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Simultaneous stimulation of arsenic methylation and inhibition of cadmium bioaccumulation in rice grain using zero valent iron and alternate wetting and drying water management

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ABSTRACT. Studies aiming to limit bioaccumulation of arsenic (As) and cadmium (Cd) in rice grain has attracted global attention. In this study, simultaneous impact of zero valent iron (Fe^0) and various water management regimes (continuous flooding (CF), alternate wetting and drying (AWD) and low water (LW)) on rice grain yield (GYM) and bioaccumulation of As and Cd in unpolished rice grain (URG) were investigated. Compared to respective control treatments, Fe^0 significantly reduced GYM under LW by 32% ($p < 0.001$) and significantly increased GYM under AWD by 24% ($p = 0.009$; $F = 5.9$) but had no significant effect on GYM under CF water management regime ($p > 0.05$). The grain harvest index was significantly higher in Fe^0 amended rice under AWD (67%; $p < 0.001$) and CF (35%; $p = 0.001$) compared to those without Fe^0 amendment. Fe^0 significantly reduced tAs in URG

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under LW by 32% ($p < 0.017$) and significantly increased tAs in URG under AWD by 37% ($p = 0.007$) but had no significant effect on tAs in URG under CF ($p > 0.05$). The Cd concentrations were significantly reduced by 51% ($p = 0.002$) and 61% ($p < 0.003$) in URG under LW and AWD respectively compared to the respective control treatments. The Dimethylarsinic acid (DMA) in unpolished rice (URG) under AWD($+Fe^0$) was significantly higher ($p < 0.01$; $F = 11.3$) compared to that accumulated in URG under AWD(-Fe°). Despite increasing As accumulation in AWD water management, simultaneous use of AWD water management and Fe° increased grain yield, enhanced accumulation of less toxic methylated As in rice grains and accumulated low Cd concentrations comparable to that attainable with CF water management indicating that simultaneous use AWD and Fe° can be effective in controlling Cd accumulation in paddies highly contaminated with Cd.

KEYWORDS. Arsenic; cadmium; Rice cultivars; zero valent iron (Fe°); Faya cv; continuous flooding; alternate wetting and drying; low water

1. Introduction

Rice (*Oryza sativa L.)* is a main staple carbohydrate source for over half of the world population (Gnanamanickam, 2009) sustaining about 35-60% dietary caloric intake for over 3 billion people worldwide, predominately in Asia (Fageria, 2007; Gnanamanickam, 2009). In addition, rice is an important source of essential nutrients including iron (Fe) and selenium for humans (Gnanamanickam, 2009). Unfortunately, rice plants also efficiently up-take arsenic (As) and cadmium (Cd) from soil solution, and bio-accumulate them in roots, shoots, panicles and grains to a higher degree compared to other cereal crops. Arsenic, being ubiquitous element in the environment, is found in rice in both organic and inorganic forms, with inorganic As (iAs) species being more toxic compared to organic arsenic (oAs) species

(Islam et al., 2017b). Cadmium, another abundant toxic trace element in the envirnoment, is highly mobile and highly transferable from soil to plants with a high bioconcentration factor (bcf) (Song et al., 2015). Both As and Cd are classified as Group 1 non-threshold carcinogens to humans (Islam et al., 2017a). Consumption of large amounts of rice containing elevated levels of carcinogenic elements exposes humans to increased health risks (Rahman et al., 2006; Syu et al., 2015). The risks include kidney damage/tubular renal dysfunction (Simmons et al., 2003), cardiovascular respiratory effects and skeletal lesions (Simmons et al., 2003) for Cd exposure; and lungs cancer, urinary tract cancer and skin cancer such as skin lesions, hyperkeratosis and pigmentation changes for ingestion of food contaminated with iAs (Honma et al., 2016; Makino et al., 2016). Infants and young children are at high risk of ingesting elevated levels of As and Cd primarily considering they have higher consumption rates of rice products per kg body weight compared to adults, various food regulatory bodies including The Commission of the European Communities have legislated the maximum contaminant limits (MCL) for As and Cd in rice including rice destined for production of baby food (The Commission of the European Communities, 2015) in order to protect infants and young children from ingesting elevated levels of the carcinogens (As and Cd). The Commission of the European Communities legislated the maximum contaminant limit (MCL) of 250 and 200 µg/kg for iAs and Cd respectively in husked rice (brown rice) and 100 µg/kg for iAs concentrations in rice destined for production of baby food.

In response, various agronomical practices and techniques have been suggested and tested to identify techniques that significantly limit accumulation of these carcinogens in rice. One of these techniques manipulates the rice paddy water management regime by shifting rice production from traditional continuous flooding (CF) water management to alternate wetting and drying (AWD) water management regime. Rice plants, under AWD water management receive alternate treatments of flooded (anaerobic) and unflooded (anaerobic) environments;

whereas rice plants grown under CF water management are exposed to continuous flooded conditions throughout the growing period. Several studies (Makino et al., 2016; Norton et al., 2017; Yang et al., 2017) have reported that AWD water management reduces As concentration in rice grains and shoots compared to CF water management but increases Cd accumulation in rice grains. The cited differences have been linked to impacts of oxic and anoxic conditions on bioavailability and mobility of As and Cd as well as on their conversion from one oxidation state to the other. For instance, under anaerobic conditions, arsenate (AsV) readily converts into arsenite (AsIII) due to solubility differences (Henke and Hutchison, 2009) thereby increasing abundance and availability of AsIII compared to AsV. In contrast, AsV species are dominant in aerobic conditions due to oxidation of AsIII to AsV which increases availability of AsV for plant uptake. Nevertheless, AsV converts faster into AsIII in reducing environments than AsIII does into AsV under oxidizing conditions (Henke and Hutchison, 2009) which simultaneously increase availability of AsIII in reducing environments hence increased plant uptake. Furthermore, AsV and AsIII species are exported into the plant by different transport proteins: AsIII (a silicate analogue) is taken up through the aquaporin Lsi1 membrane protein (silicon transporters) whereas AsV (an analogue of phosphate) is taken up through the phosphate pathway (Ahmed, 2009; Ma et al., 2008).

Cadmium, unlike As, is readily released into soil solution under oxidizing conditions but not under reducing conditions hence Cd availability is elevated under oxic condition for rice plants up-take (Rinklebe et al., 2016). In contrast, there is a decreased availability of Cd under reducing conditions (anaerobic) soil/water solutions which is linked to high soil pH and/or metal-sulfide precipitation (Rinklebe et al., 2016).

Soil amendment with sorbents is another agronomical practice suggested to reduce As and Cd accumulation in rice. Considering that bioavailability and mobility of As and Cd in soil is mainly controlled by adsorption/desorption processes and/or co-precipitation with metal oxides (Kumpiene et al., 2008), use and application of materials that immobilize these elements in rice fields reduce bioavailability and bio accessibility of these elements by rice plants. Amendments of soil with iron (Fe) containing materials as sorbents have been extensively studied for As immobilization (Farrow et al., 2015; Kumpiene et al., 2008; Qiao et al., 2018). It has been reported that the mobility of As in soil is inversely proportional to Fe and Al contents in soil (Kabata-Pendias and Pendias, 2001). Arsenic in soil or soil porewater is immobilized through adsorption onto Fe oxides by replacing the surface hydroxyl groups with the As ions, in addition to the formation of amorphous FeIII arsenates (Kumpiene et al., 2008). Types and amounts of sorbents present in soil porewater influence mobility, bioavailability and solubility of elements such as arsenic and Cd in soil porewater behaves differently. For instance, zero-valent iron (Fe°) as sorbent provides high adsorption capacities for As especially AsV (Farrow et al., 2015; Qiao et al., 2018) in oxic soils, since AsV, a dominant species of As in oxic soils, strongly sorbs onto Fe° minerals. In contrast, in submerged soils, mobility of AsIII is higher than AsV because of reductive dissociations of iron oxides in addition to consequent reduction of AsV to AsIII which is prevalent in reducing environments. Qiao *et al.* (2018) showed that Fe° amendment significantly decreased As bioavailability in CF water regime. However, the impact of Fe° amendment under AWD water management is unknown. Due to increasing demand for production of food worldwide, there is a need to identify agronomical practices that significantly reduce accumulation of toxic trace elements without negatively affecting yield and the quality of the food produced. The objectives of the present study were (i) to investigate the simultaneous impact of Fe° amendment and different water management on mobility and bioavailability of As and Cd in soil porewater under rice cultivation; (ii) to compare the effects of interaction of Fe° amendment and water management on roots, shoots and grain biomass and grain harvest index, and (iii) to determine simultaneous impact of Fe° amendment and different water management on As and Cd accumulation in rice shoots and grain.

2. Methods and experiment

2.1 Pot experiments

2.1.1 Soil preparations

Agricultural soil collected from Inch, Aberdeenshire, Scotland, was spread and placed in trays in a drying chamber at a temperature of 30 °C for 5 days to dry to constant weight. Large soil aggregates, stones and plant roots were removed from soil samples using a 4 mm sieve. Thereafter, a composite soil was prepared by mixing 1 part of sieved soil with 1 part of sand (to achieve a 1:1 soil to sand ratio; v/v). Initial soil sample pH (6.1) was measured in deionized water (with soil to water ratio of 1:2.5) after shaking for one (1) hour. Previously, the particle sizes and the initial As and Cd content of the soil-sand mix were determined (**Table SM1, Supplementary material**).

2.1.2 Treatments

The experiment used a 3 x 2 factorial design laid out with three (3) water management regimes (low (unflooded) water (LW), alternate wetting and drying (AWD) and continuous flooded (CF)); and two concentrations of zero valent iron (Fe°) amendments ((-Fe°): control/without Fe°, (*n*=4);) and (+Fe°): amended with 6.2 g Fe°/kg soil, (*n*=4)) (**Figure 1**). A total of 2.5 kg mass of dry soil/sand mix was placed in plastic tubes (height: 33 cm, diameter: 8.5 cm) after being homogeneously mixed with the correct amounts of Fe°. The Fe° used in this experiment had an average diameter of 3.9±1.01 mm. Rice, (cv. Faya 14M69), a Malawi landrace, usually cultivated under continuous flooding was used, one rice seed per well were germinated in 10 by 10 wells wide plastic trays and four (4) replicates of seedlings per Fe° amendment treatment were transplanted into tubes (one seedling per tube) after four weeks. Water levels in CF and LW irrigation regimes were respectively maintained at 3-4 cm and 6-10 cm above and below soil surface by resupplying water daily, while water level in the AWD tube was cycled from 3-4 cm above soil surface to 6-10 cm below soil surface every fortnight (14 days) (**Figure 1; Figure SM 1**, **Supplementary material**). Plants were fertilized by adding 33 mL of ammonium nitrate (Sigma Aldrich; St. Louis, MO) solution (0.1 mol/L) to each pot on 28th, 56th and 70th day after transplanting (DAT).

