumulation and food web contamination.

7.2.3.2 Biodegradable and Biosynthetic Particulate Plastics

In recent years, there has been a shift toward using biodegradable polymers due to concerns surrounding the environmental persistence of non-biodegradable PPs. However, not all the additives in biodegradable plastics completely degrade.

Plastic fibers have received considerable attention regarding biodegradable and biosynthetic alternatives. A study recently reported that clothing fibers can be made from proteins that are commonly found in squid tentacles. These "biosynthetic" fibers would break down naturally and thus have no impact on organisms or the environment (Gabbatiss 2019). PLA is another example of a biodegradable polymer that can be used in the textile industry. However, this polymer can also be used in soil erosion control or for films and coatings (e.g., paper) (Lunt & Shafer 2000). PLA is produced from the fermentation of foods, such as corn or potatoes and can be produced one of two ways. The first method involves removing water through the use of a solvent (e.g., acetone or dimethylformamide), with a strong vacuum and high temperature (Lunt & Shafer 2000; Jahangir et al. 2017). The second method uses a vacuum distillation (with no solvent) to produce a cyclic intermediate dimer known as lactide. One advantage of PLA is that it does not produce toxic products when it degrades (Gupta et al. 2007). Unfortunately, PLA can be highly flammable, thereby restricting its application. However, there has been an increase in research surrounding environmentally sustainable flame retardants for PLA over the last decade; for example, one study addressed phosphorus-based flame retardants, such as melamine polyphosphate and aluminum phosphinate (Tawiah et al. 2018). There are also many alternatives to non-biodegradable plastic microbeads, including plant-based substitutes (e.g., cellulose, nuts, seeds, and grains) and mineral substitutes (e.g., silica, mica, sea salt, and quartz sand) (Coombs Obrien et al. 2017; Scudo 2017). Calcium alginate (derived from seaweed) microbeads have also been suggested as a potential alternative to plastic microbeads and will rapidly degrade in seawater (Bae et al. 2019).

However, biodegradable PPs may also act as vectors for contaminants. Zuo et al. (2019) reported that the biodegradable PPs of poly butylene adipate co-terephthalate had a higher sorption capacity for phenanthrene when compared to the non-biodegradable plastics of polyethylene and polystyrene. Additionally, Balestri et al. (2017) examined biodegradable plastic bags made from Mater-Bi (vegetable oils and corn starch) and raised concerns about its involvement in an ~~observed~~ increase in intensity for intra- and interspecific competition within marine plants. Therefore, although biodegradable PPs may be more sustainable than their non-biodegradable counterpart, there are still concerns surrounding their use.

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On the other hand, oxo-plastic is another type of biodegradable plastic, which is considered as a potential solution to reduce the accumulation of plastic waste and litter in the soil (Chiellini & Corti 2016). The presence of an additive (pro-oxidant) facilitates the molecular structure of oxo-plastic to break down when exposed to heat or sunlight (Napper & Thompson 2019). Eventually, the degraded plastics are digested by microorganisms until they are fully degraded. Hence, oxo-plastics could be an alternative for minimizing plastic waste and thereby reducing PP accumulation in terrestrial ecosystems.

7.3 IDENTIFICATION AND QUANTIFICATION OF PARTICULATE PLASTICS

Particulate plastics can enter the terrestrial ecosystems through different anthropogenic activities and natural processes. For instance, PPs can reach agricultural lands and aquatic systems due to application of biosolids. Dry and wet depositions of airborne PPs also lead to their accumulation in terrestrial ecosystems (de Souza Machado et al. 2018; Ng et al. 2018). However, our understanding of the impact of PPs in terrestrial environments, as mentioned in the introduction section, is limited. Therefore, there is an increasing demand to develop analytical methods to extract, identify, characterize, and quantify PPs in terrestrial environments. At present, there is a gap in research on PPs in these environments, as no standardized methods have been developed for extracting and quantifying PPs in soils.

Most often, the procedures for analyzing PPs in soils follow similar methods as those used in sediments and water columns. Methods used in laboratories to quantify PPs in waters and sediments are described by Masura et al. (2015). Figure 7.2 shows a schematic diagram for analytical procedures used for PP particles in terrestrial environments. First, soil samples are properly collected for the analysis of PPs. Soils from the topsoil layer and from different depths in the soil profile are usually collected. Second, the collected soil samples are dried (i.e., air dried or mechanically dried) and sieved to desired fractions. Then the soil samples are floated, filtered, and separated by density. Following this stage, there is an oxidation or digestion step to remove organic matter from PP particles. Finally, morphological and physical characterization and a quantification of PPs are carried out using optical microscopy techniques and other analytical instruments. The following sub-sections provide detailed information on the extraction process, removal of organic matter, and morphological and chemical characterization of PPs particles in terrestrial environments, thereby highlighting the efficiencies, advantages, and challenges of the different methods and analytical instruments.

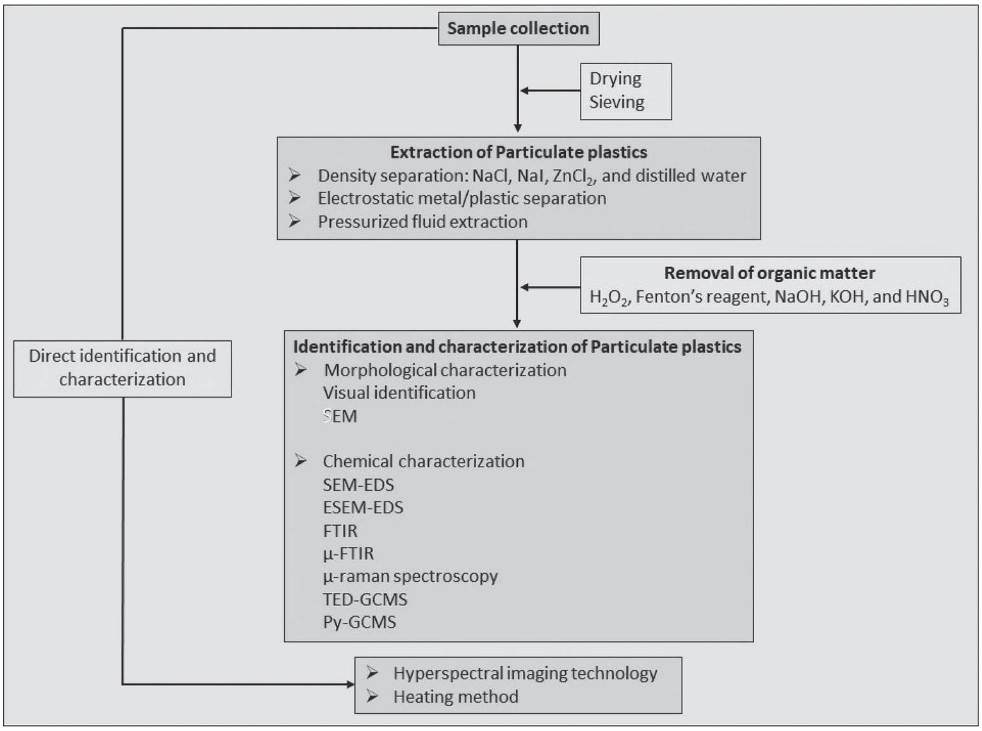

FIGURE 7.2 The procedures for analysis of microplastics in terrestrial environments.

