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# Fenton reagent reduces the level of arsenic in paddy rice grain

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4 **Fenton Reagent Reduces the Level of Arsenic in Paddy Rice Grain**  
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62 **ABSTRACT**  
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65 Hydroponic and pot experiments were conducted to examine the effects of Fenton reagent on paddy  
66 rice plant growing in arsenic-contaminated soils. Fenton reagent significantly reduced arsenic  
67 phytotoxicity, uptake by the plants and accumulation in rice grain. This is attributed to oxidation of  
68  $As^{3+}$  to  $As^{5+}$  by hydroxyl radicals and immobilization of arsenate by reacting with precipitating  $Fe^{3+}$   
69 to form practically insoluble compounds. Although this process enhanced the formation of Fe-  
70 enriched coatings on root surface, it appears that root plaque had limited effects on inhibiting As  
71 uptake since most of the young roots were not covered by iron plaque. It is more likely that As  
72 immobilization in the bulk soils play a major role in reducing As flux towards rhizosphere. The  
73 findings have implications for understanding As behavior in paddy field receiving rainwater-borne  
74 hydrogen peroxide and developing cost-effective techniques for reducing As level in rice grain  
75 produced from As-contaminated soils.  
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90 **Keywords:** Paddy rice, arsenic, iron plaque, soil, Fenton reaction  
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## 1 INTRODUCTION

Consumption of rice is a major pathway of human arsenic exposure, which could affect billions of people around the world (Schoof et al., 1999; Meharg, 2004; Williams et al., 2006; Zhu et al., 2008; Syu et al., 2015; Sinha and Bhattacharyya, 2015; Clemens and Ma, 2016). The anaerobic soil conditions associated with water inundation required for paddy rice farming favour reduction reactions, leading to formation of highly toxic arsenite ions (Xu et al., 2008; Li et al., 2009; Somenahally et al., 2011; Spanu et al., 2012). Arsenite tends to be predominantly present in undissociated form ( $\text{H}_3\text{AsO}_3^0$ ) under pH conditions encountered in most paddy rice soils (Zhao et al., 2009), and therefore it may be more resistant to immobilization by soil adsorbents. In addition, under reducing conditions the arsenic-scavenging capacity of soil is weakened due to reductive dissolution of various iron compounds that play a key role in binding soluble arsenic species through either formation of practically insoluble iron arsenate minerals or adsorption of arsenate to iron oxyhydroxides (Zhao et al., 2010; Zhu et al., 2014). As such, arsenite is readily available for uptake by rice plants and accumulation in rice grain (Williams et al., 2007; Su et al., 2010; Wang et al., 2015).

Iron-enriched root plaque plays an important role in reducing the entry of As present in the soil pore water (soil solution) into rice plant roots (Lee et al., 2013; Syu et al., 2013). The formation of root plaque is believed to be mediated by oxidation of ferrous iron ( $\text{Fe}^{2+}$ ) using molecular oxygen released from rice plant roots (Armstrong, 1964), and it is likely that the root-released oxygen also promotes microbially mediated oxidation of arsenite to form arsenate (Hu et al., 2015). As arsenate has the stronger affinity to  $\text{Fe}^{3+}$ , it is likely that arsenate-As tends to be intercepted more easily by the root plaque, as compared to arsenite-As (Chen et al., 2005; Liu et al., 2005).

It has been demonstrated that Fenton process involving reaction between hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and ferrous iron ( $\text{Fe}^{2+}$ ) resulted in enhanced oxidation of arsenite to form less toxic arsenate

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179  
180 (Hug and Leupin, 2003).  $\text{Fe}^{2+}$  is available in flooded soils like paddy rice soils (Becker and Asch,  
181 2005; Kögel-Knabner et al., 2010).  $\text{H}_2\text{O}_2$  is also commonly present in rainwater (Cooper et al., 1988;  
182 Willey et al., 1996; Gonçalves et al., 2010; Guo et al., 2014). In areas with abundant rainfall, Fenton  
183 reaction may be a naturally-occurring process that can affect the biogeochemical behaviour of  
184 arsenic in paddy rice soils. Where the enrichment of arsenic in rice grain becomes a significant  
185 health concern, it may be worthwhile to consider the use of Fenton reagent (a mixture of  $\text{H}_2\text{O}_2$  and  
186  $\text{Fe}^{2+}$ ) for reducing As uptake by rice plants.  
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189 The objective of this study was to examine the effects of Fenton reagent on reducing As uptake  
190 by rice plants. The impacts of Fenton reagent on plant growth are also evaluated. In addition, the  
191 major biogeochemical mechanisms responsible for the observed phenomena are explored.  
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## 203 **2 MATERIALS AND METHODS**

### 204 **2.1 Materials**

#### 205 **2.1.1 Hydroponic Nutrient Solution**

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213 The hydroponic nutrient solution used for the solution culture experiment consisted of the  
214 following chemical compounds: 5 mM  $\text{NH}_4\text{NO}_3$ , 2 mM  $\text{K}_2\text{SO}_4$ , 4 mM  $\text{CaCl}_2$ , 1.5 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  
215 1.3 mM  $\text{KH}_2\text{PO}_4$ , 50  $\mu\text{M}$  Fe(II)-ethylenediaminetetraacetic acid (EDTA), 10  $\mu\text{M}$   $\text{H}_3\text{BO}_4$ , 1.0  $\mu\text{M}$   
216  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0  $\mu\text{M}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 5.0  $\mu\text{M}$   $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.5  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , and 0.2  $\mu\text{M}$   
217  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ . The pH of the solution was adjusted to 5.5 using 0.1 M KOH or HCl.  
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#### 225 **2.1.2 The Experimental Soil**

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228 The soil sample used for the greenhouse experiment was taken from the paddy rice field of  
229 the experimental farm at the South China Agricultural University (Guangzhou, China). The soil  
230 samples were air-dried after collection and then crushed to pass a 2 mm sieve prior to the use in the  
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239 experiments. The soil had a pH of 6.52 and contained 2.38% of organic matter. Total nitrogen,  
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241 phosphorus and potassium were 1.06, 1.04 and 19.6 g/kg, respectively. Available nitrogen,  
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243 phosphorus and potassium were 114, 77.8 and 122 mg/kg, respectively. The soil contained 15.6  
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245 mg/kg of arsenate-As and no other arsenic species were detected.  
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### 248 249 **2.1.3 The Rice Seedlings Used in the Experiment**

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252 The seeds of rice (*Oryza sativa* cultivar: Tianyou 122) used in the experiment were provided  
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254 by the Guangdong Academy of Agricultural Sciences. Prior to sowing, the seeds *were* surface-  
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256 sterilized by soaking in 30% H<sub>2</sub>O<sub>2</sub> for 15 min. The sterilized seeds were then rinsed with deionized  
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258 water and placed in a container with moistened sands for germination. The pre-germinated seeds  
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260 were sown into the seed bed that was covered by a plastic sheet to maintain the temperature at 28 ±  
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262 2 °C. Healthy seedlings with 4 leaves were selected for the experiment.  
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## 266 **2.2 Experimental Design**

