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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}

^aSchool of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, Toowoomba, Queensland, Australia; ^bCenter for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, Toowoomba, Queensland, Australia; ^cNational Institute of Fundamental Studies, Kandy, Sri Lanka; ^dKorea Biochar Research Center & Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Korea; ^eInstitute for Global Food Security, Queen's University Belfast, Belfast, UK; ^fUNESCO 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 world-wide 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

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 ($\text{Si}(\text{OH})_4$)), and the activity of microorganisms also influence the mobility, bioavailability and speciation of As in paddy agroecosystems (Bhattacharya et al., 2007; Kumarathilaka, Seneweera, Meharg, & Bundschuh, 2018a; Xu, Chen, Wang, Kretzschmar, & Zhao, 2017). Arsenite (As(III)) and arsenate (As(V)), and monomethylarsonic 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 ($\mu\text{g kg}^{-1}$)					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	—	—	Basu et al. (2015)
	Continuous flooding (5 cm of water applied at every 3-day interval)	627	—	—	6790	—	6790	
—	Intermittent (5 cm of water applied if cracks were found)	495	—	—	5930	—	5930	Honma, Ohba, Kaneko, et al. (2016)
	Aerobic (1 cm of water applied on alternative days to aeration)	549	—	—	6450	—	6450	
	Koshihikari	Continuous flooding	390	290	20	—	—	—
IAPAQ-9	Rainfed	110	70	4	—	—	—	
	Continuous flooding	210	—	—	—	—	—	
	Aerobic (water was discharged to leave a water layer ~5 cm deep)	140	—	—	—	—	—	
37 genotypes	Intermittent (3 cm of water applied if was dried)	140	—	—	—	—	—	Spanu, Daga, Orlandoni, and Sanna (2012)
	Conventional (flooded until full tillering stage followed by intermittent irrigation)	150	—	—	—	—	—	
	Continuous flooding	95–235	—	—	—	—	—	
—	Sprinkler irrigation	1.3–5.1	—	—	—	—	—	

^aSum of As(III) and As(V) as inorganic As species.

^bSum 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 (S²⁻) 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 CdCO₃ (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 (SO₄²⁻) 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 ($6\text{--}8\text{ mg kg}^{-1}$ per a growing season) into As contaminated paddy soils ($9\text{--}102\text{ mg kg}^{-1}$) 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

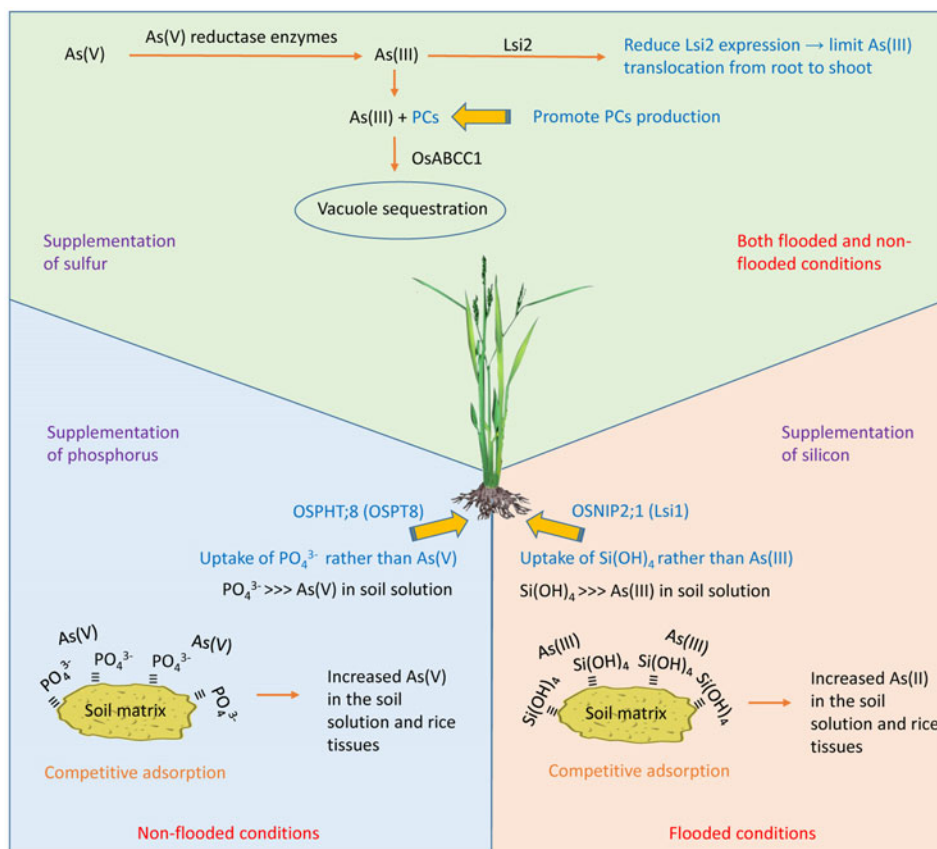


Figure 1. Effects of nutrient supplementation on As bioavailability in paddy soils and As uptake and translocation in rice plants.