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Quality control and quality assurance are important requirements in PP analysis to ensure the quality of the research results. Since there are no well-established QC and QA systems for PP investigations, some existing methods can be adopted to ensure the quality of the results.

Some studies report that analytical errors, such as misidentification and background contamination, can occur during PP analysis. For instance, insufficient spectroscopic measurements may lead to misidentification of synthetic fibers with artificial cellulose or lignin fibers (Remy et al. 2015; Wesch et al. 2016). Moreover, analysis of PPs may also be confounded by post sampling contaminants during laboratory processing. Thus, some precautionary measures, such as use of tools without any particle contamination (Lusher et al. 2013), avoiding air circulation in the working area, and wearing clothes made of cotton (Masura et al. 2015; Frias et al. 2016), would be helpful to avoid contamination by aerial PPs during analysis. Nevertheless, determination of airborne contamination levels in laboratories is an important step prior to the analysis (Hidalgo-Ruz et al. 2012). To avoid PP contamination in laboratories, isolation of the working space (such as by using pyramid glove boxes and hermetic enclosure devices) (Torre et al. 2016), use of clean-air devices (such as laminar flow cabinets) (Van Cauwenberghe & Janssen 2014; Wiesheu et al. 2016), and use of fume hoods (De Witte et al. 2014, 2015) have been used in some studies.

7.3.1 EXTRACTION

The initial PP extraction process involves sieving of dry soils to remove large particles (i.e., macroscopic debris) (Figure 7.2). In general, soil samples are initially passed through a 2 mm sieve. The densities of sand and sediments are both approximately 2.65 g cm^{-3}. Thereafter, density flotation can be used to isolate lighter PPs from the heavier sand and sediments (Figure 7.2). Salt-saturated solutions of known densities are used to separate PPs from the soil matrix. When the soils are mixed with high-density salt solutions, PP particles float to the surface of the solution or remain in suspension; however, the densest sand or sediment particles settle at the bottom of the solution. The supernatant is then collected for further analysis of the PPs.

Different types of salt-saturated solutions have been examined for their extraction efficiencies for PPs. The salt NaCl has been used to extract PPs from terrestrial environments. However, the density of the NaCl (1.2 g cm^{-3}) is too low to enable the floatation of some polymers, such as those containing additives (Coppock et al. 2017). High-density solutions, such as NaI and $ZnCl_2$ (1.6–1.8 g cm^{-3}) are preferred to separate the PP polymers containing additives (Coppock et al. 2017). Those alternatives are relatively cost-expensive compared to NaCl. Therefore, developing cost-effective methods with an excellent recovery rate is essential. Zhang et al. (2018) demonstrated that light-density PPs (i.e., polyethylene and polypropylene) in agricultural soils can be extracted using distilled water, which is a simple and cost-effective method. Since the densities of both polyethylene and polypropylene are <1 g cm^{-3}, saturated salt solutions can be replaced by distilled water (density of 1 g cm^{-3}) for agricultural soils. Liu et al. (2018) developed a method that has a number of extraction and ultrasonic treatments with NaCl for separating PPs. They found that their method can successfully extract seven out of nine tested PPs (polyethylene, polypropylene, polycarbonate, polymethyl methacrylate, polyamide, polystyrene, and acrylonitrile butadiene styrene). The use of a $CaCl_2$ solution (density = 1.5 g cm^{-3}) also shows a greater extraction recovery of PPs in floodplain soils compared to a solution of NaCl (Scheurer & Bigalke 2018). Overall, the use of salt-saturated solutions might lead to a partial or complete degradation of PPs, thereby influencing their detection and quantification. To avoid the negative impacts of salt-saturated solutions, Felsing et al. (2018) investigated the electrostatic behavior of PPs to facilitate their separation from a sample matrix using a modified electrostatic metal/plastic separator. The recovery rate for PPs under a modified electrostatic metal/plastic separator was approximately 100% (Felsing et al. 2018). Nevertheless, still there is uncertainty regarding the efficacy of this method for large-scale extractions of PPs from terrestrial environments. A pressurized fluid extraction technique has also been developed as a promising alternative for assessing the concentration and identification of PPs in terrestrial environments (Fuller & Gautam 2016). This technique utilizes solvents at sub-critical temperature and pressure conditions to recover semi-volatile organics from solid materials. The method was developed and recovered 85%–94% of the spiked PPs in a municipal waste sample (Fuller & Gautam 2016). The method efficiently extracted PP particles at <30 µm. Limitations of this method include the inability to measure the fractional sizes of PP particles and the change in morphology of the PPs (Fuller & Gautam 2016).

7.3.2 REMOVAL OF ORGANIC MATTER

Another challenge involved in analyzing PPs in soils is the presence of soil organic matter. Most commonly, PPs particles are extracted based on their densities. However, this approach is not effective for analyzing PPs in soil samples that may contain organic matter up to 99%. The densities of soil organic matter typically range between 1.0 and 1.4 g cm^{-3}. These density values are mostly similar to several other PPs, such as nylon and polyethylene terephthalate. Visual examination and sorting of the concentrated samples constitute the initial steps separating PPs from organic matter (i.e., animal and plant residues) and other non-plastics (i.e., tar and glass). Obviously, visual inspection has a minimal effect on distinguishing organic debris from PPs. Therefore, additional procedural steps are needed to remove organic matter from PP samples (Figure 7.2). Various methods, including acidic, alkaline, or oxidizing treatments, as well as enzymatic digestions, have been investigated for removing organic matter from PP samples. For example, Hurley et al. (2018) found that Fenton's reagent effectively removed organic matter from PP samples, when compared with alkaline digestion with NaOH and KOH and oxidation with H_2O_2. Scheurer and Bigalke (2018) demonstrated that an HNO_3 solution can remove organic matter in a short period of time; however, the morphology of some PPs can be affected due to the acidity of HNO_3. Alkaline solutions may also cause surface degradation of PPs (Hurley et al. 2018). Currently, H_2O_2 is the most commonly used chemical for removing organic matter from the PP samples, even though H_2O_2 can slightly change the shape of the PPs. Recent studies have focused on the development of efficient methods to remove organic matter, while maintaining the shape of the PPs. The heat bleach method has been used to reduce the H_2O_2 volume needed for removing organic matter from PP samples. For instance, Sujathan et al. (2017) demonstrated that the H_2O_2 volume required for organic matter removal from return activated sludge is approximately 6% of the sample volume. Elevated temperatures (~ 70°C) may also enhance the degradation of organic matter, thereby minimizing the time required for purification steps to 24 hours or less, and leaving the shape of the PPs unaffected (Sujathan et al. 2017). Overall, the establishment of a reliable and standardized method for the precise extraction of PPs is necessary, since this precision is a crucial pre-requisite to avoid misidentifications or underestimations of PPs in terrestrial environments.

