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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

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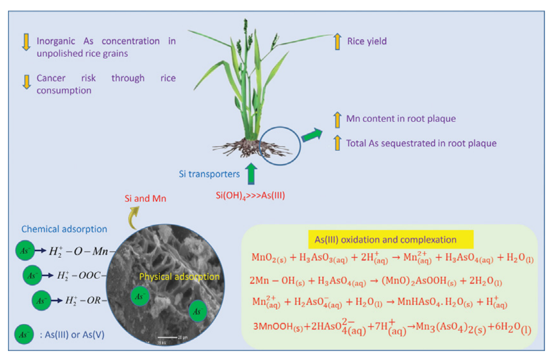
HIGHLIGHTS

- 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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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).

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

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).

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

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).

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

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.

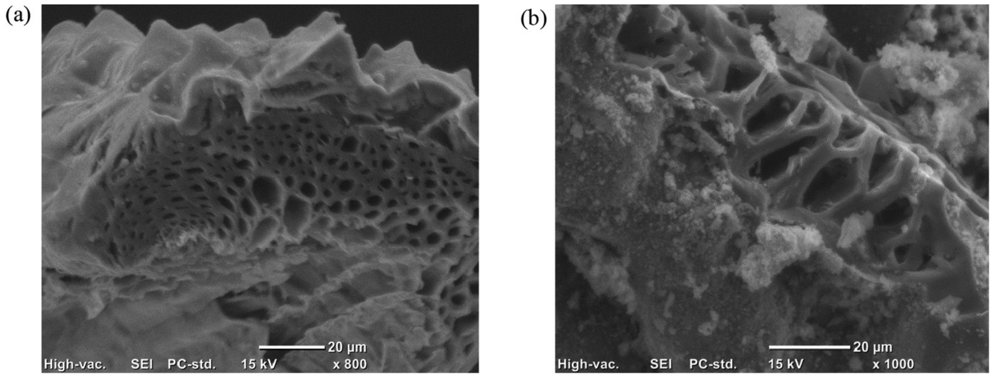

Fig. 1. Scanning electron microscopy images before (a) and after modification to Mn-RBC (b).

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)

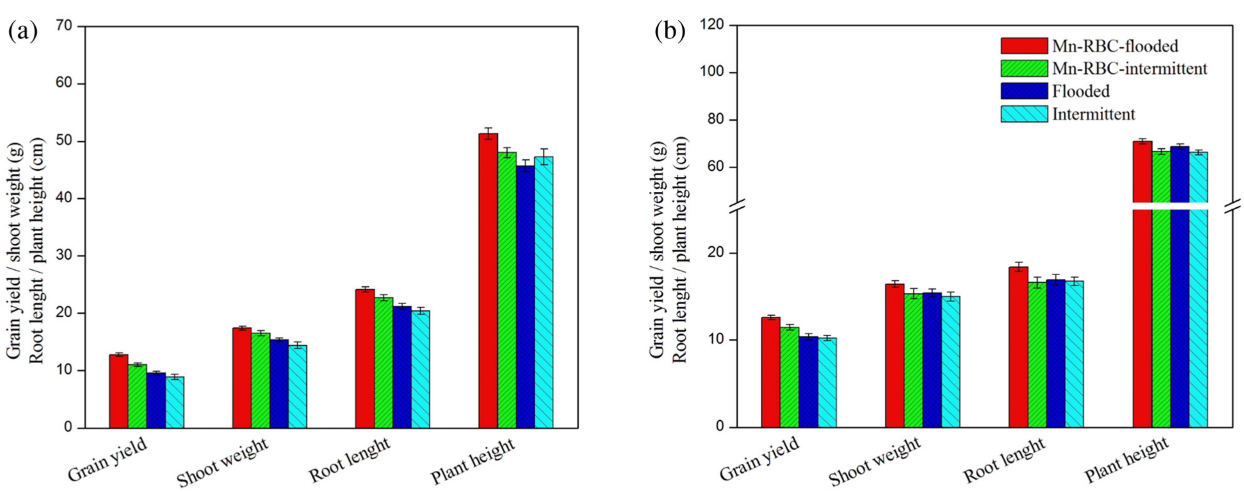

Fig. 2. Plant growth parameters under different treatments in two different rice varieties (a) *Jayanthi*, (b) *Ishikari*.