2.2 Sampling

2.2.1 Measurements of rice plant height (PHT) and pore water collection

Plant growth was determined by measuring plant height (PHT), root biomass (RBM), shoot biomass (SBM) and number of tillers (TL). PHT measurements and porewater collection were performed on the 30th, 59th, 99th, 124th, 142nd and 170th and 200th day after transplanting (DAT) which represented optimum DAT for active tillering (30th and 59th DAT), approximate on-set DAT and end-point DAT for booting/heading (99th and 110th DAT respectively), approximate on-set DAT and end point DAT of grain filling (110th and 142nd DAT respectively) (Fageria, 2007), and maturity (170th DAT) stages. Number of tillers per plant were counted on 14th, 28th, 56th, 70th and 170th DAT. The percentages of productive tillers were calculated as number of panicles that developed from tillers per plant (Wang et al., 2016). PHT were measured using a 1-m measuring ruler. Porewater was collected using rhizon samplers. The rhizon samplers, attached to 10 ml syringe were inserted into soil at an angle of 45°. Rhizon samplers were tightly capped to limit further oxidation of As species. Only 1 ml of porewater was immediately acidified with and diluted to 10 ml with 1% HNO_3 in ultra-pure water (milli-Q water, 18.2 MΩ cm) for determination of porewater total-As and

-Cd concentrations. Arsenic speciation analyses were conducted on porewater collected on 170th DAT only.

2.2.2 Harvesting and Plant biomass determination.

The rice plants were harvested on 210th DAT with shoots cut at 3 cm above soil level to avoid soil contamination. The plants were separated into roots, shoots and rice grains and roots and shoots were washed carefully with deionized water before being oven dried to constant weight at 80 °C for 72 h. Rice roots biomass (RBM), shoots biomass (SBM) and rice grains yield (GYM) were determined were using analytical balance. Total plant biomass dry weight (dw) was reported as sum of weight of roots, shoots and grains.

2.3 Trace elements analyses

2.3.1 Determination of total As and Cd in pore water

The total As and Cd concentrations in porewater were determined using inductively coupled plasma mass spectroscopy (ICP-MS, Agilent 7900 series). Three blanks that contained 1% HNO_3 (prepared from 70%, analytical grade obtained from Mallinckrodt Chemicals, USA) in ultra-pure water (milli-Q water, 18.2 MΩ cm) and certified reference materials (CRM) NCS DC73319 were prepared and measured alongside the porewater. Rhodium with a concentration of 10 μg/L was used as an internal standard. The mass charge ratio of 75 and 110 for analytes (As and Cd) were selected for measurement.

2.3.2 Determination of total As and Cd in rice plant samples

Shoots and unpolished rice grain (URG) samples were ground into fine powder using a ball mill (Retsch, MM-301, Germany) to obtain a composite sample. Exactly 0.10 g of each shoot and URG sample plus certified reference material (CRM) (NIST1568a, long grain rice flour) (in triplicates) were predigested in 2 mL concentrated nitric acid (HNO_3) (70%, analytical grade, obtained from Mallinckrodt Chemicals, USA) overnight followed by microwave digestion (MARS 5 digestion system at 95°C) the following day as described by Mlangeni et al., (2018) and Williams *et al.*, (2007). Samples were made up to 50.0 g weight with milli-Q water (18.2 MΩ cm) immediately after microwave digestion in readiness for measurement with inductively coupled plasma mass spectrometry (ICP-MS).

2.3.3 Arsenic speciation in URG and porewater

Arsenic species in URG samples plus certified reference materials (CRM) (NIST 1568b and BCR 211) were extracted using a 10 mL solution of 2% hydrogen peroxide (H_2O_2) (prepared from 30% H_2O_2, Fisher Scientific Co., Boston, U.S.) and 1% nitric acid in milli-Q water (18.2 MΩ cm) assisted by MARS 5 extraction (Mlangeni et al., 2018; Williams et al., 2007). For porewater, 1 mL of porewater was diluted with 9 ml of milli-Q water (18.2 MΩ cm) before being centrifuged at 3500 g for 12 min. Arsenic species (arsenites (AsIII), arsenates (AsV), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA)) were determined using high performance liquid chromatography (HPLC) (Agilent technologies, 1200 Series; using a Hamilton PRPX100 10-µm anion-exchange column (250 × 4.1 mm)) tandem inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, 7900). The instrumental analytical parameters were optimized as reported previously by Mlangeni et al. (2018).

2.4 Data analyses

2.4.1 Statistical analyses

All data were analyzed using analysis of variance (ANOVA), general linear model, with Minitab 18 Statistical Software and Microsoft Office Excel. Fisher pairwise comparisons were used for multiple comparisons between treatment means at $p < 0.001$, $p < 0.01$ and/or $p < 0.05$ significant levels. Pearson's correlations between URG-As and URG-Cd were also determined using Microsoft Office Excel and tested for significance using Minitab 18 Statistical Software.

2.4.2 Arsenic and cadmium transfer factor

The As and Cd bioconcentration factors (As-bcf and Cd-bcf) in shoots and URG were determined as ratios of As and Cd concentration in shoots and URG to that in porewater (Equation 1 and 2) whereas the As and Cd transfer factors (As-TF and Cd-TF) in URG were determined as ratio of As and Cd concentration in URG to that in shoots. In this study, bcf and TF were calculated as shown in (Equation 1 and 2) (Song et al., 2015; Xie et al., 2016).

$$\mathrm{bcf} = \frac{\mathrm{M(R)}}{\mathrm{M(pw)}} \qquad \text{Equation 1}$$

$$\mathrm{TF} = \frac{\mathrm{M(R1)}}{\mathrm{M(R2)}} \qquad \text{Equation 2}$$

Where bcf is either As-bcf or Cd-bcf; M(R) is As or Cd concentration in either URG (R1) or shoots (R2), and M(pw) is tAs or Cd concentration in porewater. TF is either As-TF or Cd-TF.

2.4.3 Harvest index

To investigate impact of water management, Fe amendments and interaction of water management by Fe amendment on rice grain yield, harvest index (HI) was calculated as the ratio of GYM to the above ground biological biomass as sum of GYM, and SBM and panicle masses as shown in equation 3 (Fageria, 2007).

$$HI = \frac{GYM}{GYM + SBM} \qquad \text{Equation 3}$$

2.5 Quality assurance, recoveries and limit of detection (LOD)

For greenhouse experiments, quality assurance and quality control (QA/QC) were achieved by performing pot experiments in 4 replicates whereas QA/QC analytical procedures were achieved by using standard reference materials (SRM) NIST1568a rice flour (National Institute of Standards and Technology (NIST), Maryland, USA) and certified reference material (ERM-BCR211) to verify accuracy of analytes determination as well as analytical methods. The certified value for tAs and provisional values for DMA and sum of iAs in SRM 1568a are given in **Table S2; Supplementary material**). The Cd, -tAs, -DMA and -iAs concentrations (NIST1568a) and ERM-BCR211-tAs, -DMA and -iAs concentrations obtained in this study (at different days) were not significantly different from the certified values ($p = 0.399$; **Table S2, Supplementary material).** The recoveries obtained in this study for experiments were also comparable to values reported by Narukawa and Chiba (2010) who reported 98 ± 2 µg/kg (sum of iAs), 175 ± 2 µg/kg (DMA) and 13 ± 1 µg/kg (MMA). All the 56 shoots samples from each experiment were measured in duplicates whereas only 30% (17) of total unpolished rice grain samples were measured in duplicates. The concentration means varied between ±7%. For each batch, two blanks were processed and measured alongside the samples.

3. Results

3.1 Agronomical analyses of rice plant growth

3.1.1 Effect of Fe° amendment on rice plant height (PHT)

On the final day PHT were measured (170th DAT), the tallest and shortest plants were found under AWD(+Fe^0) (1617 mm) and LW(-Fe^0) (1169 mm) respectively (**Figure 2A; Figure 2B**). All PHT gradually increased with increasing age of plants regardless of water management and/or Fe amendment with no signs of significant growth inhibition (**Figure 2A; Figure S1, Supplementary material**). There was a significant effect of Fe° amendment ($p < 0.001$; $F = 15.2$), water management ($p < 0.001$; $F = 105.7$) and Fe° amendment by water management interaction ($p = 0.019$; $F = 5.98$) on PHT (**Figure 2B**). The mean PHT under AWD (1534 mm) (regardless of Fe° amendment) was 27% significantly taller compared to that under LW (1206 mm) (**Figure 2A; Figure 2B**) but was not significantly different from that under CF (1505 mm). For Fe° amendment, regardless of water management, the mean plant heights of rice plants under (+Fe^0) treatment (1446 mm) was 6% significantly taller compared to that under (-Fe^0) treatment (1359 mm) whereas Fe° amendments increased PHT of rice plants under AWD by 13% (from 1401 - 1585 mm) (**Figure 2B**) but had no significant effect on PHT of rice plants under CF and LW.

3.1.2 Effects of Fe° amendment and water regime on productive tillers

At 59th DAT, the number of tillers significantly varied with water management ($p < 0.001$; F = 25.7), Fe° amendments ($p = 0.010$; F = 8.5), and interaction between these two factors ($p = 0.030$; F = 4.) (Data not shown). The number of tillers under AWD (5.1 tillers/plant) (regardless of Fe° amendments) were 18% and 35% significantly lower than CF (6.3 tillers/plant) and LW (7.8 tillers/plant) respectively. For Fe° amendment, regardless of water management, number of tillers under (+Fe°) treatments were 16% significantly higher than those under (-Fe°) treatment. For water management by Fe° amendment interaction impact, Fe° amendments significantly increased number of tillers under CF and significantly reduced number of tillers under LW but had no significant impact on number of tillers under AWD (Data not shown).