### 267 268 269 **2.2.1 Solution Culture Experiment**

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272 The rice seedlings were grown in the hydroponic nutrient solution for 3 weeks. The seedlings  
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274 were then rinsed with deionized water and transplanted into a beaker containing 500 mL of 20 mg  
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276 Fe<sup>2+</sup>/L solution (pH being adjusted to 5.5) for 24 h to allow the formation of iron plaques on the root  
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278 surfaces of the seedlings. After this, the seedlings were rinsed to remove any soluble Fe attached to  
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280 the plant surface before being used in the experiments.  
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285 Two sets of the experiments were performed aiming to collect data at the end of two different  
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287 lengths of growth period: 1 day (24 h) and 30 days (720 h). For each set of the experiment, one  
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289 control and one treatment were set; (a) control: plant growing in the hydroponic nutrient solution  
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291 with added arsenite-As at a dose of 1 mg/L; (c) Treatment: plant growing in the hydroponic nutrient  
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298 solution with added arsenite-As at a dose of 1 mg/L plus Fenton reagent (100  $\mu\text{M}$   $\text{H}_2\text{O}_2$  and 100  $\mu\text{M}$   
299  $\text{Fe}^{2+}$ ). For the 1-day experiment, the control and treatment were labelled as C1d and T1d,  
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301 respectively. For the 30-day experiment, the control and treatment were labelled as C30d and T30d,  
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304 respectively.  
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308 A 500 mL plastic cup (diameter: 8 cm; height: 15 cm) was used as a hydroponic container,  
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310 which was placed into a black nylon bag to avoid exposure of the plant roots to light. The lid with  
311  
312 holes was used to support the plants. Six rice plants were grown in each hydroponic container. The  
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314 plant growth units were placed randomly in a climate chamber with the daily light-dark cycle being  
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316 set at 16 h : 8 h. The light density during the photoperiod was fixed at 2500 lx. Temperature during  
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318 the dark and light periods was set at 20 °C and 28 °C, respectively. Relative humidity was  
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320 maintained at a range of 80-85 %. All the experiments were performed in 4 replicates.  
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324 For the 30-day experiment, the culture solution in each hydroponic container was replenished  
325  
326 every 3 days. This included addition of arsenite-As for the control and addition of arsenite-As plus  
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328 Fenton reagent for the treatment.  
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332 At the end of the 1-day experiment, samples of the spent culture solution were taken to  
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334 determine various As species. For the 30-day experiment, only the first (3 days or 72 hours) spent  
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336 culture solution was used for analysis of As species. These spent solution samples were labelled as  
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338 CS1d and TS1d for the control and treatment of the 1-day experiment, respectively, and CS3d and  
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340 TS3d for the control and the treatment of the first spent solution of the 30-day experiment,  
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342 respectively.  
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346 At the end of each experiment, the plants were harvested for determinations of biomass, various  
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348 As species in the plant tissues, and Fe and various As species in the root plaques. Since all the six  
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350 plants growing in each hydroponic container had very similar growth performance, only three of the  
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357 six plants were randomly selected: (a) the first one was used for determination of the biomass; (b) the  
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359 second one was used for measurement of As in various plant organs; and (c) the third one was used  
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361 to extract iron plaque.  
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### 364 365 **2.2.2 Pot Experiment**

366  
367 A greenhouse experiment was conducted to observe the growth performance of the rice plants  
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369 and uptake of As by the rice plants. The experiment lasted for more than 9 months, including two  
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371 continuous crops with a fallow period of about 3 months. The first crop commenced on September 8,  
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373 2013 and the rice plants were harvested on January 7, 2014; the second crop commenced on April 3,  
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375 2014 and the rice plants were harvested on July 22, 2014.  
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379 The soil without added As was used as the control (Ck); Treatments 1 and 2 (T1 and T2,  
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381 respectively) were the artificially contaminated soils without and with added Fenton reagent (100  
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383  $\mu\text{M H}_2\text{O}_2$ :100  $\mu\text{M Fe}^{2+}$ ), respectively. The dose of added arsenite-As in the contaminated soils was  
384  
385 set at 50 mg/kg. The thickness of the overlying water layer was maintained at approximately 2 cm.  
386  
387 For T2, an appropriate amount of standardized  $\text{H}_2\text{O}_2$  and  $\text{FeSO}_4$  solution was added to the overlying  
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389 water to maintain a theoretical concentration of  $\text{H}_2\text{O}_2$  and  $\text{Fe}^{2+}$  at 100  $\mu\text{M}$  each at the beginning of  
390  
391 Fenton reagent addition for each 3-day cycle.  
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394  
395 Two seedlings were transplanted to a soil column consisting of alternating layers (1 cm thick) of  
396  
397 quartz sand and a relevant soil material. This design was to allow easy separation of the root  
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399 materials from the soils upon harvest. The soil column was contained in a nylon mesh bag (#400  
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401 mesh; diameter: 8 cm; depth: 12 cm). Four soil columns were placed in a plastic bucket (Diameter:  
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403 22 cm; Height: 15 cm) that was filled with the same soil material. This design allowed the separation  
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405 of rhizospheric soil from the bulk soil by confining the rice plant roots within the nylon mesh bag or  
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407 so-called rhizo-bag.  
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416 Compound fertilizer (N:P:K=15:15:15) was applied at a rate of 19 g per pot at the 7<sup>th</sup> day of the  
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418 experiment. Additional fertilizers were added at a rate of 6.8 g/pot for compound fertilizer and 9.6  
419  
420 g/pot for urea in the early tillering stage of the first crop. In the second crop, 6.8 g/pot and 7 g/pot  
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422 were added 7 days after transplanting of the rice seedlings and in the heading stage, respectively.  
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426 In the first crop, one of the four rhizo-bags (together with the above-ground portion) was  
427  
428 randomly removed from each bucket in the heading stage. A second rhizo-bag was removed in the  
429  
430 maturity stage. For the second crop, sampling was carried out in the tillering, heading and maturity  
431  
432 stages. After collection, the soil materials in each rhizo-bag were recovered by separation from the  
433  
434 quartz sands. One of the two rice plants from each rhizo-bag was used for measurement of biomass  
435  
436 and another one was used for determination of various As species in the plant tissues.  
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### 439 **2.3 Sample Preparation and Analytical Methods**

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443 For biomass measurements, the straw and root portions of the rice plant were separated. The  
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445 roots were rinsed with water and the excess moisture on the root surfaces was removed using  
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447 absorbent paper towels. Fresh biomass of the two portions was obtained before they were oven-dried  
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449 at 60 °C until constant weight was attained.  
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452 For measurements of various As species in plant tissues, different organs of the rice plant (leaf,  
453  
454 stem, root and grain) were deep-frozen at -40 °C immediately after collection. The samples were then  
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456 freeze-dried using a VirTis freeze dryer. The dried plant tissue samples were pulverized (For the rice  
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458 grains, the hulls were removed but no polish was applied prior to pulverization; for the roots, iron  
459  
460 plaque was not removed) and then stored at -20 °C before being analyzed. Four As species were  
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462 determined. These include arsenate-As, arsenite-As, monomethylarsonic acid-As (MMA-As) and  
463  
464 dimethylarsinic acid-As (DMA-As). Measurements of various As species were performed using a  
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466 HPLC-ICP-MS system. For HPLC (Agilent1260) separation, Athena C18-WP column and guard  
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475 column were used. The mobile phase was a mixed solution of citric acid and sodium sulfonate. The  
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477 flow rate was set at 1.0 mL/min with an injection volume of 20  $\mu$ L. For ICP-MS (Agilent 7700),  
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479 argon was used as carrier gas and make-up gas. Details on the instrumental operating conditions are  
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481 given in Supplementary Table S1.  
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485 Iron plaque attached on the root surface was extracted by dithionite-citrate-bicarbonate (DCB,  
486  
487 [Liu et al., 2005](#)). Briefly, fresh roots were rinsed with deionized water and then dried with adsorbent  
488  
489 paper towels. For each rhizo-bag, an appropriate amount of root materials were randomly taken and  
490  
491 placed in a beaker containing 30 mL of mixed solution of 0.03 M  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  and 0.125 M  
492  
493  $\text{NaHCO}_3$ . 1 g of  $\text{Na}_2\text{S}_2\text{O}_4$  was then added into the beaker. After mixing, the beaker with its content  
494  
495 was allowed to stand for 30 min. The root materials were removed from the beaker and washed with  
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497 deionized water three times. The extract, together with the spent washing water, was transferred into  
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499 a 100 mL volumetric flask, followed by adding an appropriate amount of water to the mark. The  
500  
501 washed roots were then oven-dried at 70  $^\circ\text{C}$  to constant weight.  
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504  
505 The iron in the DCB extract was determined by atomic absorption spectrometry (ZEEnit 700 P).  
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507 Measurements of various As species in the DCB extract were performed using a HPLC-ICP-MS  
508  
509 system. The total As in the root plaque was estimated by the sum of various As species.  
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## 512 **2.4 QC/QA and statistical analysis**