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 ($\text{HAsS}_n\text{O}_{4-n}^{2-}$, $n = 1-4$) in As contaminated rice agroecosystems (Kerl, Rafferty, Clemens, & Planer-Friedrich, 2018). More precisely,

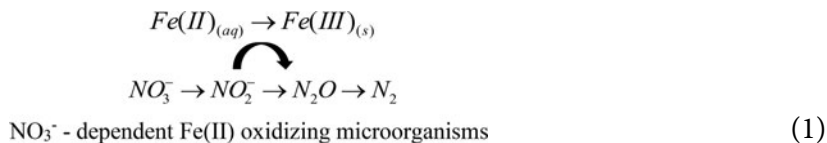
thioarsenates are formed spontaneously under sulfate reducing conditions from As(III) through the exchange of OH^-/SH^- -ligands and the oxidative addition of S (Planer-Friedrich et al., 2015; Planer-Friedrich, Suess, Scheinost, & Wallschläger, 2010). However, different factors such as S(-II)/As(III) 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.



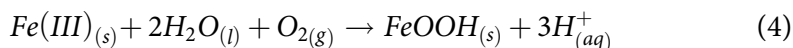
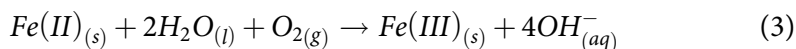
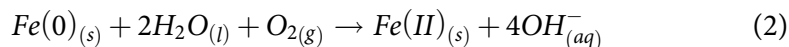
Detailed investigations on the influence of N supply on As mobility, bioavailability, and accumulation in rice tissues are lacking. Since addition of N fertilizer indirectly affects the total As concentration in rice tissues, the abundance of microorganisms and indigenous Fe content in paddy soils may 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 ($\text{Fe}_5\text{HO}_8 \cdot 4\text{H}_2\text{O}$), lepidocrocite ($\gamma\text{-FeOOH}$), hematite ($\alpha\text{-Fe}_2\text{O}_3$), and goethite ($\alpha\text{-FeOOH}$) (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).



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 O₂ 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 sequestering 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 ₃ O ₄ : 80% and Fe ₂ O 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.

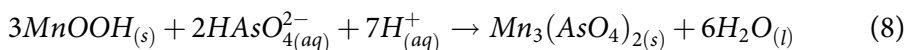
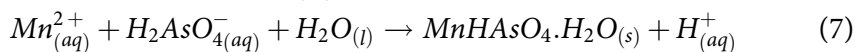
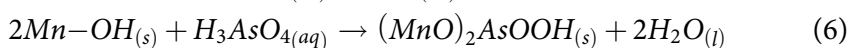
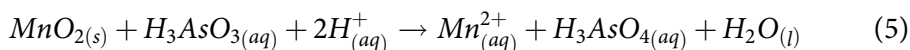
The effect of Fe amendment on As accumulation in rice grains also depends on the growth stages of the plant. Studies related to Fe amendment have not focused on As accumulation in rice grains for a fully rice growing cycle and were mainly performed in hydroponic cultures. In a recent study, Yu, Wang, 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 $1200 \text{ mg Mn kg}^{-1}$ 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.



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 to be examined. For instance, contamination of drinking water sources with Mn ($400 \mu\text{g L}^{-1}$) 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 bio-availability 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.

Biomass type	Pyrolysis temperature (°C)	Agent/s used for modification	Physico-chemical properties of BC				Arsenic increment (↑) / decrement (↓) compared to the control in soil solution / rice tissues (%)	Reference
			Application rate (%)	pH	pH _{ZPC}	Specific surface area (m ² g ⁻¹)	Elements concentration (mg kg ⁻¹)	
Rice straw	500	—	3	10.50	—	—	K – 23,684 Fe – 750 As – 1.4 P – 46.9 Fe – 740 As – 10.6 P – 55.1 Fe – 35,500 As – 9.3	↑ 202 – total As (soil solution) Wang et al. (2017)
Rice straw	450	—	1–3	10.70	—	—	—	↑ 8.1–39 total As (root) Yin et al. (2017)
Rice straw	450	Fe	0.5–2	4.87	—	—	—	↓ ~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) ↑ 327 total As (root) Khan et al. (2014)
Rice straw, husk, and bran	500	—	5	7.20–8.2	—	—	—	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	8.93	3.18	Mn – 7.41 ^a	↓ 13.9–19.8 total As (grain) Yu, Qiu, et al. (2017)
Corn straw	600	—	0.5–2.0	—	9.60	60.97	K – 112.3	↓ 13.8–39.0 total As (root) Lin et al. (2017)
		BC:KMnO ₄ :Fe(NO ₃) ₃ ; 25:4:1			3.17	208.0	K – 261.4	↓ 11.1–20.5 total As (stem)
		BC:KMnO ₄ :FeSO ₄ ; 18:3:1				7.53	K – 259.2	↓ 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)

^aPercentage (%).