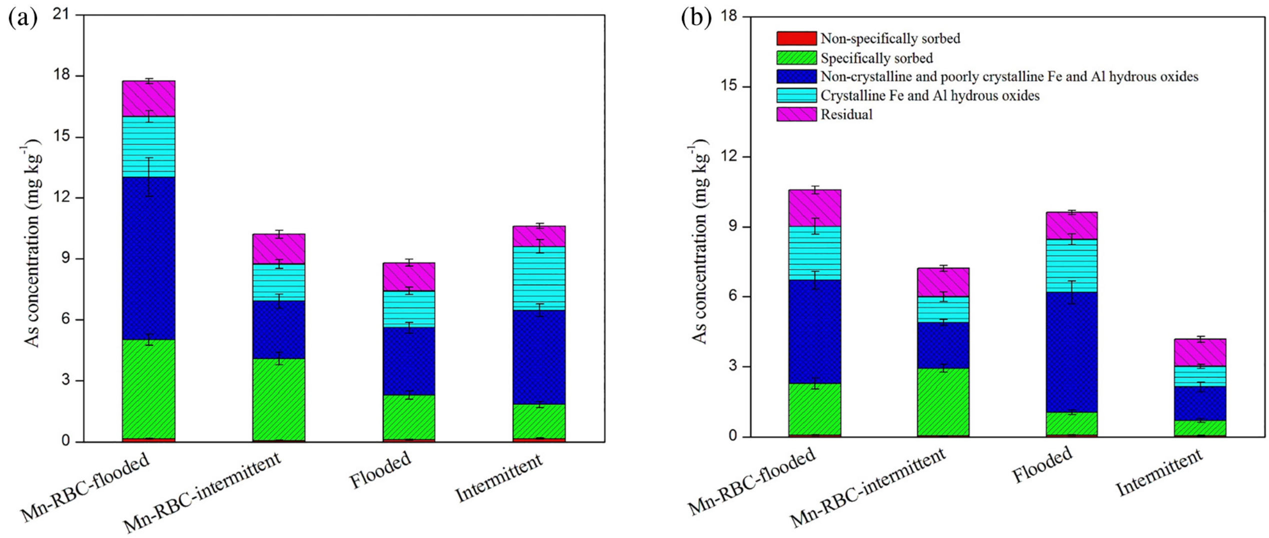

Fig. 3. Arsenic bound to different fractions (non-specifically sorbed, specifically sorbed, bound to non-crystalline and poorly crystalline Fe and Al hydrous oxides, bound to crystalline Fe and Al hydrous oxides, and residual) under different combination of Mn-RBC and water management approaches (a)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