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516 The hydroponic culture experiment was performed in 4 replicates and the pot experiment was  
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518 performed in 3 replicates. The recovery rates of matrix spike for plant tissue samples in the  
519  
520 hydroponic experiment were  $80.7 \pm 3.51$  for arsenate-As,  $92.5 \pm 2.41$  for arsenite-As,  $82.2 \pm 2.72$  for  
521  
522 MMA-As and  $123 \pm 5.98$  for DMA-As. The recovery rates of matrix spike for plant tissue samples in  
523  
524 the pot experiment were  $89.6 \pm 3.43$  for arsenate-As,  $105 \pm 3.44$  for arsenite-As,  $106 \pm 3.18$  for MMA-  
525  
526 As and  $122 \pm 6.96$  for DMA-As. Statistical difference analysis was performed using One-way  
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534 ANOVA (SPSS17.0).  
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### 537 **3 RESULTS** 538

#### 539 **3.1 Hydroponic Culture Experiment** 540 541 542

543  
544 For both CS1d and CS3d, the concentration of the originally added arsenite-As more or less  
545 remained unchanged (Fig. 1). However, for TS1d and TS3d, all the originally added arsenite-As  
546 disappeared and arsenate-As was the only As species detected. The concentration of As<sup>5+</sup> in the  
547 solution was lower than that of arsenite-As originally added into the system. In particular, TS3d only  
548 contained about 3.8% of the originally added As. Iron precipitates were observed to occur on the  
549 bottom and wall of the hydroponic containers.  
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557 The fresh biomass (either total, shoot or root) of the rice plant was significantly ( $p<0.05$ )  
558 higher in the treatment than in the control for both the 1-day and the 30-day experiments though for  
559 the dry biomass, the difference between the control and the treatment was statistically insignificant  
560 for the 1-day experiment (Table 1).  
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567 Arsenate-As dominated the As species, followed by arsenite-As. Very small amounts of  
568 methylated As species were also detected (Table 1). There was a consistent trend showing that the  
569 arsenate-As and DMA-As in the root portion was significantly ( $p<0.05$ ) higher in the control than in  
570 the treatment for both the 1-day experiment and the 30-day experiment while there was no  
571 significant difference ( $p>0.05$ ) in arsenite-As and MMA-As between the control and the treatment  
572 for both the 1-day experiment and the 30-day experiment. Overall, the sum of various As species was  
573 higher in the control than in the treatment, especially for the leaf portion.  
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583 For the stem portion, mixed results were observed. The 1-day experiment showed a higher sum  
584 of As species in the control than in the treatment (Table 1). However, the opposite was observed for  
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593 the 30-day experiment. Unlike root portion, arsenite-As dominated As species in the stem portion  
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595 and methylated As species was detected only in the 1-day experiment. There was no significant  
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597 ( $p>0.05$ ) difference in any As specie between the control and the treatment for both the 1-day  
598  
599 experiment and the 30-day experiment.  
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602  
603 For the leaf portion, there was no significant ( $p>0.05$ ) difference in any As species between the  
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605 control and the treatment except for arsenite-As in the 30-day experiment, which showed a  
606  
607 significantly ( $p<0.05$ ) higher value of arsenite-As in the control than in the treatment (Table 1).  
608  
609 Similar to the stem portion, arsenite-As dominated As species and no methylated As species were  
610  
611 detected in the treatment for the 30-day experiment.  
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614  
615 For both the 1-day and 30-day experiments, the total Fe in the root plaque was greater in the  
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617 treatment than in the control (Table 1). Total As in the root plaque was significantly greater in the  
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619 treatment than in the control for the 30-day experiment. However, the same was not observed for the  
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621 1-day experiment; there was no significant difference in root plaque-borne As between the control  
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623 and the treatment.  
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### 626 **3.2 Pot Experiment**