7.3.3 IDENTIFICATION AND CHARACTERIZATION OF PARTICULATE PLASTICS

Particulate plastics extracted from terrestrial environments must be accurately identified and quantified. Analysis of PPs in terrestrial environments can be categorized into two main categories: (1) morphological characterization and (2) chemical characterization. The following sub-sections describe these two categories in detail.

7.3.3.1 Morphological Characterization

PPs have unique properties, such as shape, color, size, and density. Visual identification is an important step in identifying and classifying different PPs in a given sample. However, there are several limitations of visual identification, such as overestimations or misidentifications of PPs. Lenz et al. (2015) confirmed through an FTIR analysis that 70% of PP particles were erroneously identified by visual observation. Eriksen et al. (2013) also demonstrated that aluminum silicate and coal ash (approximately 20%) were misidentified as PPs through visual identification. Therefore, visual identification must be combined with other analytical instrumentation methods to avoid inaccurate identifications and under/overestimations.

Morphological characteristics of PPs can be confirmed by different analytical techniques (Figure 7.2). Optical microscopy (i.e., stereomicroscopy) has been used to assess the shape and color of PP particles (Claessens et al. 2011; Eriksen et al. 2013). Scanning electron microscopy can also be used to characterize the surface morphology of PP particles, as it provides high-magnified, high resolution structural images of the particles. Prior to an analysis, scanning electron microscopy requires coating over the sample (i.e., gold coating), which may change the actual color of PPs, and particles might change the texture as well. Both scanning electron microscopy-energy dispersive X-ray spectroscopy and environmental scanning microscopy-energy dispersive X-ray spectroscopy can provide data on the surface morphology, as well as the elemental composition of PP particles (Vianello et al. 2013).

SEM images of different PPs are shown in Figure 7.3. Disintegration features of PPs can be seen on PP surfaces; for instance, patterns such as pits, fractures, flakes, and adhering particles have been observed in PPs due to changes in the surrounding environment.

Sampling and extraction methods also affect the morphological characteristics of PP particles (i.e., size distribution). For instance, using different sieve and filter-pore sizes can lead to various size categories in extracted PPs. Therefore, the establishment of a standardized method for the sampling and extraction of PPs would help to ensure precise morphological identification of PP particles.

7.3.3.2 Chemical Characterization

Chemical characterization is an important step for assessing the composition and the quantification of PPs. Chemical characterization also confirms the results obtained through visual inspection and optical analysis. A variety of analytical instrumentation methods have been used for the chemical characterization of PP particles (Figure 7.2). Infrared spectroscopy, which uses non-destructive vibrational technology, is the most commonly used analytical method for the chemical characterization of PPs. Infrared spectra of unknown PP samples can be compared with the infrared spectra of known PP samples or with the known PP polymers stored in infrared spectra libraries. μ-FTIR spectrometry and μ-Raman spectrometry have been extensively used in infrared spectroscopies. These instruments possess automated scanning coupled with microspectrometry (He et al. 2018).

μ-FTIR spectroscopy can collect spectrums in different modes, such as attenuated total reflectance (ATR), transmittance, and reflectance. In addition to the collecting of spectra, μ-FTIR spectroscopy provides simultaneous visualization and mapping of PPs particles. The ATR mode of μ-FTIR spectroscopy facilitates the chemical characterization of irregularly shaped PPs particles. There is a different spatial distinction between μ-FTIR and μ-Raman spectrometry. The assay size limit of μ-FTIR is approximately 10–20 μm, while μ-Raman spectrometry can detect PPs particles as low as 1 μm. The key disadvantage of both μ-FTIR and μ-Raman spectrometry is that organic matter in an analyzed sample may interfere with the signal of the spectrometer. Therefore, organic matter must be completely removed from the extracted PPs samples prior to μ-FTIR or μ-Raman spectrometry analysis. In addition, another major disadvantage of μ-FTIR and μ-Raman spectrometry is their time-consuming scanning procedures. Therefore, μ-FTIR or μ-Raman spectrometry is difficult to perform for routine analyses of PP particles.

LUMOS II FT-IR spectrometer introduced by the Bruker corporation is an easy-to-use technique with automated measurements and a high precision sample stage (Bruker Corporation 2020). This technique enables analyzing PPs, which are embedded in a sediment/soil matrix, without sample preparation or special filters. Moreover, ATR transmission and reflection modes can be switched instantly, and ATR measurement is further enhanced by focal-plane array imaging (Bruker Corporation 2020).