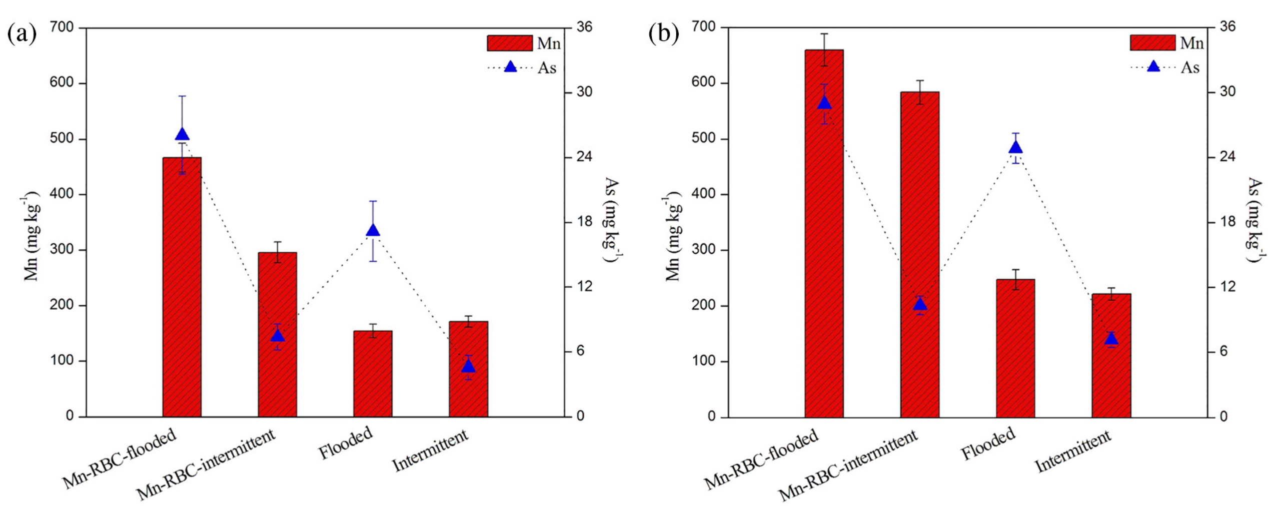

Fig. 4. Manganese and As concentration in root plaque in different combinations of Mn-RBC and water management regimes (a) *Jayanthi*, (b) *Ishikari*.