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629  
630 As expected, biomass tended to be smaller in the contaminated soils (T1 and T2) than in the  
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632 control (Ck, non-contaminated soil) due to As toxicity (Table 2). Comparison shows that treatment  
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634 of the contaminated soil with Fenton reagent (T2) resulted in a significant ( $p<0.05$ ) increase in  
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636 biomass, as compared to T1 for the first crop and the tillering stage of the second crop. For the  
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638 maturity stage of the first crop, the growth performance was even better in T2 than in Ck. However,  
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640 it is interesting to note that there was no significant difference in dry biomass of the shoot portion  
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642 between T1 and T2 for the heading stage of the second crop and the dry biomass of the shoot portion  
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644 was even greater in T1 than in T2 for the maturity stage of the second crop. For the root portion,  
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652 there was no significant difference in the dry biomass among Ck, T1 and T2. In consistent with the  
653 biomass, grain yield also tended to be in the following decreasing order: Ck (10.6 g) > T2 (8.0 g) >  
654 T1 (5.7 g).  
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659  
660 The sum of various As species in the root portion was greater in T1 than in Ck, particularly in  
661 the first crop and the tillering stage of the second crop. By comparison, the root-borne As was  
662 significantly ( $p < 0.05$ ) less in T2 than in T1 for the heading stage of the first crop and the tillering  
663 stage of the second crop. However, no significant ( $p > 0.05$ ) difference in root-borne As between T1  
664 and T2 was observed for the other sampling occasions (Table 3).  
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672 Methylated As species only accounted for a small proportion of the root-borne As (Table 3).  
673 In most of situations, arsenite-As dominated As species except in T1 for the heading stage of the first  
674 crop and in T1 and T2 for the tillering stage of the second crop when the amount of arsenate-As was  
675 close to that of arsenite-As or even slightly greater. One thing in common was that root-borne As  
676 tended to be lower in the maturity stage than in the respective earlier growing stages for either  
677 arsenite-As or arsenate-As. By comparison, root-borne As at the same growth stage tended to be  
678 higher in the first crop than in the second crop for the control and the treatments.  
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688 In comparison with the root-borne As, the concentration of As in the stem portion was  
689 relatively smaller (Table 4). Like root-borne As, stem-borne As (sum of various As species) also  
690 showed a significantly higher value in T1 than in Ck for any of the growth stages for the two crops.  
691 Unlike the root-borne As, stem-borne As was smaller in T1 than in T2 for the heading stage of the  
692 first crop and the tillering stage of the second crop while the opposite was observed for the other  
693 three sampling occasions.  
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702 The proportion of methylated As species in the sum of As species was generally small except  
703 for those in the maturity stage of the first crop (Table 4). For the first crop and the tillering stage of  
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711 the second crop, arsenate-As was greater than did arsenate-As while the opposite was observed for  
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713 the heading stage and maturity stage of the second crop.  
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717 Like the root and stem portions, leaf-borne As (sum of the As species) was consistently  
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719 greater (significantly at  $p<0.05$ ) in T1 than in Ck though the difference was not statistically  
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721 significant in the maturity stage of the second crop (Table 5). For all of the five sampling occasions,  
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723 there was no significant difference in leaf-borne As between T1 and T2. Like the root and stem  
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725 portions, methylated As species only took up a small proportion in the sum of various As species.  
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727 There was a clear trend showing that arsenite-As dominated As species in the heading and maturity  
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729 stages of the second crop. However, mixed results were observed for other sampling occasions.  
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733 The abundance of grain-borne As (sum of various As species) in both the first and second  
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735 crops had the same pattern:  $T1 > T2 > Ck$ . This was consistent with the pattern observed for the stem  
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737 portion in the maturity stage (Fig. 2). By comparison, the concentration of As in the grain portion  
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739 was consistently higher in the second crop than in the first crop. This was accompanied by the same  
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741 trend for the stem-borne As. Arsenite-As and DMA-As were the two dominant species. Depending  
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743 on individual treatments, Ck had more DMA-As; T2 contained more arsenite-As; and T1 tended to  
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745 have equal amounts of arsenite-As and DMA-As.  
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749 There were orange-colored coating materials (root plaque) on the surfaces of plant roots.  
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751 However, root coatings did not cover the entire root surface with T1 tending to have a lower  
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753 coverage of root plaque, as compared to T2. The abundance of root plaque-borne Fe, as measured by  
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755 the amount of Fe attached to the surface of per unit of root biomass (g/kg) in the different stages of  
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757 rice plant growth for the control and the two treatments is shown in Table 6. The root plaque-borne  
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759 Fe tended to be higher in T2 than in either T1 or Ck (significant at  $p<0.05$ ). There was a clear trend  
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761 showing that root plaque-borne As increased from Ck to T1 to T2 for all the five sampling occasions.  
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773 **4 DISCUSSION**  
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777 The results obtained from the hydroponic experiment suggest that, under the set experimental  
778 conditions,  $\text{As}^{3+}$  was resistant to oxidation in the presence of molecular oxygen only. However,  
781 addition of Fenton reagent markedly accelerated the oxidation of  $\text{As}^{3+}$ , resulting in formation of  $\text{As}^{5+}$ .  
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783 The decrease in As concentration in the culture solutions suggests that immobilization of As took  
784 place. The presence of iron precipitates on the bottom and wall of the hydroponic containers suggests  
785 that the  $\text{Fe}^{3+}$  formed from Fenton reaction acted as a scavenger to sequester As from the hydroponic  
786 solution, resulting in a decrease in solution-borne As. [Since the hydroponic nutrient solution](#)  
787 [contained FeII-EDTA, which is not stable in the presence of oxygen, the Fe from this source could](#)  
788 [be oxidized to  \$\text{Fe}^{3+}\$ , resulting in the formation of iron oxyhydroxide that might add to the plaque](#)  
789 [\(Seibig and and van Eldik, 1997\). This was also likely to enhance the local oxidation of  \$\text{As}^{3+}\$  to  \$\text{As}^{5+}\$](#)   
790 [\(Hug and Leupin, 2003\). In addition, the As\(III\)-oxidizing microbes could also play an important](#)  
791 [role in oxidizing As on the root iron plaque \(Hu et al. 2015\).](#)  
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803 The generally lower concentration of As in the rice plant tissue in the treatment, relative to the  
804 control, can be attributed to the reduced availability of As in the hydroponic solution. The  
805 predominant presence of arsenate-As in the root portion appears to suggest that while both arsenate  
806 and arsenite might be taken up by the rice seedlings, the uptake of As by root took place more  
807 favourably through an arsenate pathway. The change in the predominant As species from arsenate-  
808 As to arsenite-As in the above-ground portion reflects the in-plant reduction of arsenate-As ([Kramar](#)  
809 [et al., 2015](#)).  
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819 The significantly greater root plaque-borne Fe concentration in the treatment than in the control  
820 suggests that addition of Fenton reagent significantly enhanced the formation of iron compounds on  
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829 the root surfaces of the rice plants. Liu et al. (2006) suggested that root plaque-Fe was in mineral  
830 forms of iron oxyhydroxides. This work demonstrates that addition of Fenton reagent enhanced the  
831 formation of Fe<sup>3+</sup>-containing chemical compounds on the root surface.  
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837 Although no significant difference between the control and the treatment was observed for the 1-  
838 day experiment, the root plaque-borne As was significantly ( $p<0.05$ ) greater in the treatment than in  
839 the control for the 30-day experiment. This suggests that the addition of Fenton reagent could  
840 enhance retention of As by the root plaque. However it took time to incorporate solution-borne As  
841 into root plaque and a duration of 24 hours was not sufficient to allow this to take place even when  
842 Fenton reagent was added into the system. For the control, transformation of arsenite-As to arsenate-  
843 As did not take place and arsenite was the only form of arsenic in the nutrient solution (Fig. 1).  
844 Therefore, any arsenate contained in the root plaque was likely to be formed as a result of arsenite  
845 oxidation driven by root-released oxygen. For the treatment, production of arsenate was markedly  
846 enhanced due to Fenton reaction. From Fig. 1, it is clear that conversion of all arsenite-As into  
847 arsenate-As was completed within 1 day after addition of Fenton reagent. The arsenate formed was  
848 then gradually removed from the nutrient solution by deposition as iron precipitates and plant uptake.  
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863 In the pot experiment, the poorer growth performance, as indicated by smaller biomass in T1 than in  
864 Ck during the first crop and the tillering stage of the second crop suggests that an initial dose of As at  
865 50 mg/kg was sufficient to cause phytotoxicity to the rice plants under the set experimental  
866 conditions. Das et al. (2013) observed phytotoxicity to paddy rice at a dose of 40 mg As/kg, which is  
867 very similar to 50 mg As/kg in this experiment. The toxic effects of As on rice plant growth became  
868 less significant during the heading and maturity stages of the second crop. This may be attributed to  
869 reduced bioavailability of the added As due to As immobilization through formation of practically  
870 insoluble minerals such as scorodite or adsorption by soil colloids such as Fe oxyhydroxides (Lin  
871 and Puls, 2000; Campbell and Nordstrom, 2014; Serrano et al., 2015). Contamination of the soils by  
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889 As led to increased uptake of As by the plants, which impedes the physiological functions of the  
890 plants (Hughes, 2002; Islam et al., 2015). However, the application of Fenton reagent effectively  
891 reduced this harmful effect and significantly enhanced the growth of the rice plants grown in the As-  
892 contaminated soils. It is interesting to note that the significant increase in biomass in the first crop  
893 and the tillering stage of the second crop in T2, as compared to T1, was accompanied by a significant  
894 reduction in root-borne As in T2, relative to T1 while the insignificant difference in biomass between  
895 T1 and T2 in the heading and maturity stages of the second crop was consistent with the insignificant  
896 difference in root-borne As between T1 and T2. It is noted that the biomass tended to be greater in  
897 the first crop than in the second crop (Table 2). The rice cultivar (TY122) used for the experiment  
898 was the one that is more suitable for being grown during the period from autumn to early winter (the  
899 first crop) than during the period from late spring to summer (the second crop). In addition, the  
900 application rate of chemical fertilizers was relatively lower in the second crop than in the first crop,  
901 and this might also affect the growth performance of the rice plants in the second crop.  
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917 The relatively low level of root plaque-borne As in Ck reflected the limited availability of As in  
918 the non-contaminated soil. A significantly higher level of root plaque-borne As in T2, as compared  
919 to T1 is attributable to the enhanced formation of iron plaques on the root surfaces of the rice plants  
920 due to application of Fenton reagent, which in turn allowed more As being intercepted when As in  
921 the soil solution moved towards the surfaces of the plant roots, and consequently reduced the  
922 amounts of As being taken by the plant roots.  
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931 The trend that  $As_{Stem}/As_{Root}$  and  $As_{leaf}/As_{Root}$  increased over time (Table 7 and Table 8) suggests  
932 that the root-to-shoot translocation of As was enhanced as the rice plants became more mature,  
933 possibly due to intensified transpiration. The much higher  $As_{Stem}/As_{Root}$  at the maturity stage in the  
934 second crop than in the first crop indicates that the efficiency of root-to-stem As translocation was  
935 improved due to the reduced As phytotoxicity, which allowed better growth performance of the rice  
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947 plants being achieved. This explains the much higher rice grain-borne As in the second crop than in  
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949 the first crop.  
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952 The capacity of root plaque to impede As flux towards the root surfaces in T1 were limited,  
953 leading to substantial uptake of As by the roots. This could also be due to that younger roots and the  
954 younger parts of the old roots that play key role in plant uptake of nutrients and metals were hardly  
955 coated by iron plaque, as also pointed out by other workers (Seyfferth et al., 2010; Yamaguchi et al.,  
956 2014). The addition of Fenton reagent led to production of  $\text{Fe}^{3+}$  and hydroxyl radical that enhanced  
957 formation of iron precipitates and  $\text{As}^{3+}$ - $\text{As}^{5+}$  conversion. This effect was not limited to rhizosphere  
958 but also the bulk soils. As demonstrated in the hydroponic experiment, solution-borne arsenite can be  
959 oxidized and removed from the culture solution within a relatively short period of time after addition  
960 of Fenton reagent. It is therefore likely that arsenite in the soil pore water could experience the same  
961 process for the pot experiment. The immobilization of As in the bulk soil could markedly reduce the  
962 supply of dissolved As for the plant root, leading to reduced uptake of As by the rice plants. The  
963 effect of  $\text{FeSO}_4$  addition on enhancing formation of iron plaque on rice root surfaces was previously  
964 observed by Hossain et al. (2009)  
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981 The concentration of As (0.26 mg/kg for the 1<sup>st</sup> crop and 0.55 mg/kg for the 2<sup>nd</sup> crop) in the  
982 grain of rice plants grown in the contaminated soils (T1) far exceeded the maximum limit of 0.1  
983 mg/kg set by the European Union for the rice destined for the production of foods for infants and  
984 young children (Signes-Pastor et al. 2017) though the level of As could be lower than these values if  
985 the rice grains are polished (Meharg et al., 2008). The significant reduction in rice grain-borne As in  
986 both the first and second crops due to addition of Fenton reagent sheds some light on the possible  
987 role of rainwater-borne  $\text{H}_2\text{O}_2$  in alleviating As contamination in rice grain. In our recent experiment  
988 examining the paddy soils receiving natural rainwater containing hydrogen peroxide, a similar effect  
989 like what was showed in this microcosm experiment was observed, suggesting that rainwater-borne  
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1006 hydrogen peroxide does affect arsenic chemistry in paddy soils (unpublished data). This raises a  
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1008 question on whether rice produced from areas receiving abundant rainfall tends to contain less  
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1010 arsenic. It will be interesting to establish whether there is a relationship between annual rainfall and  
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1012 rice grain-borne As on a global scale.  
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1016 From a mitigation perspective, the research findings have implications for developing cost-  
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1018 effective management strategies and remediation techniques to reduce As uptake by rice plants and  
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1020 accumulation in the rice grain. The uses of industrial grade H<sub>2</sub>O<sub>2</sub> (US\$500/t, source: Zhengzhou  
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1022 Huize Biochemical Technology Co., Ltd) and FeSO<sub>4</sub> (US\$100/t, source: Dalian Future International  
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1024 Co., Ltd.) are not economically prohibitive. A rough calculation based on the experimental design in  
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1026 this study gives an estimated cost of US\$89 per hectare for the purchase of the required chemicals. If  
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1028 appropriate procedure for mixing the Fenton reagent into the irrigation water can be developed,  
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1030 significant reduction of As level in rice grain may be achieved cost-effectively in rice-producing  
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1032 areas where the soils contain high level of As or where As-bearing groundwater is used for irrigation  
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1034 purpose.  
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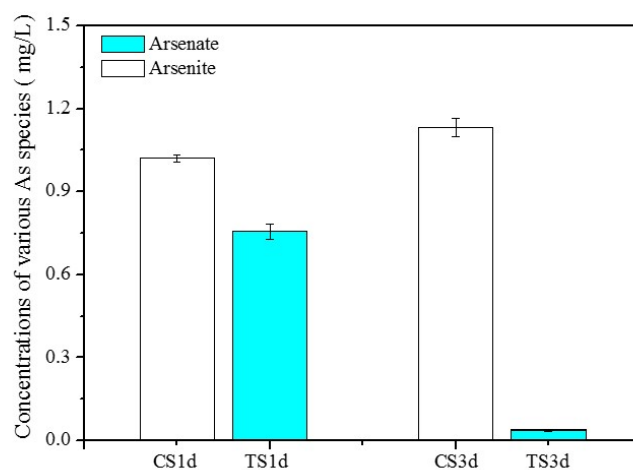
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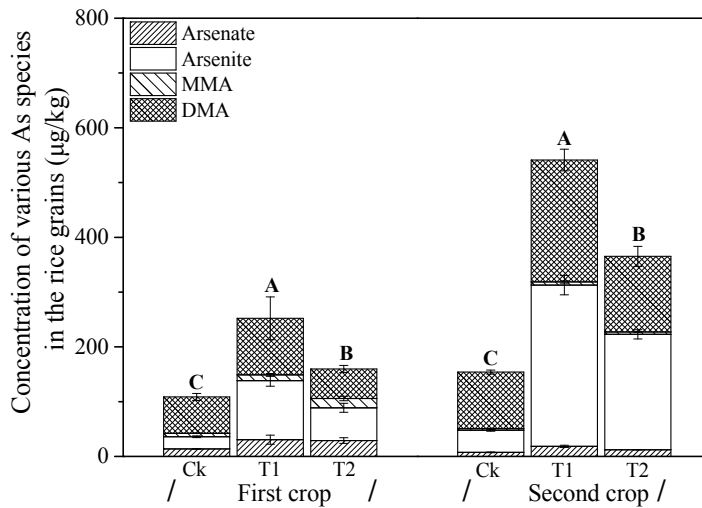
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**Fig 1 Concentration of various arsenic species in the culture solution in the control (CS) and the treatment (TS) at the end of the 1-day experiment (24 h) and at the end of the first nutrient replenishment cycle (72 h) of the 30-day experiment. All values are presented as mean  $\pm$  standard error (n=4).**