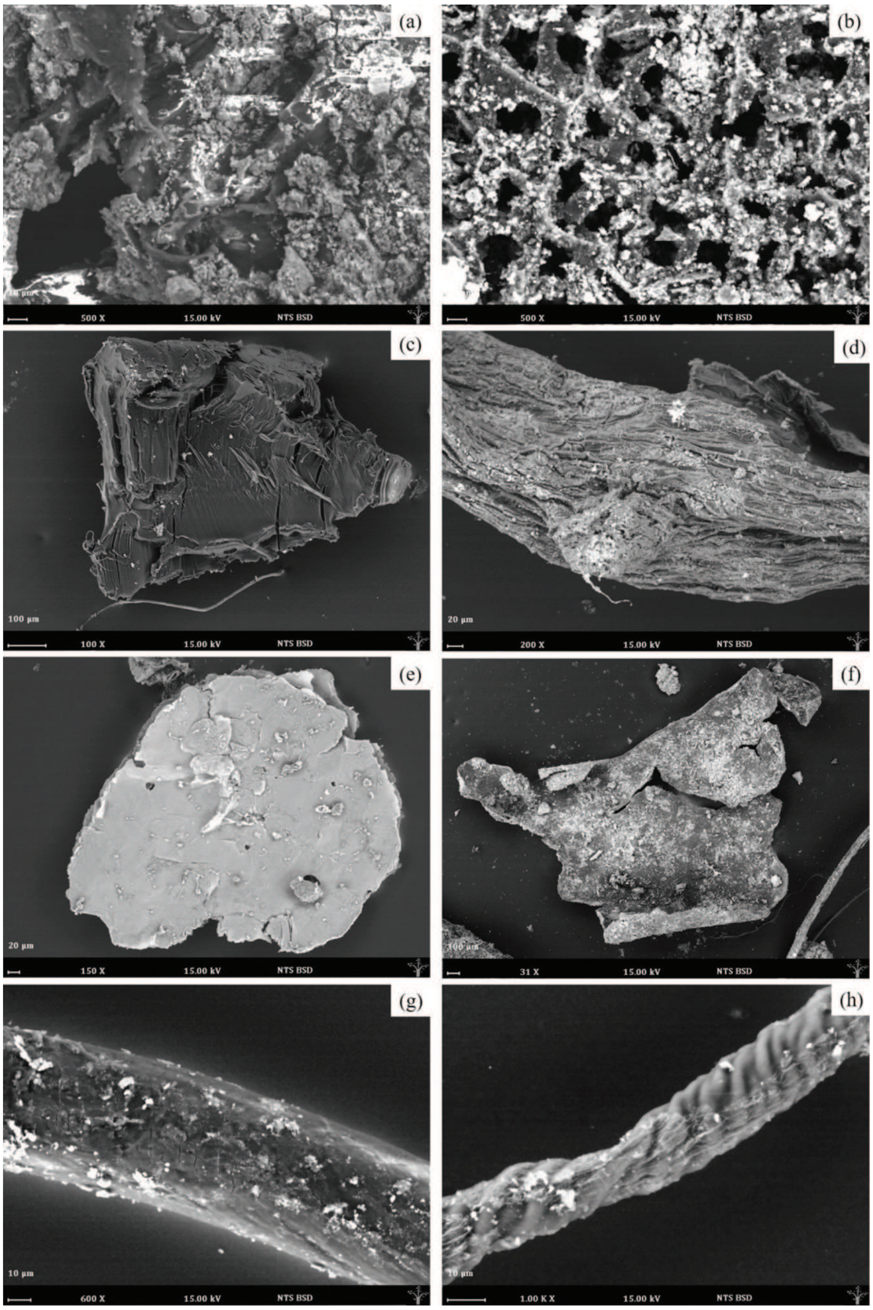

FIGURE 7.3 SEM images displaying different disintegration features in particulate plastics/microplastics (a,b) pits; (c,d) fractures; (e,f) flakes; and (g,h) adhering particles. (Reproduced from Shruti, V. et al., *Sci. Total Environ.*, 654, 154–163, 2019. With the permission from publisher).

Recent studies have focused on developing reliable and time-saving methods to determine PPs particles in terrestrial environments. Shan et al. (2018) developed a hyperspectral imaging technology as a potential method to determine and visualize the PPs with particle sizes ranging between 0.5 and 5 mm in soils. Dümichen et al. (2017) also developed a thermo-analytical method to identify PP particles in terrestrial environments. A homogenized sample of 20 mg is subjected to complete thermal decomposition. Specific degradation products are then adsorbed onto solid-phase absorbers, followed by subsequent analysis through TED-GCMS. This thermo-analytical method enabled researchers to identify polypropylene, polyethylene, and polystyrene in a biogas plant in terms of mass (Dümichen et al. 2017). Since the TED-GCMS is a quantifiable technique, it can measure the amount of PPs (micrograms) present in one liter of water in addition to the large amounts of natural particles (BAM Institute 2020). The other advantage of TED-GCMS is that the measurements can be completed within a few hours.

In general, Py-GCMS may be used to analyze polymers, as well as PP particles. In this method, single polymer particles are pyrolyzed under inert environments; consequently, thermal degraded products are cryo-trapped, separated, and quantified through the GCMS. This method works perfectly for single particles; therefore, a time-consuming preselection of a single particle is mandatory. The small amount of the sample (approximately 0.1–0.5 mg) used in Py-GCMS may not represent complex sample mixtures, such as environmental samples. Although the Py-GCMS method provides rapid measurements, information regarding the size distribution might be lost since the polymeric particles can be melted prior to degradation. A simple and cost-effective method was also developed to identify and quantify light-density PPs, such as polyethylene and polypropylene (Zhang et al. 2018). In this method, PPs and impurities are identified under a heating protocol (3–5 s at 13°C). When the sample is exposed to heat, PP particles melt and are transformed into circular and transparent particles, whereas impurities (i.e., silicates and organic matter) are not changed by the heat. The size and the number of the PP particles are evaluated using a camera connected to a microscope. An empirical model was developed to quantify PPs in the sample (Zhang et al. 2018).

In summary, analyzing PPs in terrestrial environments is still highly challenging due to the presence of complex and organic-rich substrates. There are inconsistencies of units (i.e., particles m^{-2}, particles m^{-3}, and kg of dry sediment) in different studies when the concentrations of PPs are reported. As mentioned previously, scientifically based improvements are required to standardize the methods for effective extraction and characterization of PP particles in terrestrial environments.

7.4 CONCLUSIONS

Particulate plastics are ubiquitous and persistent contaminants of increasing concern. Based on the properties of particulate plastics, their physical and biogeochemical behavior and associated risks to humans and animal health can differ. The physical and chemical properties of PPs and their abundance in terrestrial environments are the key factors that determine their bioavailability to soil organisms. The chemical composition and the availability of various chemical additives (such as trace elements, stabilizers, plasticizers, monomers, anti-oxidants, clarifiers, colorants, and flame retardants used during plastic manufacturing) in particulate plastics may determine their degradability and associated risks to terrestrial ecosystems. At present, various analytical techniques are used to extract, identify, characterize, and quantify particulate plastics in the environment. However, no standardized methods have been yet developed for the extraction and quantification of particulate plastics in soils. Generally, the procedures for analyzing particulate plastics in soils are highly similar to those used in sediments and water columns. After preparing the samples, morphological and chemical characterization can be performed with techniques, such as scanning electron microscopy-energy dispersive X-ray spectroscopy, environmental scanning microscopy-energy dispersive X-ray spectroscopy, FTIR, μ-FTIR and micro Raman (μ-Raman) spectrometry, TED-GCMS, and Py-GCMS. There is urgent need to prioritize scientific-based research on the environmental behavior and ecotoxicity of PPs in terrestrial environments, with a particular focus on the development of standard methods for extraction, identification, and quantification of PPs. This information would be useful for policy planning and to develop strategic management of PP pollution in terrestrial environments.