At 170th DAT, there was no significant effect of Fe° amendments ($p > 0.05$) and water treatment ($p > 0.05$) on number of productive tillers. However, water management by Fe° amendment interaction impact on number of productive tillers was significant ($p = 0.017$; F = 5.3). It was observed that Fe° amendments significantly reduced number of productive tillers per plant from 5.0 tiller to 3.8 tillers (25%) under LW and significantly increased number of productive tillers per plant from 4.0 tillers to 5.3 tillers (25%) under CF. At that 170th DAT, percentage of productive tillers per treatment was also significantly affected by water management ($p < 0.001$; F = 16.0) whereas Fe° amendments, and Fe° amendments by water management interaction had no significant effect on percentage of productive tillers. The percentages of productive tillers under AWD (95%) and LW (56%) were 17% higher and 21% lower respectively compared to that under CF (78%) (**Figure 3A; Figure 3B**) and AWD (+Fe°) treatment had the highest percentage of productive tillers (100%) whereas LW(+Fe°) had the least percentage (47.7%) and the percentage of productive tillers decreased in the order AWD(+Fe°) > AWD(-Fe°) ≥ CF(-Fe°) ≥ CF(+Fe°) > LW(-Fe°) >> LW(+Fe°) (**Figure 3B**).

3.1.3 Impact of water management and Fe° amendment on RBM, SBM and GYM

Root biomass (RBM). There was no significant effect of Fe° amendment ($p > 0.012$) and water treatment ($p > 0.05$) on RBM. However, Fe° amendment by water management interaction effect on RBM was significant ($p = 0.007$; F = 5.3). Fe° amendment significantly increased RBM under ADW (13%) and CF (19%) while Fe° amendment had no effect on RBM under LW (**Figure 4A**).

Shoots biomass (SBM). There was a significant effect of Fe° amendment ($p = 0.002$; F = 13.8) and water management ($p < 0.001$; F = 15.9) on SBM whereas water management by Fe° amendment interaction effect on SBM was not significant ($p > 0.05$; F = 2.0). Regardless of Fe° amendment, the mean SBM under LW (43.2 g/plant) was 13% and 18% significantly lower compared to that under AWD (49.8 g/plant) and CF (52.8 g/plant) respectively (**Figure 4B**). However, SBM of plants under AWD and CF were comparable (**Figure 4B**). Regardless of water management, mean SBM of plants under (+Fe°) amendment (51.2 g/plant) were 10% significantly higher compared to those under control (-Fe°) treatment (45.9 g/plant; **Figure 4B**).

Rice grain yield (GYM). There was a significant effect of water management ($p < 0.001$; F = 36.6) and water management by Fe° amendment interaction ($p < 0.05$; F = 5.4) on GYM whereas effect of Fe° amendment on GYM was not significant ($p > 0.05$; F = 0.04). Regardless of Fe° amendment, the mean GYM under AWD (6.3 g/plant) and CF (5.9 g/plant) were 100% and 92% respectively higher compared to those obtained under LW (3.1 g/plant) with mean GYM under AWD being comparable to that under CF (**Figure 4C**). For water management by Fe° amendment interaction effect, Fe° amendment significantly increased GYM by 33% (from 5.6 to 7.1 g/plant) under AWD but Fe° amendment had no significant effect on GYM under CF and LW (**Figure 4C**).

Correlation. In combined samples, the strongest positive correlation was observed between GYM-PHT whereas the weakest positive correlation was between GYM-RBM and decreased in the order: GYM- PHT > GYM-SBM > SBM- PHT > RBM-SBM > GYM-RBM (**Table 1; Figure S2**). Furthermore, GYM-SBM correlation ($r = 0.91$; $p < 0.01$) was larger and more significant in (+Fe°) treatments compared to (-Fe°) treatments whereas vice-versa was true to PHT-SBM correlation (**Table 1; Figure S2**).

3.1.4 Grain yield harvest index, HI

The greatest harvest index (HI) was obtained under AWD and smallest HI was obtained under LW. HI decreased in the order: LW(+Fe°) << LW(-Fe°) << CF(+Fe°) < AWD(-Fe°) < CF(-Fe°) < AWD(+Fe°) (**Table 2**). The mean HI under AWD (0.13) (regardless of Fe° amendments) was significantly greater ($p < 0.001$; F = 20.2) than that under LW (0.059) but not significantly different from that under CF (0.11) ($p > 0.05$; **Table 2**). For water management by Fe° amendment interaction effect, Fe° amendments significantly increased

HI by 24% under AWD (p 0 0.016; F = 2.0) and significantly decreased HI by 32% and 19% under LW and CF respectively ($p < 0.05$; **Table 2**).

3.2 Effect of Fe° amendment and water management on porewater tAs and Cd concentration

Arsenic. At 110th DAT, the mean porewater tAs under AWD (regardless of Fe° amendment) was 21% significantly lower and 31% significantly higher compared to that under CF and LW respectively ($P < 0.001$; F = 39) whereas the mean porewater tAs under (+Fe°) treatment (regardless of water management) was 17% significantly higher compared to that under (-Fe°) treatment ($P < 0.001$; F = 21). For water management by Fe° amendment interaction effect, Fe° amendment significantly reduced porewater-tAs by 28% under CF(+Fe°) (p = 0.001; F = 7.8) compared to that under CF(-Fe°) (**Figure SM2; Figure 5**) and significantly increased porewater-tAs concentrations under AWD(+Fe°) compared to that under AWD(-Fe°) treatment (p = 0.001; F = 7.8) (**Figure SM2; Figure 5**) but had no significant effects on porewater-tAs under LW water management.

Figure 3.5. ***Cadmium.*** At 110th DAT, the mean porewater Cd concentration under AWD (5.2 µg/kg) (regardless of Fe° amendments) was 117% (p = 0.001; F = 7.8) and 41% ($P < 0.001$; F = 39) significantly greater compared to that under LW (2.4 µg/kg) and CF (3.7 µg/kg) respectively whereas the mean porewater Cd under (+Fe°) amendment was 9% significantly greater (0.01; F=25) compared to that under control (-Fe°) treatment (**Figure 5B**). For water management by Fe° amendment interaction effect, Fe° amendments significantly increased (P = 0.004; F=18) porewater-Cd by 57% under AWD and had no significant effect on porewater-Cd under the other water management regimes ($P > 0.05$; **Figure 5B**).

3.3 Plant materials chemical analyses

3.3.1 Effect of water management and Fe° amendments on accumulation of As and Cd in rice shoots and URG

Arsenic. For shoots samples, there was a significant effect of water management ($P < 0.001$; $F = 44.6$) and water management by Fe° amendment interaction ($p < 0.001$; $F = 18$) on shoot-tAs accumulation but Fe° amendments alone had no significant effect on shoot-tAs ($P > 0.05$). The mean shoot-tAs concentration under AWD (1686 µg/kg) and LW (701 µg/kg) (regardless of Fe° amendments) were significantly reduced by 27% and 69% ($P < 0.001$; $F = 267$) compared to that under CF (2309 µg/kg) respectively (**Figure 6A**). For water management by Fe° amendments interaction, Fe° amendment significantly reduced shoot-tAs by 64% (- from 2760 µg/kg to 1650 µg/kg) under CF, and significantly increased shoot-tAs by 43% (- from 1280 µg/kg to 2100 µg/kg) under AWD and had no significant effect on shoot-tAs under LW (**Figure 6A**). Furthermore, shoot-As under AWD(+Fe°) was not significantly different from that under CF(-Fe°) ($p > 0.05$). For the URG samples, there was a significant effect of water management ($p = 0.004$, $F = 7.6$) and water management by Fe° amendment of interaction ($p = 0.03$, $F = 2.3$) on accumulation of URG-tAs with URG-tAs under AWD (310 µg/kg) being comparable to that under CF (408 µg/kg). However, both URG-tAs under AWD and CF water management were significantly higher compared to that under LW (250 µg/kg). For water management by Fe° amendment of interaction effect, Fe° amendment significantly decreased URG-tAs under LW and increased it under AWD and but had no significant effect on URG-tAs under CF water management.

Cadmium. The shoots-Cd under AWD (235µg/kg) and LW (222 µg/kg) (regardless of Fe° amendments) were both significantly higher ($p < 0.001$; $F = 32.1$) compared to that under CF

(70.0 µg/kg) but no significant shoots-Cd differences between AWD and LW were observed (**Figure 6C**). Shoots-Cd under (+Fe°) treatment (138 µg/kg) (regardless of water management) was 54% significantly lower ($p < 0.001$; F = 17.5) compared to that under (-Fe°) treatment (213 µg/kg; **Figure 6C**). There was a significant water management by Fe° amendment interaction effect on shoots-Cd ($p = 0.003$; F = 6.7) of which Fe° significantly reduced shoots-Cd by 50% under AWD (from 316 µg/kg to 154 µg/kg) but had no significant effects on shoots-Cd under LW and CF (**Figure 6c**).

URG-Cd under AWD water management (105.2 µg/kg) and LW (94.4 µg/kg) (regardless of Fe° amendments) were 360% and 410% respectively significantly higher ($p < 0.001$; F = 12.4) compared to that under CF water management (20.7 µg/kg) but there were no significant differences between URG-Cd under AWD and URG-Cd under LW water management. The URG-Cd accumulation under (+Fe°) treatment (49.1 µg/kg) (regardless of water management) was 50% significantly lower ($p = 0.005$; F = 10.2) compared to URG-Cd accumulation under control (-Fe°) treatment (97.8 µg/kg; **Figure 6D**). For interaction between water management and Fe° amendments, Fe° amendments also significantly decreased URG-Cd by 61% (151 to 59 µg/kg) ($p < 0.01$; F = 3.9) under AWD and 51% (126 to 62 µg/kg) under LW ($p < 0.05$) but had no significant effect on URG-Cd under CF.

3.3.2 Impact of water management and Fe° amendment on As speciation in unpolished rice grain (URG)

Inorganic As (as AsV) and DMA were the main peaks detected in the chromatograms for all URG samples and traces of MMA peaks were detected only in chromatograms of CF samples (**Figure 7**). There was no significant effect of Fe° amendments ($P > 0.05$) and water management ($P > 0.05$) on As speciation of URG samples (i.e. iAs and DMA accumulation) (**Figure 7; Figure 8A; Figure 8B**). However, water management by Fe° amendment interaction effect on URG-DMA was significant ($p < 0.05$; F = 3.65; **Figure 8A**). Thus, Fe°

amendment significantly reduced URG-DMA by 40% under LW water management, i.e. from 82 µg/kg in LW(-Fe°) to 49 µg/kg) in LW(+Fe°) treatments (**Figure 8A**). Conversely, Fe amendment significantly increased URG-DMA by 368% under AWD water management, i.e. from 28 µg/kg under AWD(-Fe°) to 131 µg/kg in AWD(+Fe°) treatments (**Figure 8A**).