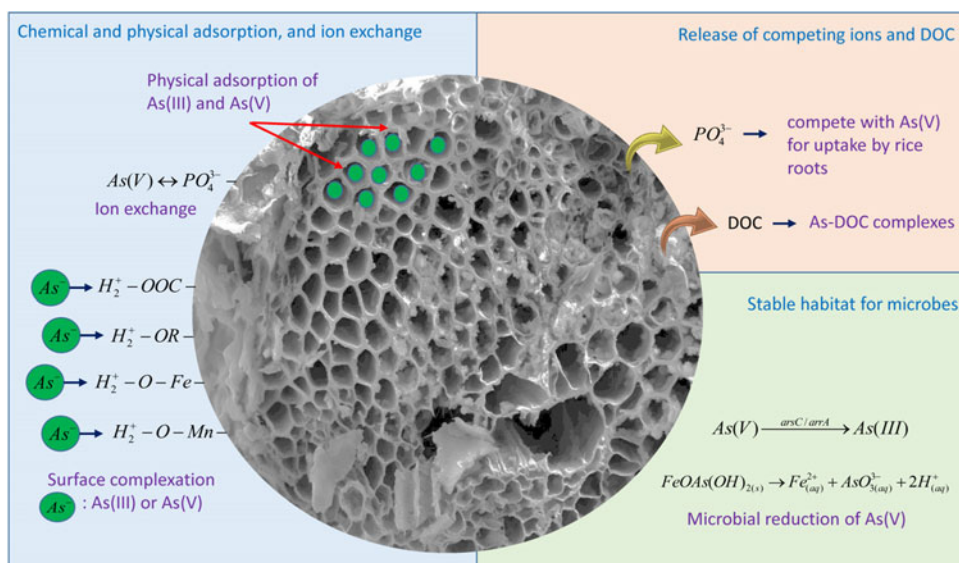


Figure 2. Possible interactions between BC (pristine and modified) and As in the paddy agroecosystem.

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 physico-chemical 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): $\text{MMA(V)} \rightarrow \text{monomethylarsonous acid (MMA(III))} \rightarrow \text{DMA(V)} \rightarrow \text{dimethylarsinous acid (DMA(III))} \rightarrow \text{arsines (monomethylarsine ((CH}_3\text{)AsH}_2\text{), dimethylarsine ((CH}_3\text{)}_2\text{AsH), trimethylarsine (As(CH}_3\text{)}_3\text{, and arsine (AsH}_3\text{))}$ (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

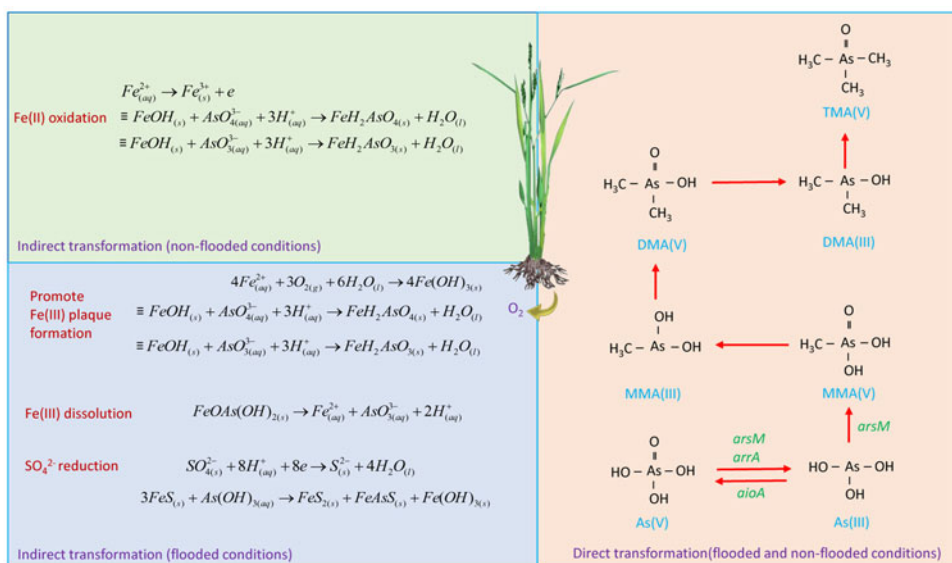


Figure 3. Direct and indirect As transformation pathways mediated by microbes in the rice rhizosphere.

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 $483.2 \mu\text{g kg}^{-1}$ 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 sequester 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. *Anaeromyxobacter*, *Desulfuromonas*, *Desulfocapsa*, *Desulfobulbus*, *Geobacter*, *Lacibacter*, and *Ohtaekwangia*) were present in higher abundance under flooded conditions, while the Fe(II) and S^{2-} -oxidizing genera (i.e. *Acinetobacter*, *Lysobacter*, *Ignavibacterium*, 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 PO₄³⁻ fertilizers (i.e. (NH₄)₂HPO₄) 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 mosseae*) 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-Sabaris 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 through 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 O₂ 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 O₂ 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 O₂ 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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