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Appendix D

Graphical abstracts of published articles

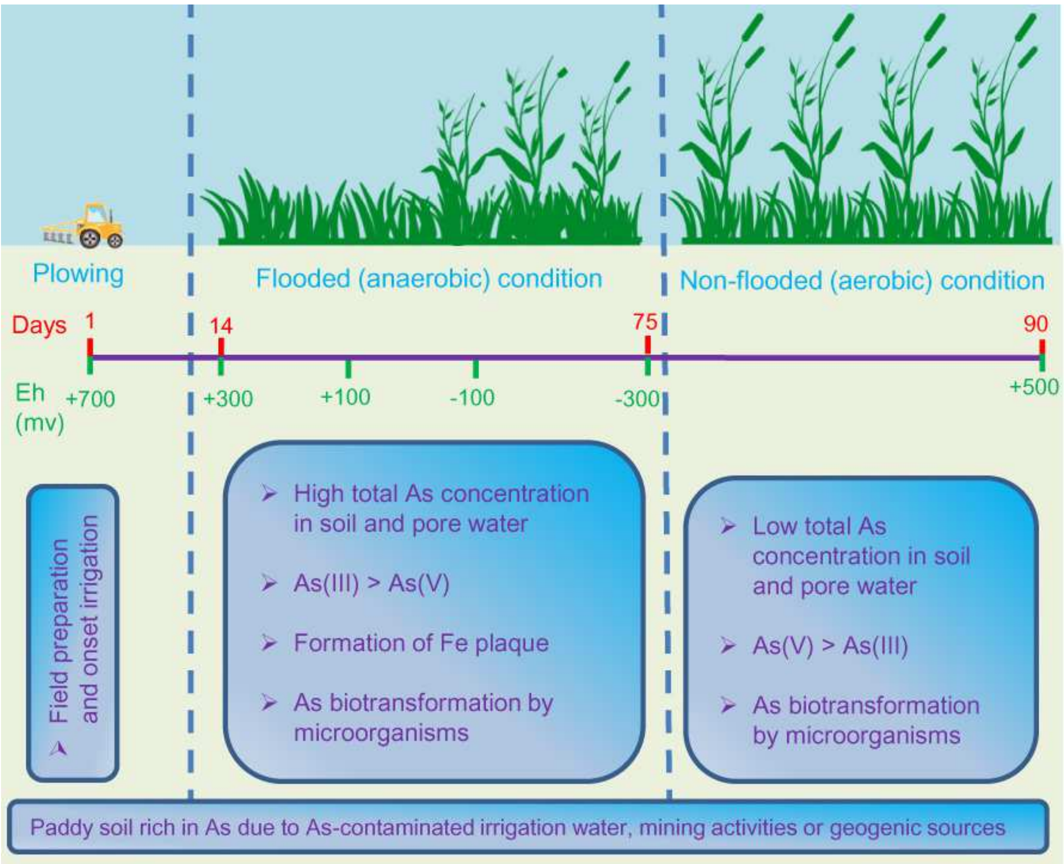


Fig. D. 1 The graphical abstract of article I: Arsenic speciation dynamics in paddy rice soil-water environment: sources, physico-chemical, and biological factors – a review.

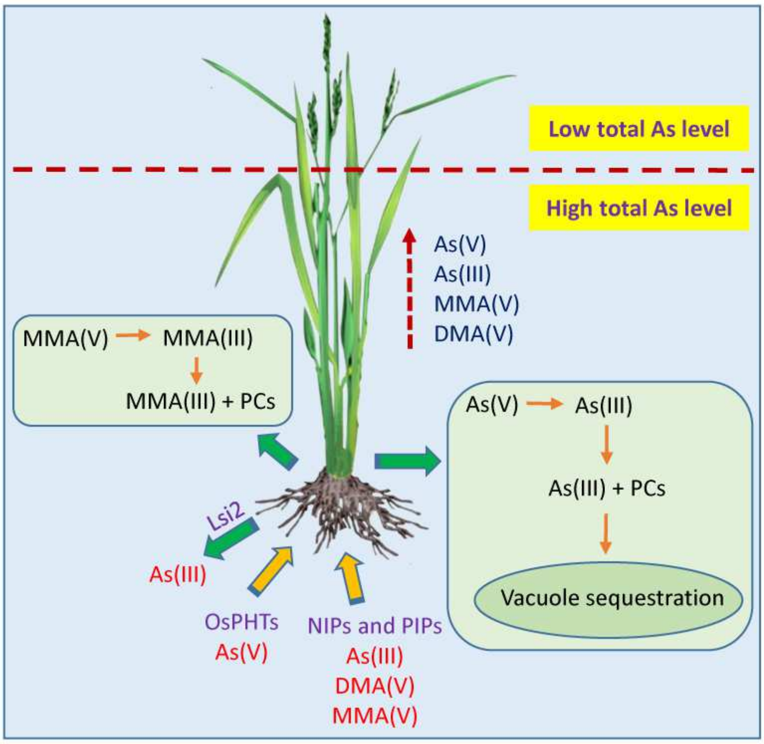


Fig. D. 2 The graphical abstract of article II: Arsenic accumulation in rice (Oryza sativa L.) is influenced by environment and genetic factors.

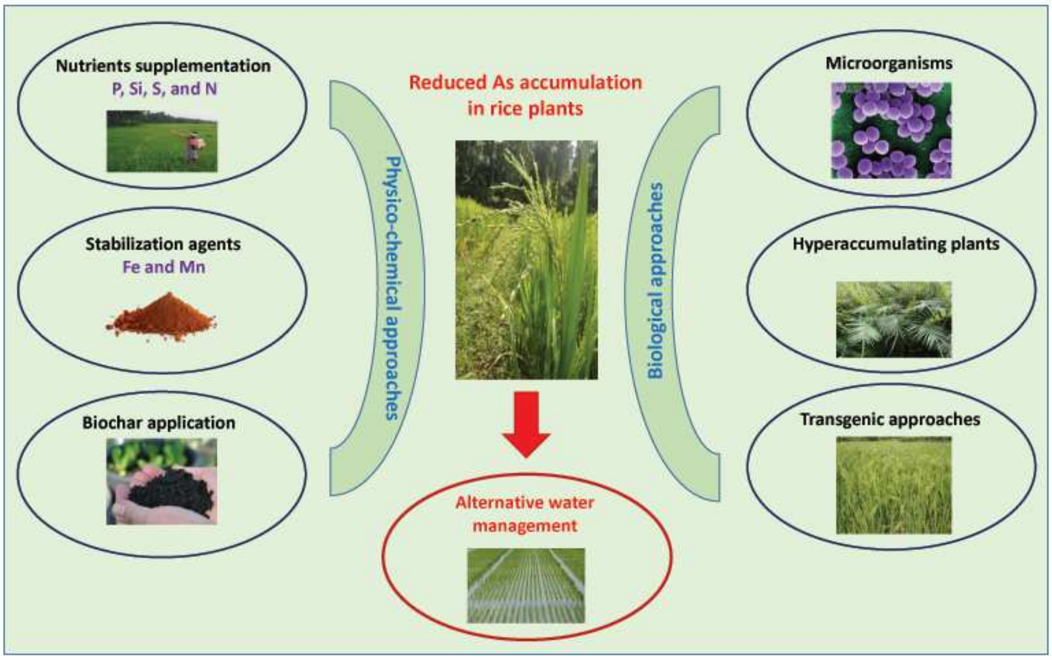


Fig. D. 3 The graphical abstract of article III: Mitigation of arsenic accumulation in rice: an agronomical, physico-chemical, and biological approach - a critical review.