**Fig 2 Concentration of various arsenic species in the rice grain harvested in the first crop and second crop for the control (Ck) and the two treatments (T1 and T2) in the pot experiment. All values are presented as mean  $\pm$  standard error (n=3) and bars with different letters indicate significantly ( $P < 0.05$ ) different means for the sum of various arsenic species (arsenate-As, arsenite-As, MMA-As and DMA-As).**



**Table 1 Dry biomass, arsenic species in plant tissues, and iron and arsenic species in root plaque for the 1-day and 30-day hydroponic experiments**

		C1d	T1d	C30d	T30d
Dry biomass (g)	Straw	0.20±0.01	0.26±0.05	0.56±0.05	0.78±0.06
	Root	0.07±0.01	0.06±0.00	0.23±0.06	0.30±0.02
As (root portion, mg/kg)	Arsenate-As	98.5±3.04*	70.0±1.85	315±9.74*	238±6.30
	Arsenite-As	18.4±0.63	18.7±3.02	59.0±2.03	59.9±9.67
	MMA-As	2.08±0.44	0.82±0.10	4.99±1.06	1.96±0.24
	DMA-As	3.40±0.78*	1.65±0.15	8.84±2.04*	4.29±0.38
	Total As	122	91.1	387	304
As (stem portion, mg/kg)	Arsenate-As	5.14±0.61	4.42±0.68	15.9±0.21	19.5±1.49
	Arsenite-As	11.4±0.73	8.86±0.63	22.5±1.70	25.7±3.29
	MMA-As	ND	ND	ND	ND
	DMA-As	1.1±0.12	0.64±0.31	ND	ND
	Total As	17.6	13.9	38.4	45.2
As (leaf portion, mg/kg)	Arsenate-As	1.85±0.38	2.04±0.26	20.6±2.56	11.3±1.47
	Arsenite-As	3.13±0.37	3.48±0.12	45.7±6.11*	27.6±2.69
	MMA-As	ND	ND	ND	ND
	DMA-As	0.14±0.02	0.06±0.02	ND	ND
	Total As	5.12	5.58	66.3	38.9
Root plaque-Fe (g/kg)	Total Fe	5.86±1.38*	8.29±0.46	2.00±0.18*	8.45±0.58
Root plaque-As (mg/kg)	Total As	60.9±1.26	56.2±1.12	76.6±0.51*	115±5.59

All values are presented as mean ± standard error (n=4). Independent sample t-test was used to determine whether the two mean values obtained for the control and the treatment differ significantly. Pairs marked with an asterisk indicate significant (P <0.05) difference between the control and the treatment for each harvest time. ND: not detectable.