3.3.3 Correlation analyses shoots-As versus URG-As and shoots-Cd versus URG-Cd

Regardless of any treatment, shoot-As strongly and positively correlated with URG-tAs ($r = 0.71$; $p = 0.001$; **Figure SM3**). Regardless of Fe° amendments, shoot-tAs strongly and positively correlated with URG-tAs under AWD water management ($r = 0.83$; $p = 0.001$) and LW water management ($r = 0.80$; $p = 0.01$) whereas no significant correlations were observed under CF water management (**Figure SM3**). Regardless of water management, there was also strong positive linear correlation of shoots-tAs with URG-tAs under control treatment but none under (+Fe°) treatment. Regardless of any treatment, stronger and positive linear correlation between URG-DMA and URG-sum of extracted As species ($r = 0.88$, $p < 0.001$) and between URG-iAs and URG-sum of extracted As species ($r = 0.65$, $p < 0.001$).

3.3.4 Bioconcentration factors (bcf) and transfer factors (TF) of As and Cd in rice

The porewater-to-shoots bcf and shoots-to-URG TF were calculated to evaluate impact of water management and Fe amendment on rice plants' As and Cd transfer potentials (**Figure 9A**). The highest porewater-to-shoots As-bcf were found under CF management decreased in the order: CF > AWD > LW whereas the highest shoots-to-URG-TF was found under LW management decreased in the order: LW > AWD > CF (**Figure 9B**). There was a significant effect of water management on porewater-to-shoots As-bcf ($p = 0.003$; $F = 9.0$) but there were no significant effects of Fe° amendments and water management by Fe° amendment interaction on porewater-to-shoots As-bcf ($p > 0.05$; **Figure 9A**). Regardless of Fe° amendment, mean porewater-to-shoots As-bcf under AWD (189) was comparable to that under CF (186) whereas pw-to-shoots As-bcf under both AWD and CF water managements were significantly higher compared to that under LW (77) (**Figure 9A**). For shoots-to-URG As-TF, there was a significant effect of water management on shoots-to-URG As-TF ($p = 0.008$; $F = 7.3$) whereas Fe° amendments and water management by Fe° amendment interaction had no significant effect on shoots-to-URG As-TF ($p > 0.05$; **Figure 9B**). The mean shoots-to-URG As-TF under AWD (0.20) (regardless of Fe° amendment) was comparable to that under CF (0.17) but shoots-to-URG As-TF under both AWD and CF were significantly lower compared to that under LW (0.55; **Figure 9B**).

For Cd, there was a significant effect of water management ($p < 0.001$; $F = 28.4$), Fe° amendment ($p = 0.002$; $F = 16.7$) and interaction between these two factors ($p = 0.002$; $F = 11.6$) on porewater-to-shoots Cd-bcf (**Figure 9C**). Across Fe° amendments, the mean porewater-to-shoots Cd-bcf under AWD (26.4) and LW (26.5; $P > 0.05$; **Figure 9C**) was not significantly different from each other; whereas Cd-bcf determined in both AWD and LW were at least 363% significantly greater compared to that determined in CF water management (5.7; $p < 0.001$; $F = 15.1$). Across water management regimes, the mean porewater-to-shoots Cd-bcf under (+Fe°) amended shoots (14.2) was 43% significantly lower than that under control (24.8; **Figure 9C**). For water management by Fe° amendments interaction effect, Fe° amendment significantly reduced porewater-to-shoots Cd-bcf under AWD and LW from 39.4 and 31.7 respectively to 13.4 (66%) and 21.2 (33%) respectively but Fe° amendments had no significant effect on porewater-to-shoots Cd-bcf under CF water management. For shoots-to-URG Cd-TF, effect of water management on shoots-to-URG Cd-TF was significant ($p = 0.008$; $F = 7.3$) whereas the rest of the treatments were not significant ($p > 0.05$; **Figure 9D**). Thus, across Fe° amendment, mean shoots-to-URG Cd-TF under

AWD water management (0.20) was comparable to that under CF water management (0.17) whereas shoots-to-URG Cd-TF under both AWD and CF were at least 63% significantly less compared to that under LW water management (0.54) (**Figure 9D**).

4. Discussion

4.1 Impact of AWD and Fe amendment and their interactions on agronomical parameters

Alternating wetting and drying (AWD) and Fe^0 amendments have been reported to significantly affect rice PHT. Khairi *et al.* (2015) and Norton *et al*. (2017) reported that AWD water management significantly decreased PHT by 9% and 10% respectively compared to CF water management. In this study, contrary to results reported by Khairi *et al.* (2015) and Norton et al. (2017), we found that PHT under AWD water management were comparable to those under CF water management ($p > 0.05$; **Figure 2A; Figure 2B**). Nevertheless, PHT of rice plants under AWD were 27% significantly taller compared to those under LW (Figure 3.2A; **Figure 2B**). We also observed significant PHT increase due to iron amendments (**Figure 2A; Figure 2B**) which agrees with Irem *et al*. (2019) who reported that application of soil amendments such as iron (Fe) at 50 mg/kg soil improved the PHT. However, Irem *et al.* (2019) used iron sulfate, not necessarily zero valent iron (Fe°). Despite having AWD alone inducing non-significant effect on PHT, the combination of AWD and Fe° i.e. (AWD(+Fe^0)) significantly increased PHT (13%) compared to (CF(+Fe^0)) (**Figure 2A**) which suggests synergistic effect of Fe° and AWD on PHT.

In this study we found that plants under AWD had comparable number of productive tillers with those under CF (**Figure 3B**) which is consistent with findings of Norton *et al.*, (2017). However, we also found that percentage of productive tillers in AWD were 11% significantly higher (**Figure 3B**) compared to that under CF which agrees with Norton *et al.* (2017) who reported that AWD had 6% more productive tillers compared to those under CF water management. Despite reporting that moderate AWD significantly reduced maximum number of tillers by 21-23% and comparable number of productive tillers compared to that under CF, Yang *et al.* (2017) also reported that moderate AWD markedly increased the percentage of productive tillers which is consistent with our observations.

Many studies have reported that AWD increased SBM and GYM compared to CF (Islam et al., 2017a; Norton et al., 2017; Yang et al., 2017) while others have reported contrary results (Sudhir-Yadav et al., 2012; Yao et al., 2012). Considering that larger and enhanced root biomass (RBM) supports greater rate of aboveground biomass production (Chu et al., 2018; Yang et al., 2009), the aboveground biomasses (SBM and GYM) among water regimes in this study showed no significant differences which is consistent to comparable RBM observed in plants under CF and AWD ($p > 0.05$). These observations are consistent with Xu *et al.* (2008) who reported that straw biomasses (RBM) were not affected by the water management regimes. In agreement to Yao *et al.* (2012), GYM under AWD water management was comparable to that under CF water management (**Figure 4C**) but GYM of both AWD and CF were significant higher compared to that under LW (**Figure 4C**) that possibly indicates prolonged low water under LW may have consistently and significantly limited bioavailability of plant nutrients.

The greater RBM observed in plants under Fe^0 amendment of ADW and CF treatments compared to those under control treatments may have influenced greater SBM and GYM production observed under amended AWD(+Fe^0) and CF(+Fe^0) treatments indicating that Fe^0 amendment indirectly stimulated both SBM and GYM. The observed greater RBM, SBM and GYM in plants under (+Fe^0) amendment could be linked to low porewater-As and Cd due to elevated abstraction by oxidized iron hydroxides produced after oxidation of Fe^0 that limited toxic effect of As and Cd in porewater that could have inhibited plant growth. Dixit *et al.* (2016) linked the enhanced plant growth to low As accumulation in their sulfur experiments. Impact of Fe amendment on HI appear to be dependent on water management regime with stimulating HI effect under AWD (+24%) and inhibiting HI effect under CF (-19%) and LW (-32%).

The observed stimulatory effects of Fe amendment on PH, SBM, percentage of tillers and GYM under AWD(+Fe°) treatments compared to that under AWD(-Fe°) could also be specifically linked to reduced toxic effect of AsV due to greater oxidation of iron (FeII to FeIII) and AsIII to AsV which probably enhanced adsorption capacity for AsV, since AsV is dominant under oxic environment and strongly sorbs onto oxidized iron (Farrow et al., 2015; Qiao et al., 2018). Besides, elevated oxidation of Fe° in intermittent oxic and anoxic conditions under AWD may have increased nutrient reservoir capacity during time of diminished supply (Farrow et al., 2015; Qiao et al., 2018). The GYM increases under AWD(+Fe^0) could also be attributable to higher HI and increased percentage of productive tillers than that observed under CF, which has a strong and positive correlation with GYM ($r = 0.91$, $p < 0.001$) under AWD (**Table 2**).

4.2 Impact of AWD and Fe amendment on porewater tAs and Cd concentrations

The lower porewater-tAs observed in this study under CF amended with Fe° (CF(+Fe°)) compared to that without amendment (CF(-Fe°)) agrees with reports by Norton et al. (2017) and Makino *et al.* (2016) who reported that iron-bearing materials decrease porewater-As but disagrees with Qiao *et al.* (2018) who reported that iron bearing materials have no effect. Probably, continuous anoxic conditions under CF enhanced continuous immobilization of AsIII (Farrow et al., 2015). The greater tAs concentration was observed in porewater under AWD amended with Fe° (AWD(+Fe°)) compared to that under AWD(-Fe°) which suggested that intermittent oxic and anoxic condition may have increased release of As into porewater

(Farrow *et al.*, 2015; Qiao *et al.*, 2018; Makino *et al.*, 2016). These observations further suggest that impact of Fe° amendment on As mobilization in porewater is water management regime dependent. For Cd, Wan et al. (2018) found greater Cd concentrations mobilized in porewater under aerobic (LW) water compared to that under CF water management regime. In agreement to Wan *et al.* (2018), we found higher Cd in porewater under AWD (5.2 µg/kg) compared to that under LW (2.4 µg/kg) and CF (3.7 µg/kg) which confirms that greater cadmium, unlike As, is released into soil solution under oxidizing condition (Rinklebe et al., 2016).