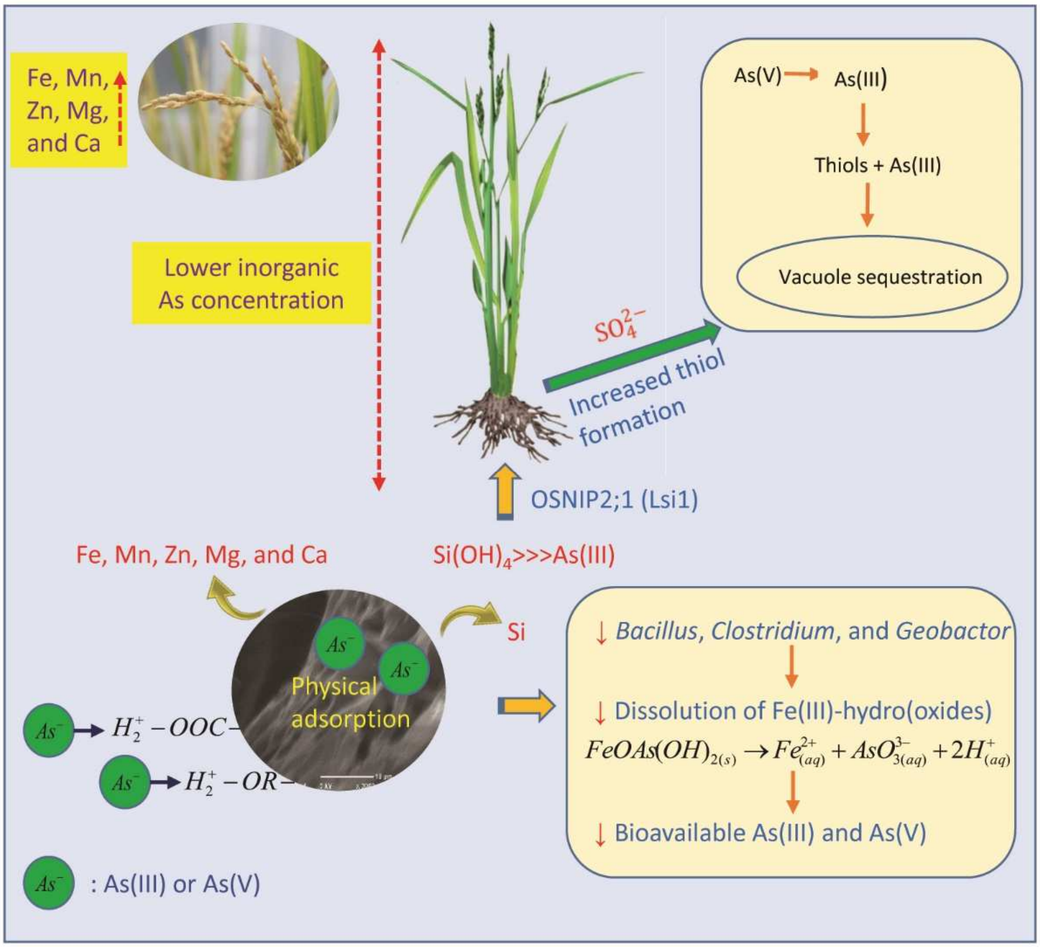


Fig. D. 4 The graphical abstract of article IV: An integrated approach of rice hull biochar-alternative water management as a promising tool to decrease inorganic arsenic levels and to sustain essential element contents in rice.

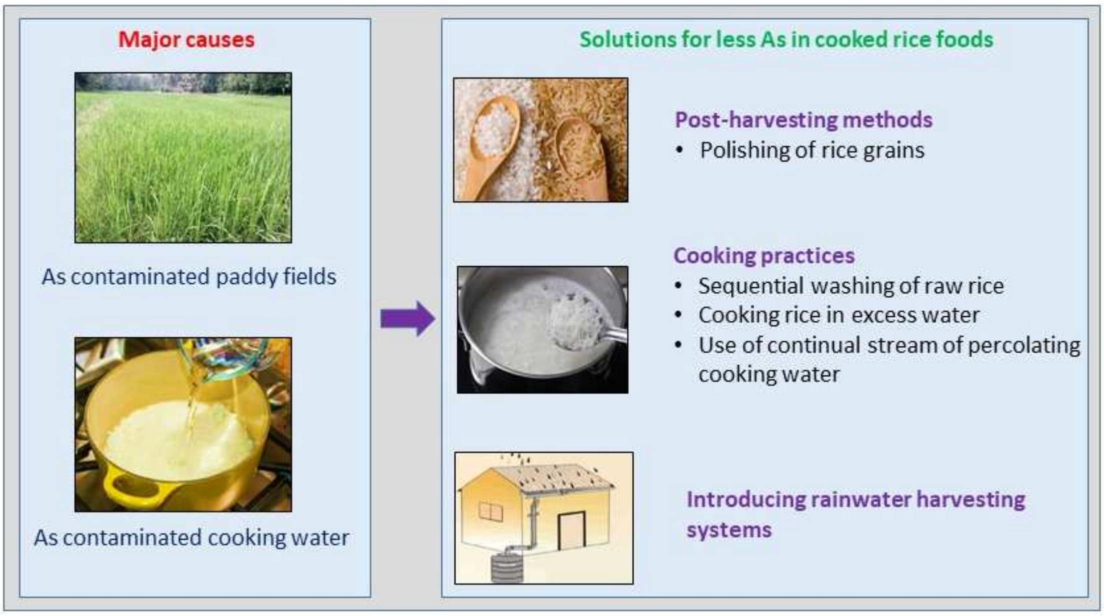


Fig. D. 5 The graphical abstract of article VII: Arsenic in cooked rice foods: Assessing health risks and mitigation options.