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**Table 2 Fresh and dry weight (g) of the shoot and root in the first crop and second crop for the control and the two treatments for the pot experiment**

Growth stage	Treatments	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
Heading (1 <sup>st</sup> crop)	Ck	47.4±1.11a	8.88±0.59a	20.5±1.35a	4.48±0.74a
	T1	14.5±1.32c	2.88±0.3c	6.04±0.97c	0.94±0.21c
	T2	28.8±1.84b	6.14±0.27b	13.9±0.68b	2.76±0.24b
Maturity (1 <sup>st</sup> crop)	Ck	29.1±4.20ab	14.7±1.45a	24.0±0.91b	8.52±0.91b
	T1	21.1±0.45c	8.24±1.05c	4.11±0.29c	1.64±0.32c
	T2	31.0±1.82a	13.2±0.60ab	31.0±4.21a	14.6±1.32a
Tillering (2 <sup>nd</sup> crop)	Ck	5.40±0.58a	0.71±0.11a	0.52±0.05a	0.12±0.02a
	T1	1.57±0.13c	0.23±0.02c	0.14±0.00c	0.07±0.02ab
	T2	2.75±0.53bc	0.41±0.09bc	0.30±0.05bc	0.08±0.01ab
Heading (2 <sup>nd</sup> crop)	Ck	24.7±2.72a	8.07±1.23a	13.3±1.70ab	1.73±0.17a
	T1	18.5±1.29ab	6.52±0.31ab	17.8±0.80a	2.12±0.11a
	T2	16.8±2.17b	6.42±0.76ab	18.4±2.01a	2.25±0.64a
Maturity (2 <sup>nd</sup> crop)	Ck	15.9±1.46a	6.63±0.68ab	14.0±1.50a	1.77±0.24a
	T1	16.7±1.87a	7.27±0.78a	11.8±2.87a	2.43±0.67a
	T2	12.8±1.68ab	4.74±0.76b	9.36±0.58ab	1.87±0.12a

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p<0.05$ ).

**Table 3 Concentration (mg/kg) of various As species in the root portion of the rice plant during different growth stages for the pot experiment**

Growth stage	Treatment	Arsenate-As	Arsenite-As	MMA-As	DMA-As	Sum
Heading (1 <sup>st</sup> crop)	Ck	12.7±2.10bc	19.1±1.94c	1.20±0.14a	0.10±0.01c	33.1±3.74b
	T1	73.2±0.78a	60.1±1.57a	1.21±0.12a	1.41±0.22a	136±2.16a
	T2	11.8±0.37c	32.8±5.95b	0.65±0.04b	0.79±0.11b	46.1±6.16b
Maturity (1 <sup>st</sup> crop)	Ck	1.21±0.05c	7.04±1.08c	0.79±0.02c	0.89±0.09ab	9.93±1.10b
	T1	2.03±0.31b	30.0±1.56a	1.19±0.15b	0.60±0.03b	33.8±1.11a
	T2	3.82±0.24a	22.1±2.96b	1.52±0.10a	1.28±0.23a	28.7±3.38a
Tillering (2 <sup>nd</sup> crop)	Ck	8.56±0.68c	11.1±1.15b	0.03±0.01b	0.59±0.03b	20.3±1.45c
	T1	82.6±4.15a	85.9±2.15a	2.90±0.37a	2.51±0.29a	174±6.72a
	T2	27.8±1.27b	18.2±1.26b	0.17±0.02b	0.66±0.04b	46.9±0.38b
Heading (2 <sup>nd</sup> crop)	Ck	3.04±0.39b	14.6±0.64b	0.14±0.00a	1.33±0.23a	19.1±0.53b
	T1	4.91±0.64ab	49.7±5.64a	0.35±0.05a	0.77±0.17a	55.7±6.11a
	T2	6.52±0.94a	52.4±2.92a	0.22±0.01a	1.27±0.23a	60.4±4.01a
Maturity (2 <sup>nd</sup> crop)	Ck	1.60±0.21a	4.94±0.32c	0.34±0.04b	0.50±0.08b	7.38±0.57b
	T1	1.89±0.55a	9.30±0.69b	1.42±0.20a	0.73±0.13ab	13.3±1.05a
	T2	1.61±0.39a	12.4±1.06a	0.82±0.04ab	0.88±0.05a	15.7±1.43a

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p < 0.05$ ).

**Table 4 Concentration (mg/kg) of various As species in the stem portion of the rice plant during different growth stages for the pot experiment**

Growth stage	Treatment	Arsenate	Arsenite	MMA	DMA	Sum
Heading (1 <sup>st</sup> crop)	Ck	1.40±0.09c	1.02±0.13c	0.19±0.01b	0.13±0.01c	2.74±0.15c
	T1	6.47±0.82ab	4.03±0.29ab	0.28±0.01ab	0.44±0.07b	11.2±0.49b
	T2	7.70±0.48a	5.12±0.16a	0.34±0.00a	0.60±0.01a	13.7±0.35a
Maturity (1 <sup>st</sup> crop)	Ck	2.16±0.21b	1.58±0.20ab	0.36±0.06c	0.47±0.03c	4.57±0.22c
	T1	3.75±0.30a	2.28±0.13a	1.30±0.21a	1.54±0.16a	8.87±0.34a
	T2	3.01±0.21ab	1.84±0.06ab	0.94±0.07ab	1.26±0.1ab	7.06±0.08b
Tillering (2 <sup>nd</sup> crop)	Ck	0.43±0.07c	0.34±0.03b	0.00±0.00ab	0.04±0.00a	0.80±0.05c
	T1	1.33±0.30b	0.55±0.01a	0.03±0.00a	0.04±0.00a	1.94±0.30b
	T2	2.45±0.13a	0.64±0.08a	0.02±0.00ab	0.07±0.00a	3.17±0.06a
Heading (2 <sup>nd</sup> crop)	Ck	0.56±0.13ab	7.03±0.78c	0.03±0.00b	0.06±0.00b	7.68±0.81c
	T1	0.88±0.19a	18.21±1.77a	0.08±0.02a	0.23±0.03a	19.3±1.84a
	T2	0.57±0.03ab	12.58±1.02b	0.05±0.02ab	0.24±0.05a	13.4±0.93b
Maturity (2 <sup>nd</sup> crop)	Ck	0.57±0.22a	8.78±0.55b	0.03±0.00a	0.08±0.02ab	9.46±0.60b
	T1	0.67±0.06a	20.46±2.65a	0.05±0.02a	0.23±0.06a	21.4±2.78a
	T2	0.91±0.37a	10.75±2.19b	0.03±0.00a	0.18±0.00a	11.8±2.52b

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p < 0.05$ ).