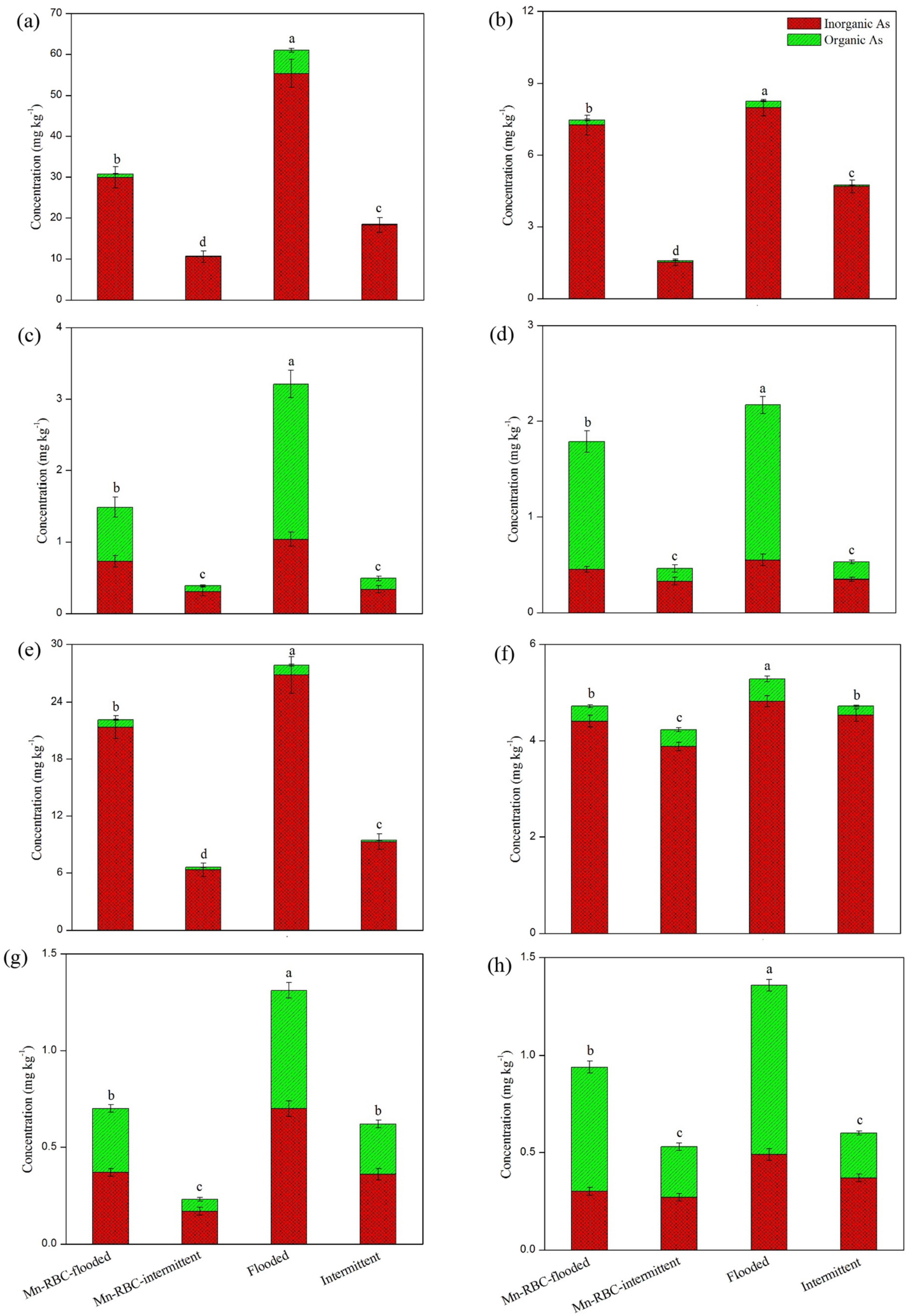

Fig. 5. Arsenic content in rice tissues under different combinations of Mn-RBC and water management approaches; *Jayanthi*: (a) roots, (b) shoots, (c) husks, and (d) unpolished grains, *Ishikari*: (e) roots, (f) shoots, (g) husks, and (h) unpolished grains. Treatments labelled with the same letter are 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 ($p \leq 0.05$) between each treatment. In variety *Jayanthi*, total As concentrations in husks and unpolished rice grains were significantly different ($p \leq 0.05$) in each treatment except between Mn-RBC-intermittent and intermittent treatments. In variety *Ishikari*, the total As concentration in husks was significantly different ($p \leq 0.05$) in each treatment whereas total As concentration in unpolished rice grains was not significantly different ($p \leq 0.05$) between Mn-RBC-intermittent and intermittent treatments. Moreover, total As contents in unpolished rice grains were not significantly different ($p \leq 0.05$) 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%,

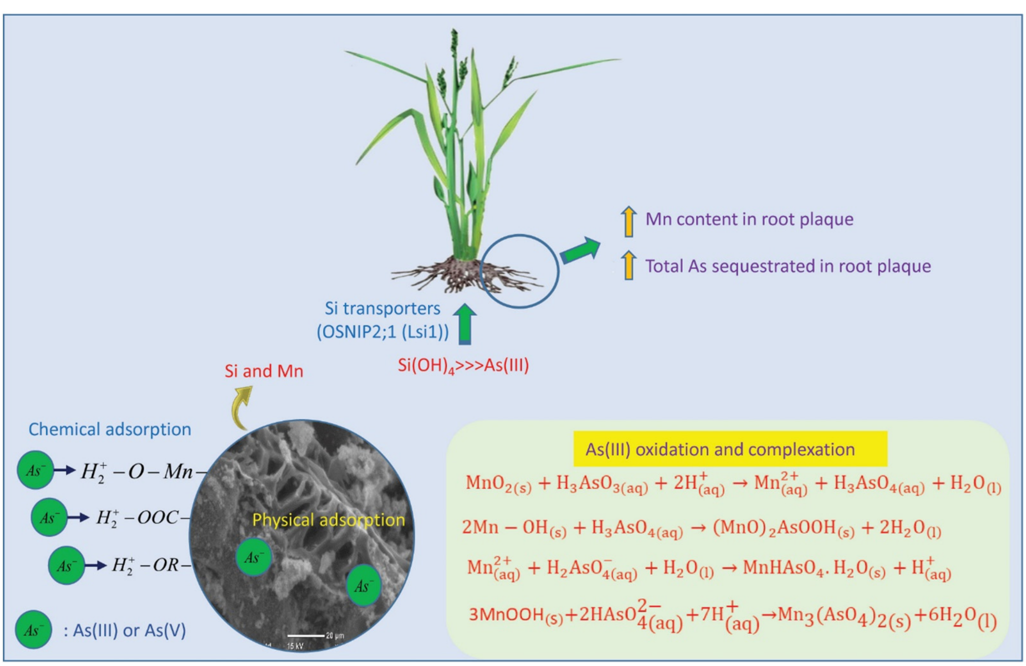

Fig. 6. Potential mechanisms involved in decreasing As bioavailability in the paddy soil-water system following the application of Mn-RBC.