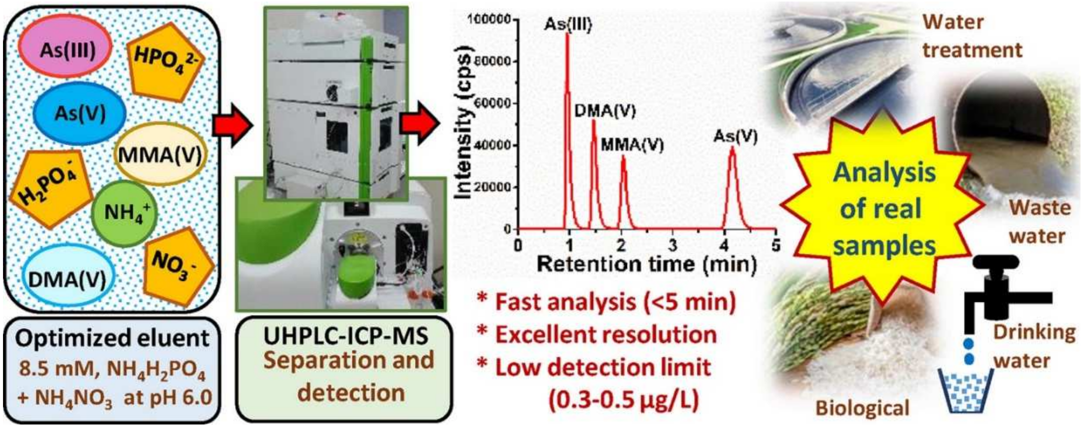


Fig. D. 6 The graphical abstract of article VIII: A fast analytical protocol for simultaneous speciation of arsenic by Ultra-High Performance Liquid Chromatography (UHPLC) hyphenated to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as a modern advancement in liquid chromatography approaches.

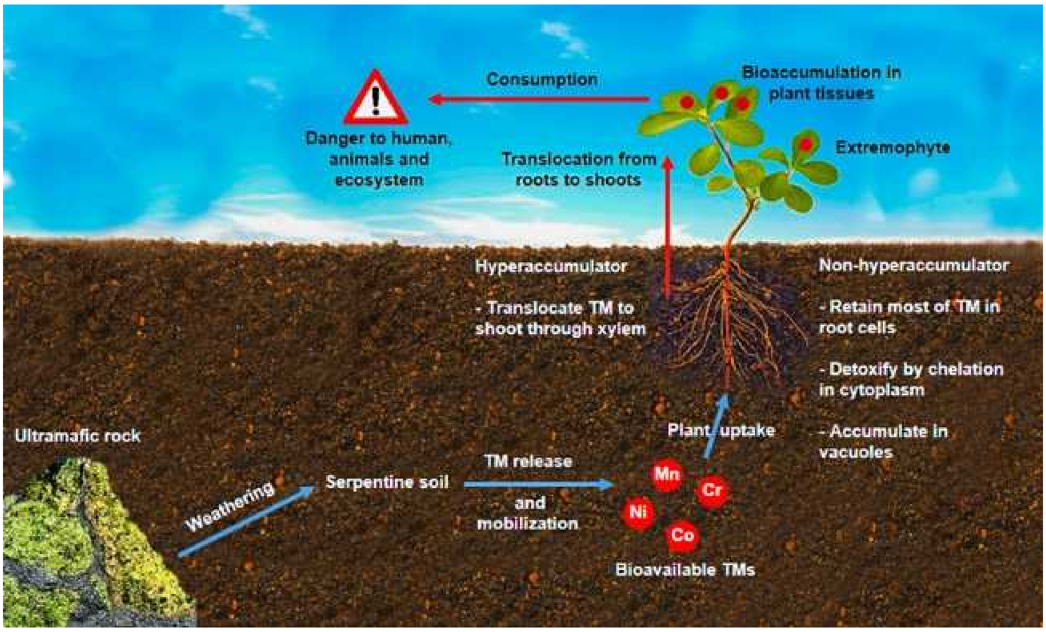


Fig. D. 7 The graphical abstract of article IX: Occurrence and cycling of trace elements in ultramafic soils and their impacts on human health: a critical review.

Appendix E

Presentations in international conferences

7th International Congress on Arsenic in the Environment. Beijing, China. 1-6 July 2018.

International Society of Groundwater for Sustainable Development

ISGSD

7th International Congress & Exhibition

Arsenic in the Environment

Environmental Arsenic in a Changing World (As2018)

Beijing, China

July 1-6, 2018

As China 2018

Certificate of Participation

This is to certify that Prasanna Kumarathilaka **has participated in the 7th International Congress on Arsenic in the Environment – *Environmental Arsenic in a Changing World* (As2018) organized in Beijing, China from 1-6 July, 2018.**

Yong-Guan Zhu, PhD
Professor, Chair –As2018
Institute of Urban Environment, Chinese Academy of Sciences

Appendix F

Invited talks in international webinars

Invited speaker in international webinar on Emerging Materials and Technology for Water Purification, Department of Physics, Jawaharlal Nehru Collage Pasighat, Arunachal Pradesh, India, 12th August 2020.

INTERNATIONAL WEBINAR

"EMERGING MATERIALS AND TECHNOLOGY FOR WATER PURIFICATION"

Department of Physics
in collaboration with IQAC,
Jawaharlal Nehru College Pasighat,
Arunachal Pradesh, India.

Dr. Milorai Modi
Patron & Principal
JNC Pasighat,
Arunachal Pradesh,
India

DATE AND TIME: 12th August 2020 at 10 am to 5 pm

Online platform:
Zoom

SPEAKERS

Dr. PRATIMA SOLANKI
Assistant Professor,
Special Centre for Nanoscience,
Jawaharlal Nehru University
New Delhi

Dr. ROBIN KUMAR DUTTA
Professor,
Department of Chemistry,
Tezpur University,
Assam

Dr. ANKUR SARASWAT
Senior Manager
Research and Testing
Development alternatives,
New Delhi

Dr. PRASANNA RUMESH KUMARATHILAKA
School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences,
University of Southern Queensland, Australia

Target Audience
Faculties, Academician, Research Scholars, Students.

E-Certificate will be awarded to all the participants

Free Registration:
Link: https://forms.gle/9CSQYMiWxWSMDFEP9

Join WhatsApp group:
https://chat.whatsapp.com/F0hvYUh9wf1Joi7sKfp5pM

For Further Information Contact:
7005697268 / 9643639435 E-mail: webinarinternational20@gmail.com

Dr. D.P. Panda
Coordinator, IQAC
JNC Pasighat,
Arunachal Pradesh

Dr. S. Jayanthi
Head of Dept. Physics
JNC Pasighat,
Arunachal Pradesh

Webinar Coordinators:

Dr. Yana Bagbi
Assistant Professor
JNC Pasighat,
Arunachal Pradesh

Mr. Tanik Libang
Assistant Professor
JNC Pasighat,
Arunachal Pradesh

CERTIFICATE

IQAC
J.N.C. PASIGHAT

International Webinar on Emerging Materials and Technology for Water Purification

DEPARTMENT OF PHYSICS, JAWAHARLAL NEHRU COLLEGE, PASIGHAT
INTERNAL QUALITY ASSURANCE CELL, JAWAHARLAL NEHRU COLLEGE, PASIGHAT

This is to certify that *Dr. Prasanna Rumesh Kumarathilaka* of *School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, Australia* delivered a lecture on *Physio-Chemical and Biological Approaches to mitigate Arsenic Bioavailability in Paddy Soil-Water Systems.* in the International Webinar on Emerging Materials and Technology for Water Purification.