4.3 Impact of AWD and Fe° amendments on shoots and unpolished rice -tAs, -iAs and -DMA accumulation

The tAs concentration found in URG under AWD (310 µg/kg) and CF (408 µg/kg) exceeded the European Commission's legislated MCL (250 µg/kg) of unpolished rice by 48% and 46% respectively. The higher URG-tAs under AWD and CF could be linked to higher soil As content (5.4 mg/kg; **Table SM1**). Previous studies have reported that AWD water management decreases both shoots and grain As in comparison with CF water management (Islam et al., 2017a; Norton et al., 2017; Xu et al., 2008). Islam *et al.* 2017a) reported a grain-As decline of 17–35% under AWD water management (215 µg/kg) when compared to CF water management (304 µg/kg). Xu *et al.* (2008) reported 10 to15-folds higher As increase in rice grown under CF water management compared to aerobically grown rice. In this study, we found significantly lower tAs in shoots and URG under AWD compared to CF which is consistent with results reported by others (Islam et al., 2017a; Norton et al., 2017; Xu et al., 2008). Moreover, the low shoots and URG -As under AWD water management could be linked to the reduced As mobilization observed in porewater under AWD and LW water managements compared to that under CF water management (**Figure SM2**).

The increase of tAs accumulation in shoots and URG under AWD(+Fe^0) compared to AWD(-Fe^0) could be linked to intermittent oxic and anoxic condition that induced intermitted and interrupted accessibility and bioavailability of As in porewater and consequently increased its uptake by rice plants. On the contrary, the decrease of tAs accumulation in shoots and URG under CF(+Fe^0) enhanced uninterrupted As immobilization that limited As accessibility to rice plants under amended CF due to availability of probably unlimited sites on oxidized iron that sorbed As. (Farrow et al., 2015; Honma et al., 2016; Makino et al., 2016). The low shoots-As and URG-As under AWD (**Figure 6A; Figure 6B**) could also be linked to lower bcf and TF observed in AWD compared to that under CF considering that the larger the bcf and/or TF the more metals the rice plant is capable of up-taking from the soil (Xie et al., 2016). Higher tAs accumulation resulted under CF followed by AWD. Despite having highest shoots-to-URG As-TF under LW water management, low amounts of As accumulated in corresponding URG, which suggest that the low URG-tAs could be attributable to low porewater-to-shoots As-bcf.

The correlation analyses showed strong and positive linear correlation between porewater and tAs and shoots-tAs and URG-tAs (**Figure SM2**) which agrees with Kumarathilaka *et al.* (2018) who reported significant positive porewater-tAs and URG-tAs correlations. Since uptake of As by rice plants is partly dependent on bioavailable As in porewater (Carolina et al., 2019), higher tAs found under AWD(+Fe^0) demonstrates that Fe° amendment strongly enhanced mobilization and bioavailability of As through oxidative reaction in oxic conditions of AWD water management that released/freed previously sorbed As, thereby increasing As accessibility to and uptake by rice plants. On the contrary, Fe amendments appeared to have simultaneously inhibited porewater-to-shoots As-bcf and stimulated shoots-to-URG As-TF in rice plants under CF (**Figure 9A; Figure 9B**).

Arsenic species significantly varied with water management with higher percentage of DMA observed in URG under AWD(+Fe°) compared to that under AWD(-Fe°) suggesting Fe° amendments significantly enhanced As methylation in AWD water regime while iAs remained approximately low relative to DMA across all water management regimes which reduced its percentage.

Increased bioavailable tAs in porewater under AWD(+Fe°) could result from dissolution and remobilization of As within iron plaque and that previously adsorbed onto sorbents such as Fe and Al when water management is switched from low to flooded considering that under flooded (anaerobic) condition microbial transformation of AsV to AsIII is largely favored that leads to marked release of AsIII into soil porewater (Wang et al., 2019). Moreover, reductive dissolution of iron oxyhydroxides reduces binding sites of As on minerals (Jia et al., 2015). Arsenic species in paddy soil directly influence As speciation in rice grain with methylated As species in rice being mainly derived from microbial methylation in paddy soil (Zheng et al., 2013). Furthermore, combination of AWD water management and Fe° amendments increased DMA concentration in URG. Simultaneous use of Fe° amendment and AWD water management may have also increased total arsenic concentration in soil pore water considering that due to iron promoting microbial methylation of arsenic in soil during the flooded anoxic period considering that microorganisms play critical roles in As transformation and mobility in paddy soil, such as through interactions with iron oxides or organic matters (Zheng et al., 2013). The methylated arsenic such DMA may have lower affinity for adsorption onto oxidized iron compared to AsIII and AsV with AsV being strongly adsorbed on most mineral constituents such as iron or aluminium (hydr)oxides and aluminosilicates) which may have resulted in increased uptake and accumulation of methylated arsenic in URG. Considering that nitrate is an important oxidant in the natural environment and can participate in microbial-catalyzed AsIII oxidation under anaerobic conditions (Lin et al., 2018), nitrates addition might have probably limited predominance of AsIII and enhanced formation of AsV through oxidative reactions (Lin et al., 2018; Wang et al., 2019). However, considering that nitrate addition was uniform in zero valent iron amended and non-amended CF water management regime, interaction effect of nitrate by Fe° is likely to have resulted in significant oxidation of Fe° and eventually affect speciation of AsIII and AsV which requires further investigation.

4.4 Impact of AWD and Fe° amendments on Cd accumulation in shoots and URG

Yang *et al.* (2017) and Islam *et al.* (2017a) reported that AWD increases shoots-Cd (20%) and grain-Cd (25%) accumulation compared to CF whereas Ishikawa *et al.* (2016) reported that Cd decreased as follows : Water saving conditions (WAS) > AWD > CF in most sites studied. In this study, shoots-Cd and grain-Cd under AWD were higher than that under CF but lower than that LW (**Figure 6C; 6D**) which is in agreement with Yang *et al.* (2017), Islam *et al*. (2017a) and Ishikawa *et al.* (2016).The observed higher shoots- and URG-Cd accumulation under LW and AWD could be linked to higher bioavailable Cd observed in their respective porewater.

The observations that Fe° significantly reduced URG-Cd under AWD to the extent that URG-Cd under AWD(+Fe°) was comparable to those under both CF(-Fe°) and CF(+Fe°) demonstrates that simultaneous use of Fe° amendment and AWD could be used to achieve low Cd accumulation in rice grain under AWD water management compared to that attained with CF water management likely to mitigate antagonistic behavior of As and Cd accumulation in rice grains. Besides, the observation also demonstrates that use of Fe° amendment strongly inhibited Cd uptake by rice plants under AWD and LW (**Figure 6C; Figure 6D**).

5. Conclusion

This study has demonstrated that combination of AWD water management and (+Fe°) amendments could markedly increase grain yield (GYM) by at least 33% compared to all other treatments. The application of Fe° amendments also resulted in significant URG-As and -Cd concentrations reductions in rice cultivated under LW water management. However, yield under LW was significantly reduced by 47 and 51% compared to CF and AWD respectively. Besides, Fe° amendment increased grain yield and harvest index under AWD (from 0.11 to 0.14) and significantly reduced accumulation of Cd (from 151 µg/kg to 59 µg/kg and 162 µg/kg to 62 µg/kg in rice cultivated under AWD and LW water managements respectively) compared to non-significant reduction under CF water management. On the other hand, a combination of CF water management regime with (+Fe°) amendments simultaneously reduced GYM which is undesirable for rice production. Considering that Fe° amendments had no significant effect on URG-tAs and URG-Cd under CF water management (**Figure 6B, Figure 6D**), it could imply that amending rice under CF with 6.2 g Fe°/kg soil maintained relatively high levels of As and low levels of Cd in the URG. The study results demonstrate that positive results of reduction of either As or Cd obtained with combination of zero valent iron (Fe^0) with specific water management regime significantly depended on soil-As and -Cd problem. For instance, the combination of CF and Fe° would be effective in rice paddies seriously contaminated with high As content but without Cd problem since As accumulation, compared to Cd, is significantly reduced. On the other hand, the combination of AWD with Fe° appear promising in significantly reducing Cd, compared to As hence would be suited for rice paddies contaminated with Cd rather than As. The study also demonstrated that simultaneous use Fe and AWD water management stimulates arsenic methylation in rice grain. Thus, the observed DMA increase could be harnessed to produce relatively safer rice considering that methylated As species are less toxic for human health.

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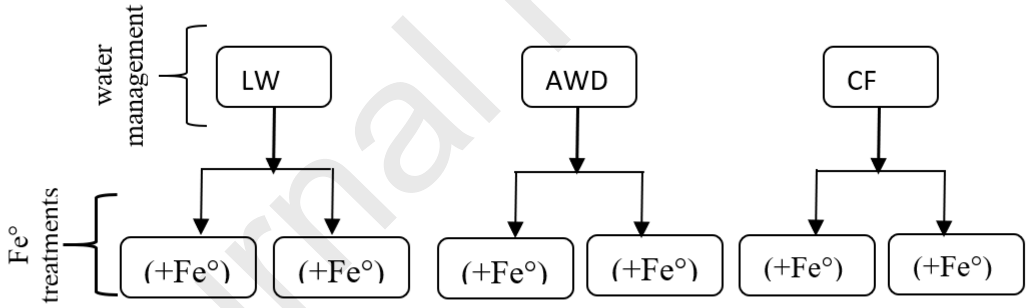


Figure 1. Design of rice experiments. CF, continuously flooded water management regime; AWD, alternate wetting and drying water management regime; LW, unflooded water management regime; (-Fe^0), without Fe^0 amendment (control) (n = 4); and (+Fe^0), amended with 6.2 g Fe^0/kg soil (n = 4).