**Table 5 Concentration (mg/kg) of various As species in the leaf portion of the rice plant during different growth stages for the pot experiment**

Growth stage	Treatment	Arsenate-As	Arsenite-As	MMA-As	DMA-As	Sum
Heading (1 <sup>st</sup> crop)	Ck	1.64±0.16b	1.76±0.22c	0.08±0.01a	0.06±0.01b	3.53±0.33b
	T1	5.16±0.36a	3.48±0.56ab	0.09±0.01a	0.23±0.03b	8.97±0.87a
	T2	4.45±0.38a	3.83±0.33a	0.11±0.02a	0.88±0.07a	9.27±0.36a
Maturity (1 <sup>st</sup> crop)	Ck	1.87±0.18c	1.66±0.07c	0.09±0.02a	0.15±0.01b	3.77±0.13b
	T1	4.10±0.29a	4.81±0.42ab	0.08±0.01a	0.28±0.01a	9.27±0.66a
	T2	2.94±0.31b	5.40±0.42a	0.12±0.02a	0.12±0.02b	8.58±0.64a
Tillering (2 <sup>nd</sup> crop)	Ck	1.70±0.07c	2.40±0.20ab	0.04±0.00a	0.06±0.00ab	4.19±0.18b
	T1	3.73±0.28b	4.47±0.81a	0.05±0.00a	0.15±0.01a	8.41±1.08a
	T2	5.57±0.50a	3.04±0.57a	0.05±0.01a	0.09±0.01a	8.76±1.03a
Heading (2 <sup>nd</sup> crop)	Ck	0.11±0.01b	1.14±0.06b	0.00±0.00b	0.02±0.00b	1.26±0.07b
	T1	0.15±0.01ab	1.88±0.18a	0.02±0.00a	0.05±0.01a	2.10±0.19a
	T2	0.19±0.02a	2.08±0.21a	0.01±0.00ab	0.04±0.01ab	2.32±0.21a
Maturity (2 <sup>nd</sup> crop)	Ck	1.13±0.25a	7.20±0.85ab	0.14±0.03ab	0.25±0.04b	8.72±1.10a
	T1	2.22±1.14a	8.65±1.00a	0.27±0.03a	0.82±0.09a	11.9±0.45a
	T2	2.22±0.49a	7.97±0.51a	0.15±0.02ab	0.80±0.05a	11.1±1.00a

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p < 0.05$ ).

**Table 6 Concentration of total iron and arsenic in the iron plaque of root surface during different growth stages for the pot experiment**

Growth stage	Treatments	Total Fe (g/kg)	Total As (mg/kg)
Heading (1 <sup>st</sup> crop)	Ck	1.61±0.10c	19.1±4.84c
	T1	2.34±0.15bc	29.7±4.19b
	T2	21.35±1.44a	55.4±12.0a
Maturity (1 <sup>st</sup> crop)	Ck	15.00±0.60a	73.7±18.5c
	T1	11.30±0.36b	330±20.5b
	T2	15.50±0.49a	711±37.1a
Tillering (2 <sup>nd</sup> crop)	Ck	17.79±0.51a	235±3.35c
	T1	9.79±0.57b	526±20.6b
	T2	19.43±1.32a	980±38.2a
Heading (2 <sup>nd</sup> crop)	Ck	28.28±3.73a	175±8.86c
	T1	16.42±0.57b	562±13.5b
	T2	25.62±1.10a	707±38.8a
Maturity (2 <sup>nd</sup> crop)	Ck	17.52±0.98ab	173±4.98c
	T1	15.57±0.43b	297±3.28b
	T2	20.07±1.17a	579±15.6a

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p<0.05$ ).

**Table 7 The ratios of stem-borne As to root-borne As ( $As_{Stem}/As_{Root}$ ) in the different growth stages of rice plants for the control and the treatments for the pot experiment**

Growth stage	Treatment	Arsenate-As	Arsenite-As	MMA-As	DMA-As	Sum
Heading (1 <sup>st</sup> crop)	Ck	0.110	0.053	0.158	1.300	0.083
	T1	0.088	0.067	0.231	0.312	0.082
	T2	0.651	0.156	0.523	0.759	0.298
Mature (1 <sup>st</sup> crop)	Ck	1.785	0.224	0.456	0.528	0.460
	T1	1.847	0.076	1.092	2.567	0.262
	T2	0.788	0.083	0.618	0.984	0.245
Tillering (2 <sup>nd</sup> crop)	Ck	0.050	0.030	0.000	0.068	0.039
	T1	0.016	0.006	0.010	0.016	0.011
	T2	0.088	0.035	0.118	0.106	0.068
Heading (2 <sup>nd</sup> crop)	Ck	0.184	0.481	0.214	0.045	0.401
	T1	0.179	0.366	0.229	0.299	0.348
	T2	0.087	0.240	0.227	0.189	0.223
Mature (2 <sup>nd</sup> crop)	Ck	0.356	1.777	0.088	0.160	1.282
	T1	0.354	2.200	0.035	0.315	1.606
	T2	0.565	0.866	0.037	0.205	0.753

**Table 8 The ratios of leaf-borne As to root-borne As ( $As_{leaf}/As_{Root}$ ) in the different growth stages of rice plants for the control and the treatments for the pot experiment**

Growth stage	Treatment	Arsenate-As	Arsenite-As	MMA-As	DMA-As	Total
Heading (1 <sup>st</sup> crop)	Ck	0.128	0.092	0.067	0.600	0.106
	T1	0.070	0.058	0.074	0.163	0.066
	T2	0.376	0.116	0.169	1.114	0.201
Mature (1 <sup>st</sup> crop)	Ck	1.545	0.236	0.114	0.169	0.380
	T1	2.020	0.160	0.067	0.467	0.274
	T2	0.770	0.244	0.079	0.094	0.298
Tillering (2 <sup>nd</sup> crop)	Ck	0.199	0.215	1.333	0.102	0.206
	T1	0.045	0.052	0.017	0.060	0.048
	T2	0.200	0.166	0.294	0.136	0.187
Heading (2 <sup>nd</sup> crop)	Ck	0.036	0.078	0.000	0.015	0.066
	T1	0.031	0.038	0.057	0.065	0.038
	T2	0.029	0.040	0.045	0.031	0.038
Mature (2 <sup>nd</sup> crop)	Ck	0.706	1.457	0.412	0.500	1.182
	T1	1.175	0.930	0.190	1.123	0.896
	T2	1.379	0.642	0.183	0.909	0.708



## Highlights

- Hydroponic and pot experiments were conducted in arsenic-contaminated systems
- The effects of Fenton reagent on immobilization of arsenic were examined
- Fenton process enhanced the growth of rice plants in arsenic –contaminated systems
- As uptake by rice plant was impeded leading to reduced level of As in rice grain
- Implications for minimizing human health risk from consumption of As-rich rice

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4 **Fenton Reagent Reduces the Level of Arsenic in Paddy Rice Grain**  
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7 **Junhao Qin<sup>1,2</sup>, Yongjun Li<sup>3</sup>, Minling Feng<sup>3</sup>, Huashou Li<sup>1\*</sup> and Chuxia Lin<sup>2\*</sup>**  
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62 **ABSTRACT**  
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65 Hydroponic and pot experiments were conducted to examine the effects of Fenton reagent on paddy  
66 rice plant growing in arsenic-contaminated soils. Fenton reagent significantly reduced arsenic  
67 phytotoxicity, uptake by the plants and accumulation in rice grain. This is attributed to oxidation of  
68  $As^{3+}$  to  $As^{5+}$  by hydroxyl radicals and immobilization of arsenate by reacting with precipitating  $Fe^{3+}$   
69 to form practically insoluble compounds. Although this process enhanced the formation of Fe-  
70 enriched coatings on root surface, it appears that root plaque had limited effects on inhibiting As  
71 uptake since most of the young roots were not covered by iron plaque. It is more likely that As  
72 immobilization in the bulk soils play a major role in reducing As flux towards rhizosphere. The  
73 findings have implications for understanding As behavior in paddy field receiving rainwater-borne  
74 hydrogen peroxide and developing cost-effective techniques for reducing As level in rice grain  
75 produced from As-contaminated soils.  
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90 **Keywords:** Paddy rice, arsenic, iron plaque, soil, Fenton reaction  
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## 1 INTRODUCTION