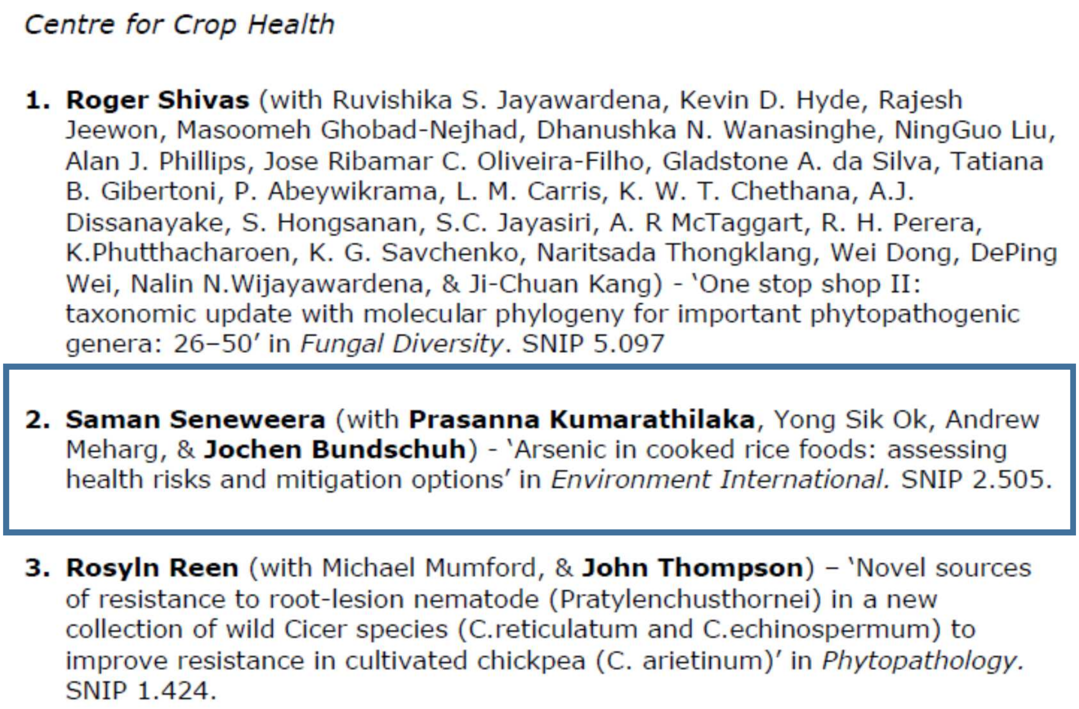
Conducted live by the Department of Physics, Jawaharlal Nehru College, Pasighat.
In collaboration with the IQA Cell, Jawaharlal Nehru College, Pasighat, on 12th August 2020.

Dr. S. Jayanthi Head, Dept. of Physics J.N. College, Pasighat	**Dr. D.P. Panda** IQAC Coordinator J.N. College, Pasighat	**Dr. Yana Bagbi** Webinar Coordinator J.N. College, Pasighat	**Mr. Enuk Libang** Webinar Coordinator J.N. College, Pasighat	**Dr. Milorai Modi** Patron & Principal J.N. College, Pasighat

Appendix G

Research awards

USQ 2020 research award, University of Southern Queensland, Australia.



Centre for Crop Health

1. **Roger Shivas** (with Ruvishika S. Jayawardena, Kevin D. Hyde, Rajesh Jeewon, Masoomeh Ghobad-Nejhad, Dhanushka N. Wanasinghe, NingGuo Liu, Alan J. Phillips, Jose Ribamar C. Oliveira-Filho, Gladstone A. da Silva, Tatiana B. Gibertoni, P. Abeywikrama, L. M. Carris, K. W. T. Chethana, A.J. Dissanayake, S. Hongsanan, S.C. Jayasiri, A. R McTaggart, R. H. Perera, K.Phutthacharoen, K. G. Savchenko, Naritsada Thongklang, Wei Dong, DePing Wei, Nalin N.Wijayawardena, & Ji-Chuan Kang) - 'One stop shop II: taxonomic update with molecular phylogeny for important phytopathogenic genera: 26–50' in *Fungal Diversity*. SNIP 5.097
2. **Saman Seneweera** (with **Prasanna Kumarathilaka**, Yong Sik Ok, Andrew Meharg, & **Jochen Bundschuh**) - 'Arsenic in cooked rice foods: assessing health risks and mitigation options' in *Environment International.* SNIP 2.505.
3. **Rosyln Reen** (with Michael Mumford, & **John Thompson**) – 'Novel sources of resistance to root-lesion nematode (Pratylenchusthornei) in a new collection of wild Cicer species (C.reticulatum and C.echinospermum) to improve resistance in cultivated chickpea (C. arietinum)' in *Phytopathology.* SNIP 1.424.

Appendix H

Recognition of the review contributed to journals

ELSEVIER

Chemosphere

Certificate of Reviewing

Awarded since November 2017 (6 reviews)
presented to

PRASANNA KUMARATHILAKA

in recognition of the review contributed to the journal

The Editors of Chemosphere

ELSEVIER

Environmental Pollution

Certificate of Reviewing

Awarded since August 2018 (1 review)
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PRASANNA KUMARATHILAKA

in recognition of the review contributed to the journal

The Editors of Environmental Pollution

ELSEVIER

Science of the Total Environment

Certificate of Reviewing

Awarded since October 2017 (2 reviews)
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PRASANNA KUMARATHILAKA

in recognition of the review contributed to the journal

The Editors of Science of the Total Environment

ELSEVIER

Food Control

Certificate of Reviewing

Awarded since March 2018 (1 review)
presented to

PRASANNA KUMARATHILAKA

in recognition of the review contributed to the journal

The Editors of Food Control

ELSEVIER

Journal of Environmental Management

Certificate of Reviewing

Awarded since July 2019 (1 review)
presented to

PRASANNA KUMARATHILAKA

in recognition of the review contributed to the journal

The Editors of Journal of Environmental Management

ELSEVIER

Environmental Technology & Innovation

Certificate of Reviewing

Awarded since August 2018 (1 review)
presented to

PRASANNA KUMARATHILAKA

in recognition of the review contributed to the journal

The Editors of Environmental Technology & Innovation

Water Research

Certificate of Reviewing

Awarded since October 2019 (1 review)
presented to

PRASANNA KUMARATHILAKA

in recognition of the review contributed to the journal

The Editors of Water Research

Process Safety and Environmental Protection

Certificate of Outstanding Contribution in Reviewing

awarded October 2018 to

Prasanna Kumarathilaka

in recognition of the contributions made to the quality of the journal

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Journal of Hazardous Materials

Certificate of Reviewing

Awarded since March 2020 (1 review)
presented to

PRASANNA KUMARATHILAKA

in recognition of the review contributed to the journal

The Editors of Journal of Hazardous Materials