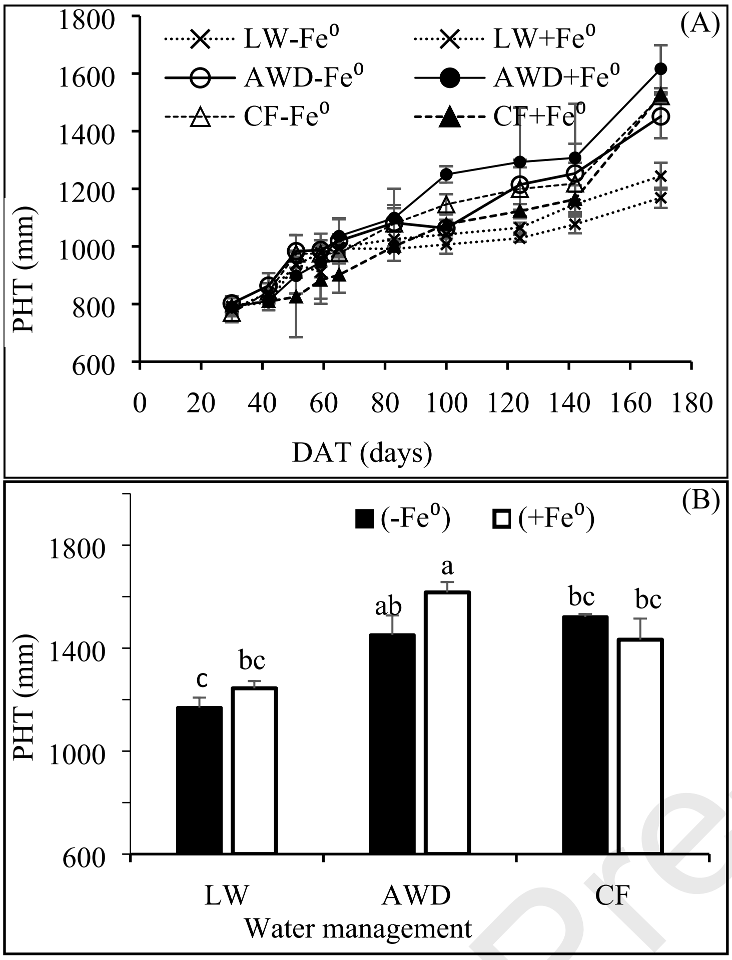


Figure 2. (A) Mean plant heights (PHT) of rice plants under three water management regimes (CF, AWD and LW) and two Fe^0 amendments ((+Fe°) and ($-Fe^0$)); and (B) Mean PHT of rice plants measured on 170th day after transplanting (DAT) depicting effects of different water management regimes and Fe^0 amendment treatments. Bars that do not share a common letter are significantly different ($p < 0.01$). Note: ($+Fe^0$) and ($-Fe^0$), refers to plants amended with and without Fe° respectively. Error bars represent ±SD (n=4).

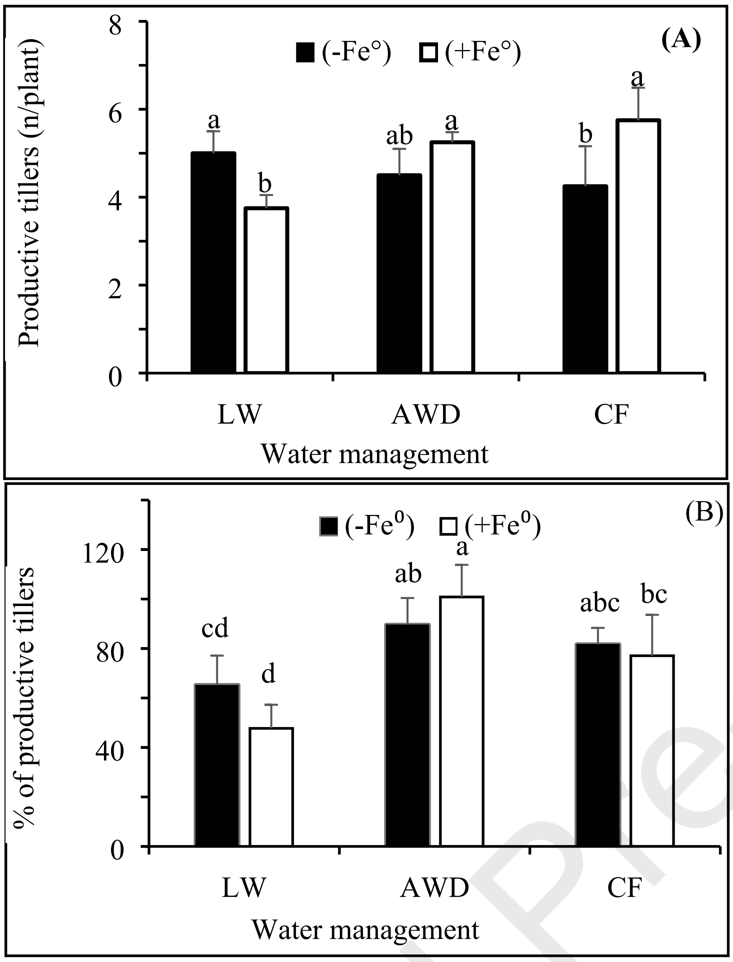


Figure 3. Interaction effects of Fe° amendment by water management on (A) mean number of productive tillers per plant at 170th DAT and (B) variation of percentage of productive tillers (%) by water management regime and Fe° amendment. Bars that do not share a common letter are significantly different ($p < 0.01$). Error bars represent ±SD ($n = 4$). (Note: mean number of productive tillers (n) per plant at 170th DAT were determined from number of tillers that produced panicles per plant; whereas percentage of productive tillers were determined by dividing number of productive tillers per plant by maximum number of tillers per plant).

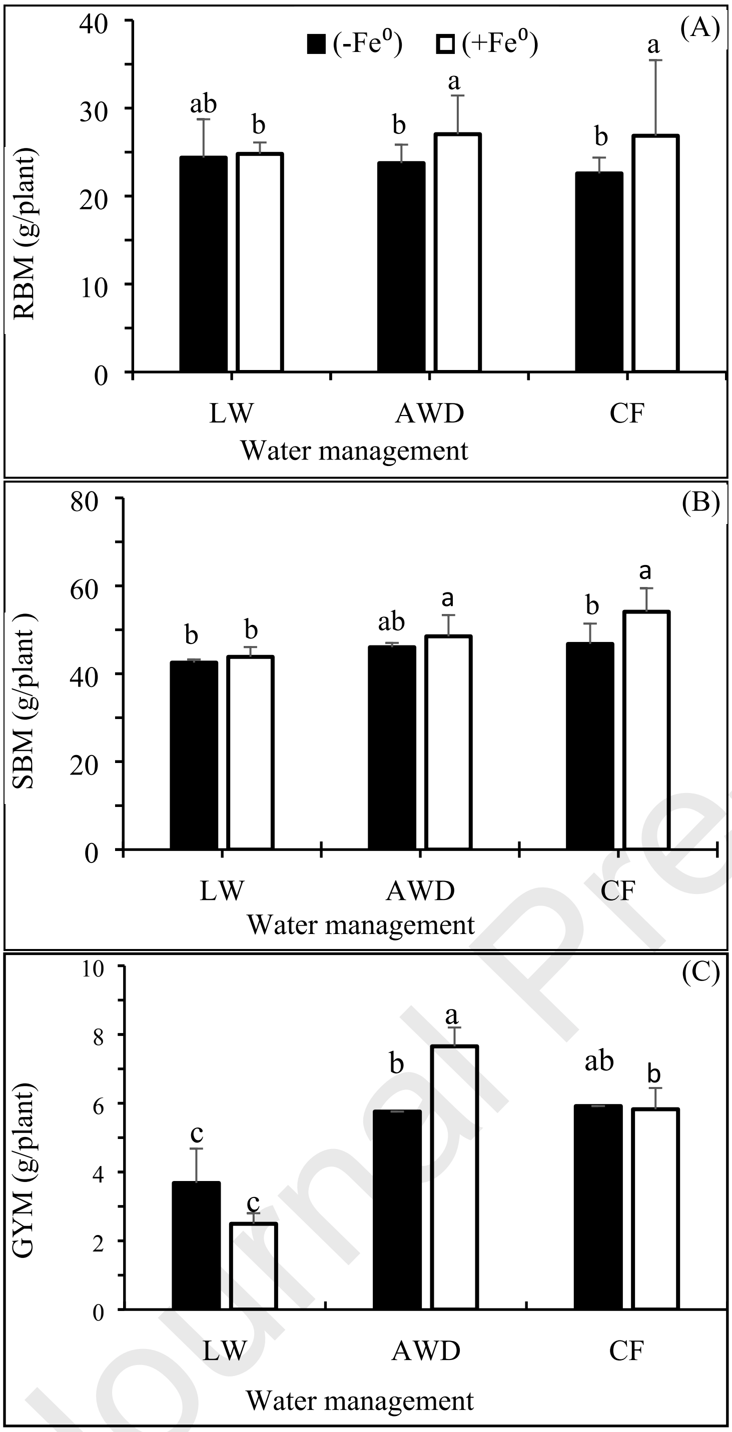


Figure 4. Effects of interaction of water management and Fe° amendment on (A) root biomass (RBM), (B) shoots biomass (SBM), (C) mass of grain yield (GYM). Bars that do not share a common letter are significantly different ($p<0.05$). Error bars represent ± SD ($n = 4$).