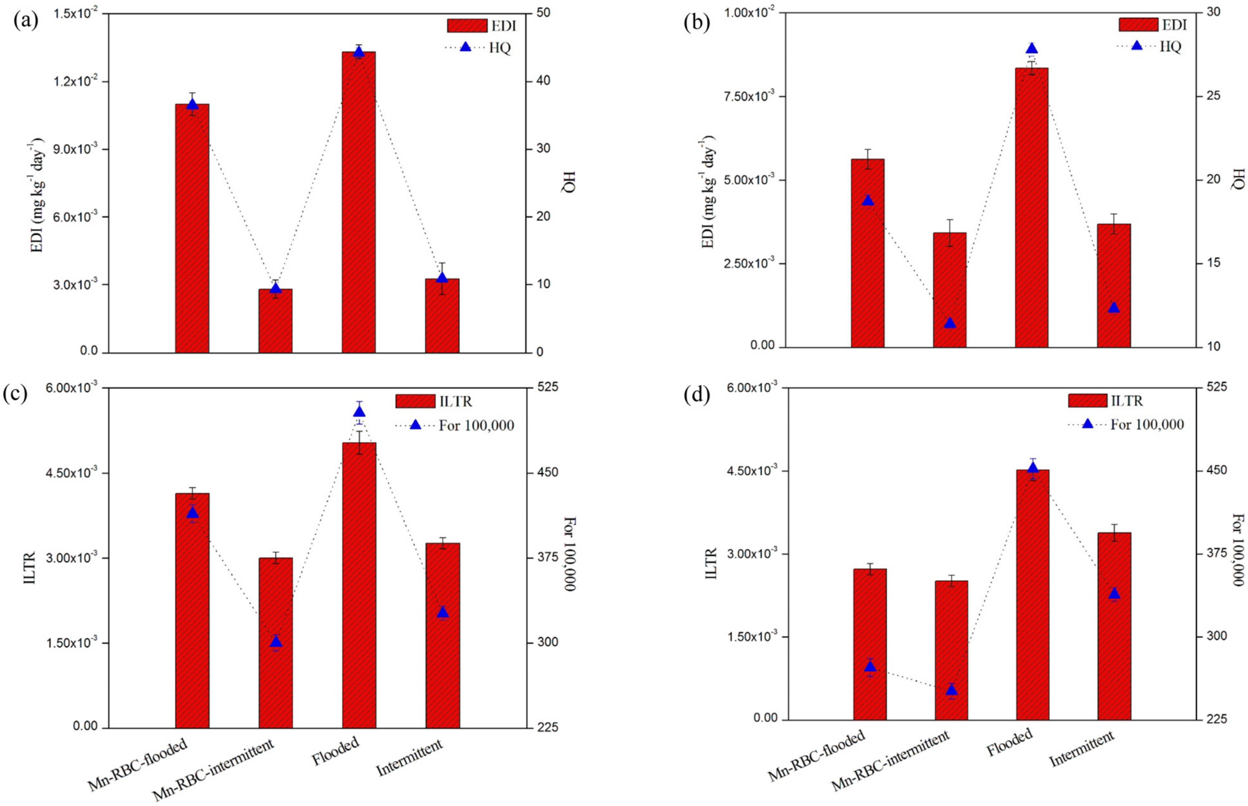

Fig. 7. Health risk indexes (EDI, HQ, ILTR, and cancer risk for 100,000 people) under different combination of Mn-RBC and water management techniques in *Jayanthi* (a and c) and *Ishikari* (b and d).

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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