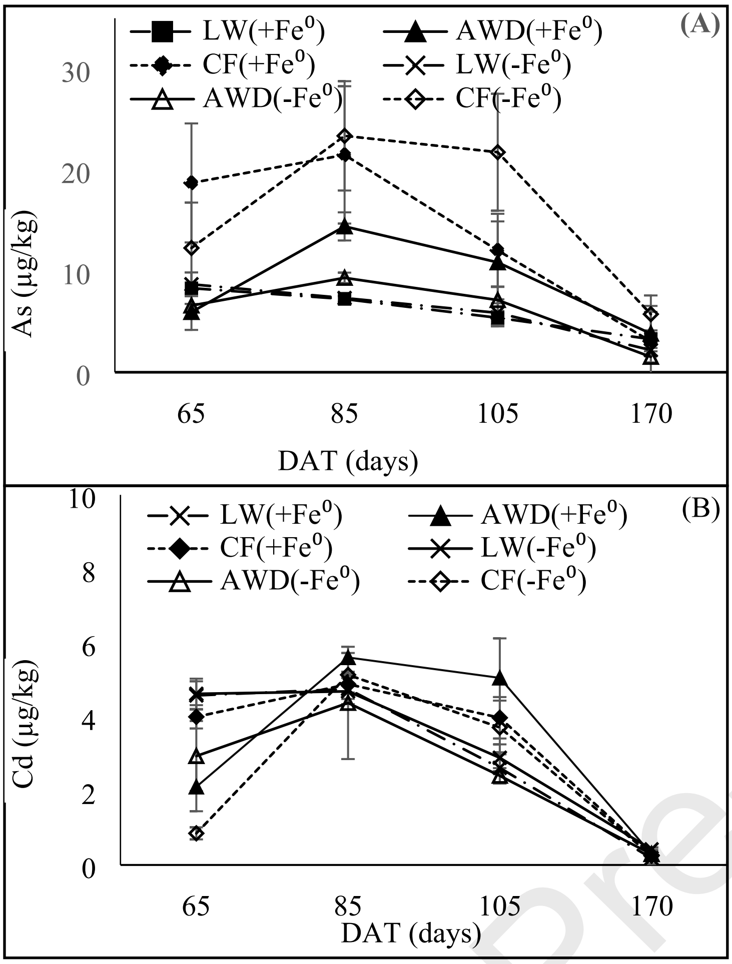
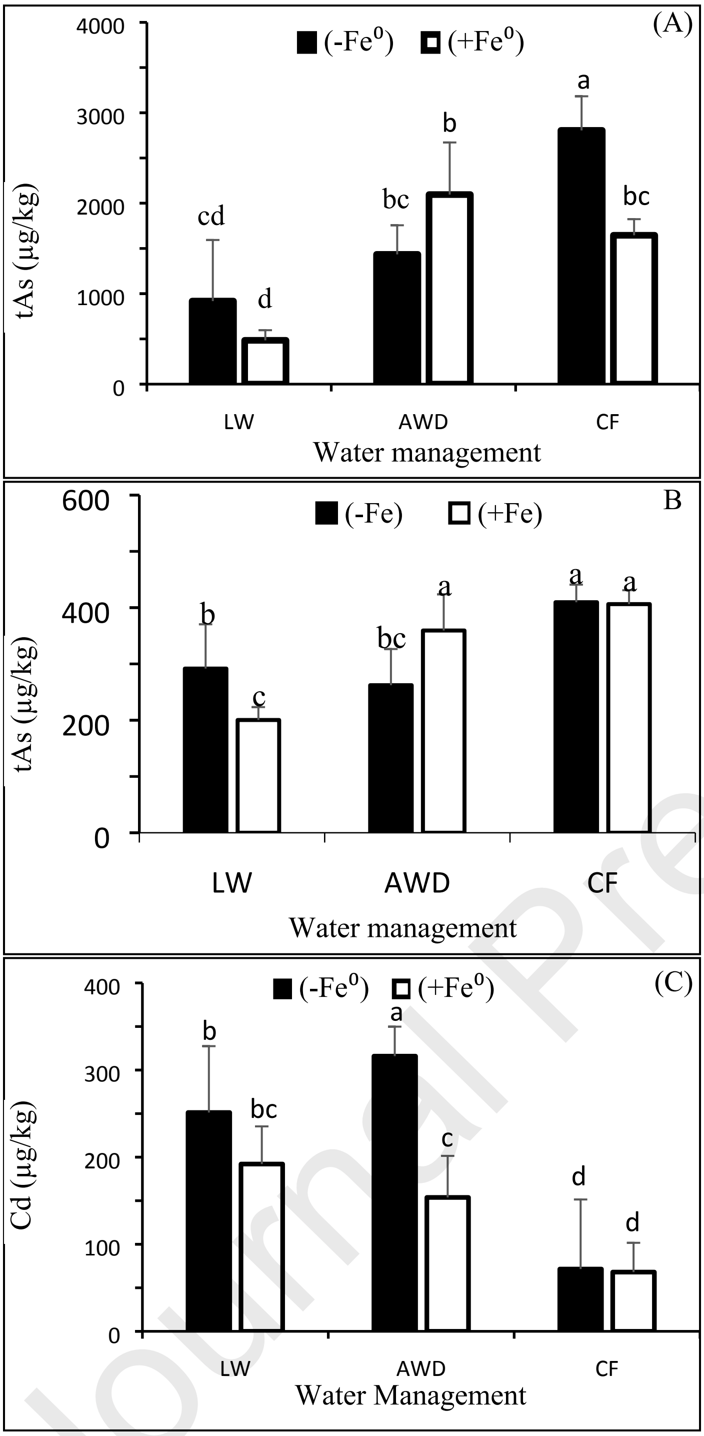


Figure 5. Effect of Fe^o amendment on (A) porewater-tAs and (B) porewater-Cd concentrations under different water management regimes at various days after transplanting (DAT). Error bars represent ±SD (n = 4).


(A)
■ (-Fe^0) □ (+Fe^0)
tAs (µg/kg)
4000
3000
2000
1000
0
cd
d
bc
b
a
bc
LW
AWD
CF
Water management
B
■ (-Fe) □ (+Fe)
tAs (µg/kg)
600
400
200
0
b
c
bc
a
a
a
LW
AWD
CF
Water management
(C)
■ (-Fe^0) □ (+Fe^0)
Cd (µg/kg)
400
300
200
100
0
b
bc
a
c
d
d
LW
AWD
CF
Water Management

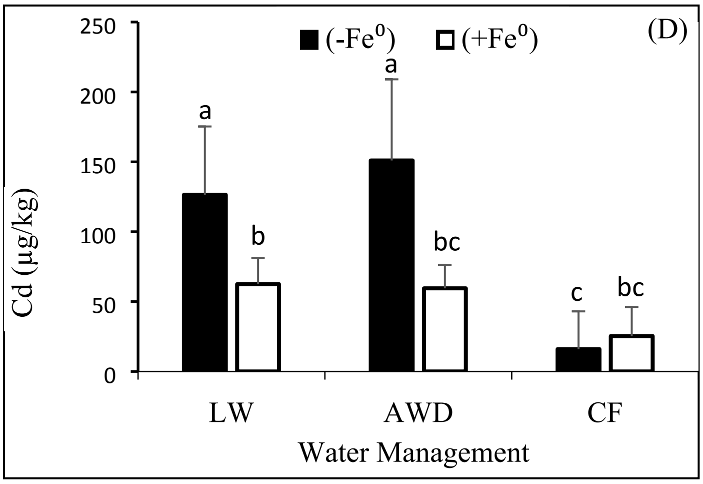


Figure 6. Effect of variation of tAs (µg/kg) (A and B) and Cd (µg/kg) (C and D) concentrations in rice shoots (A and C) and URG (B and D). Bars that do not share a common letter are significantly different ($p<0.01$). Error bars represent ±SD (n = 4).

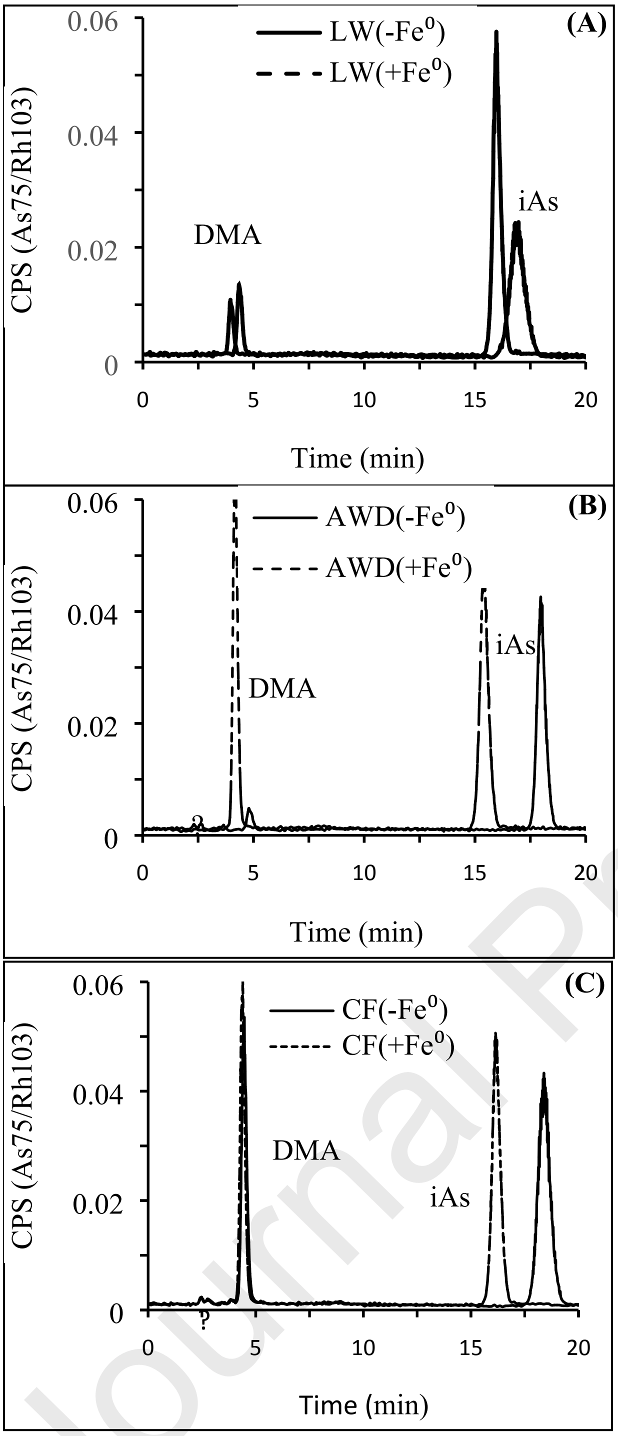


Figure 7. HPLC-ICP/MS representative chromatograms for As speciation analyses of URG showing simultaneous impact of various water management regimes and Fe° amendment treatments on As speciation URG. Retention time shifted due to matrix effect.

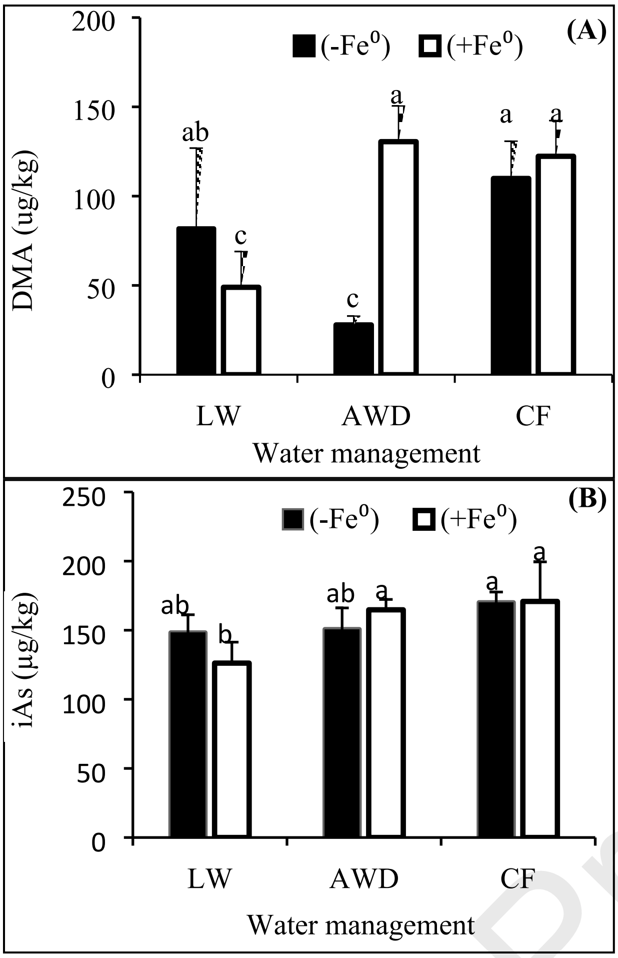
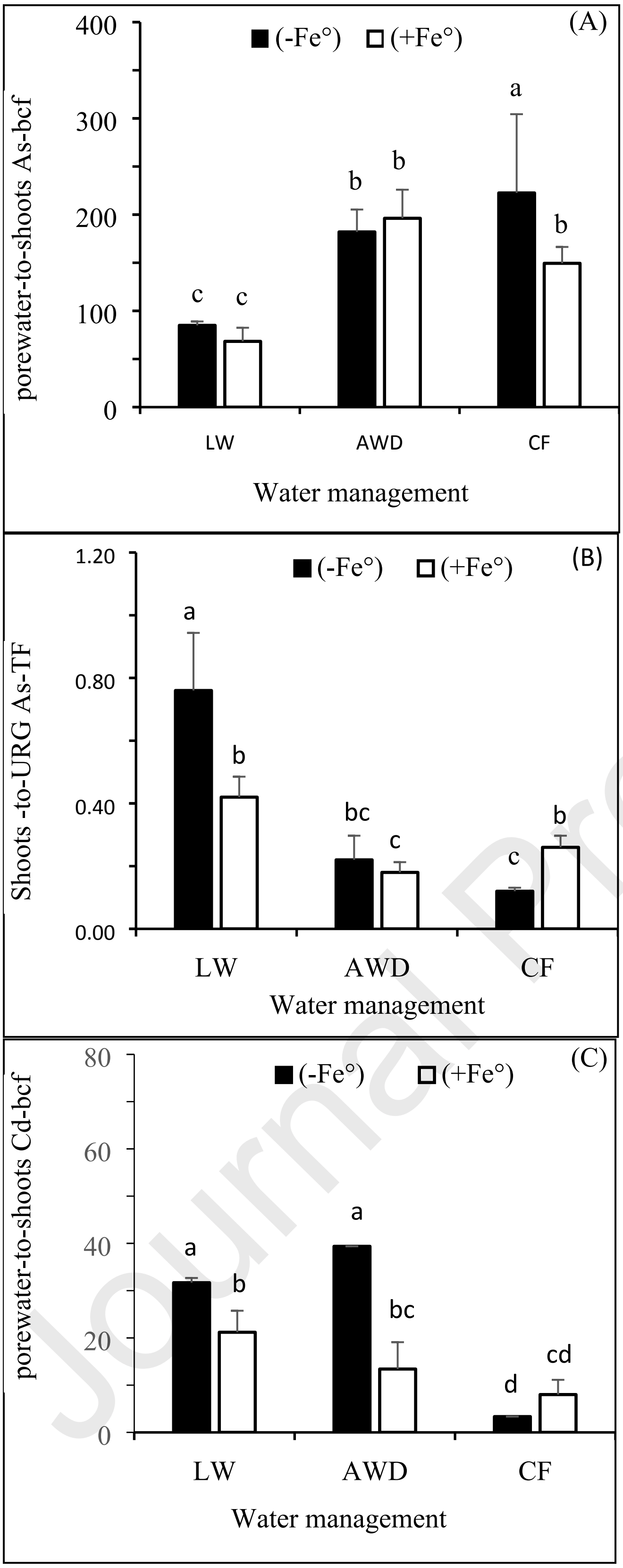


Figure 8. Effect of water management and Fe° amendment on accumulation of (A) DMA and (B) iAs in URG. Bars that do not share a common letter are significantly different ($p<0.01$). Error bars represent ±SED (n = 4).


(A)
porewater-to-shoots As-bcf
0
100
200
300
400
■ (-Fe°)
□ (+Fe°)
c
c
b
b
a
b
LW
AWD
CF
Water management
(B)
Shoots -to-URG As-TF
0.00
0.40
0.80
1.20
■ (-Fe°)
□ (+Fe°)
a
b
bc
c
c
b
LW
AWD
CF
Water management
(C)
porewater-to-shoots Cd-bcf
0
20
40
60
80
■ (-Fe°)
□ (+Fe°)
a
b
a
bc
d
cd
LW
AWD
CF
Water management

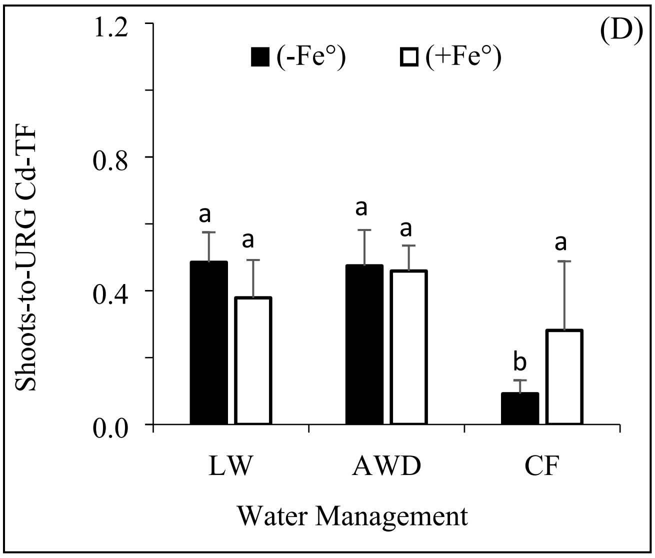


Figure 9. Effect of water management and Fe° amendment and their interaction on (A) porewater-to-shoots As-bcf, (B) shoots-to-URG As-TF, (C) porewater-to-shoots Cd-bcf, and (D) shoots-to- URG Cd-TF. Bars that do not share a common letter are significantly different ($p<0.05$). Error bars represent ±SED ($n = 4$).

TABLES

Table 1. Significant correlation between GYM, PHT, RBM and SBM in plants.

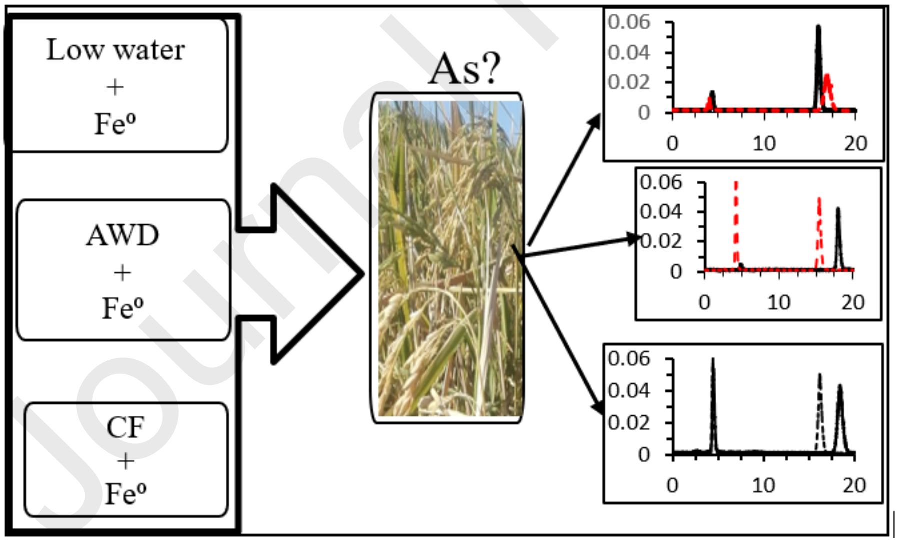
	PHT	**SBM**	**RBM**	**PHT**	**SBM**	**RBM**	**PHT**	**SBM**	**RBM**
	Combined			(+Fe°)			(-Fe°)		
GYM	**0.76 *****	**0.72*****	**0.54****	**0.70***	**0.91****	**ns**	**ns**	**ns**	**ns**
PHT		0.69**	ns	ns	0.61*	ns		0.87***	ns
SBM			0.55**			0.62*			ns

Note: * significant at 0.05, ** significant at 0.01, *** significant at 0.001 and ns, not significant

Table 2. Effects of water management and Fe^0 amendment on harvest index (HI) (mean ±SD, n=4)

Parameter	**Harvest index (HI)**		
Fe^0 amendment	(-Fe°)	(+Fe°)	% HI increase or decrease
LW	0.079 ± 0.029c	0.054 ± 0.005d	-32%
AWD	0.111 ± 0.008b	0.138 ± 0.023a	+24%
CF	0.120 ± 0.027ab	0.097 ± 0.003c	-19%

Graphic abstract



HIGHLIGHTS

1. Interaction effects of Fe° and water management on grain yield and bioaccumulation of arsenic (As) and cadmium (Cd) in rice shoots and grain were investigated.
2. Fe° significantly increased percentage of productive tillers and grain yield in rice under alternate wetting and drying (AWD) compared to continuous flooding (CF).
3. Simultaneous use of Fe° amendment and AWD water management significantly stimulated bioaccumulation of methylated As in rice grains.
4. Simultaneous use of Fe° amendment and AWD water management also significantly inhibited Cd bioaccumulation in rice grains indistinguishable to that achieved with CF