Consumption of rice is a major pathway of human arsenic exposure, which could affect billions of people around the world (Schoof et al., 1999; Meharg, 2004; Williams et al., 2006; Zhu et al., 2008; Syu et al., 2015; Sinha and Bhattacharyya, 2015; Clemens and Ma, 2016). The anaerobic soil conditions associated with water inundation required for paddy rice farming favour reduction reactions, leading to formation of highly toxic arsenite ions (Xu et al., 2008; Li et al., 2009; Somenahally et al., 2011; Spanu et al., 2012). Arsenite tends to be predominantly present in undissociated form ( $\text{H}_3\text{AsO}_3^0$ ) under pH conditions encountered in most paddy rice soils (Zhao et al., 2009), and therefore it may be more resistant to immobilization by soil adsorbents. In addition, under reducing conditions the arsenic-scavenging capacity of soil is weakened due to reductive dissolution of various iron compounds that play a key role in binding soluble arsenic species through either formation of practically insoluble iron arsenate minerals or adsorption of arsenate to iron oxyhydroxides (Zhao et al., 2010; Zhu et al., 2014). As such, arsenite is readily available for uptake by rice plants and accumulation in rice grain (Williams et al., 2007; Su et al., 2010; Wang et al., 2015).

Iron-enriched root plaque plays an important role in reducing the entry of As present in the soil pore water (soil solution) into rice plant roots (Lee et al., 2013; Syu et al., 2013). The formation of root plaque is believed to be mediated by oxidation of ferrous iron ( $\text{Fe}^{2+}$ ) using molecular oxygen released from rice plant roots (Armstrong, 1964), and it is likely that the root-released oxygen also promotes microbially mediated oxidation of arsenite to form arsenate (Hu et al., 2015). As arsenate has the stronger affinity to  $\text{Fe}^{3+}$ , it is likely that arsenate-As tends to be intercepted more easily by the root plaque, as compared to arsenite-As (Chen et al., 2005; Liu et al., 2005).

It has been demonstrated that Fenton process involving reaction between hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and ferrous iron ( $\text{Fe}^{2+}$ ) resulted in enhanced oxidation of arsenite to form less toxic arsenate

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179  
180 (Hug and Leupin, 2003).  $\text{Fe}^{2+}$  is available in flooded soils like paddy rice soils (Becker and Asch,  
181 2005; Kögel-Knabner et al., 2010).  $\text{H}_2\text{O}_2$  is also commonly present in rainwater (Cooper et al., 1988;  
182 Willey et al., 1996; Gonçalves et al., 2010; Guo et al., 2014). In areas with abundant rainfall, Fenton  
183 reaction may be a naturally-occurring process that can affect the biogeochemical behaviour of  
184 arsenic in paddy rice soils. Where the enrichment of arsenic in rice grain becomes a significant  
185 health concern, it may be worthwhile to consider the use of Fenton reagent (a mixture of  $\text{H}_2\text{O}_2$  and  
186  $\text{Fe}^{2+}$ ) for reducing As uptake by rice plants.

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189 The objective of this study was to examine the effects of Fenton reagent on reducing As uptake  
190 by rice plants. The impacts of Fenton reagent on plant growth are also evaluated. In addition, the  
191 major biogeochemical mechanisms responsible for the observed phenomena are explored.

## 202 203 **2 MATERIALS AND METHODS**

### 204 205 **2.1 Materials**

#### 206 207 **2.1.1 Hydroponic Nutrient Solution**

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209  
210 The hydroponic nutrient solution used for the solution culture experiment consisted of the  
211 following chemical compounds: 5 mM  $\text{NH}_4\text{NO}_3$ , 2 mM  $\text{K}_2\text{SO}_4$ , 4 mM  $\text{CaCl}_2$ , 1.5 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  
212 1.3 mM  $\text{KH}_2\text{PO}_4$ , 50  $\mu\text{M}$  Fe(II)-ethylenediaminetetraacetic acid (EDTA), 10  $\mu\text{M}$   $\text{H}_3\text{BO}_4$ , 1.0  $\mu\text{M}$   
213  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0  $\mu\text{M}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 5.0  $\mu\text{M}$   $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.5  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , and 0.2  $\mu\text{M}$   
214  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ . The pH of the solution was adjusted to 5.5 using 0.1 M KOH or HCl.

#### 215 216 **2.1.2 The Experimental Soil**

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219 The soil sample used for the greenhouse experiment was taken from the paddy rice field of  
220 the experimental farm at the South China Agricultural University (Guangzhou, China). The soil  
221 samples were air-dried after collection and then crushed to pass a 2 mm sieve prior to the use in the  
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239 experiments. The soil had a pH of 6.52 and contained 2.38% of organic matter. Total nitrogen,  
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241 phosphorus and potassium were 1.06, 1.04 and 19.6 g/kg, respectively. Available nitrogen,  
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243 phosphorus and potassium were 114, 77.8 and 122 mg/kg, respectively. The soil contained 15.6  
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245 mg/kg of arsenate-As and no other arsenic species were detected.  
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### 248 249 **2.1.3 The Rice Seedlings Used in the Experiment**

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252 The seeds of rice (*Oryza sativa* cultivar: Tianyou 122) used in the experiment were provided  
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254 by the Guangdong Academy of Agricultural Sciences. Prior to sowing, the seeds *were* surface-  
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256 sterilized by soaking in 30% H<sub>2</sub>O<sub>2</sub> for 15 min. The sterilized seeds were then rinsed with deionized  
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258 water and placed in a container with moistened sands for germination. The pre-germinated seeds  
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260 were sown into the seed bed that was covered by a plastic sheet to maintain the temperature at 28 ±  
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262 2 °C. Healthy seedlings with 4 leaves were selected for the experiment.  
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## 266 **2.2 Experimental Design**

### 267 268 269 **2.2.1 Solution Culture Experiment**

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272 The rice seedlings were grown in the hydroponic nutrient solution for 3 weeks. The seedlings  
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274 were then rinsed with deionized water and transplanted into a beaker containing 500 mL of 20 mg  
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276 Fe<sup>2+</sup>/L solution (pH being adjusted to 5.5) for 24 h to allow the formation of iron plaques on the root  
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278 surfaces of the seedlings. After this, the seedlings were rinsed to remove any soluble Fe attached to  
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280 the plant surface before being used in the experiments.  
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285 Two sets of the experiments were performed aiming to collect data at the end of two different  
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287 lengths of growth period: 1 day (24 h) and 30 days (720 h). For each set of the experiment, one  
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289 control and one treatment were set; (a) control: plant growing in the hydroponic nutrient solution  
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291 with added arsenite-As at a dose of 1 mg/L; (c) Treatment: plant growing in the hydroponic nutrient  
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298 solution with added arsenite-As at a dose of 1 mg/L plus Fenton reagent (100  $\mu\text{M}$   $\text{H}_2\text{O}_2$  and 100  $\mu\text{M}$   
299  $\text{Fe}^{2+}$ ). For the 1-day experiment, the control and treatment were labelled as C1d and T1d,  
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301 respectively. For the 30-day experiment, the control and treatment were labelled as C30d and T30d,  
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308 A 500 mL plastic cup (diameter: 8 cm; height: 15 cm) was used as a hydroponic container,  
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310 which was placed into a black nylon bag to avoid exposure of the plant roots to light. The lid with  
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312 holes was used to support the plants. Six rice plants were grown in each hydroponic container. The  
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314 plant growth units were placed randomly in a climate chamber with the daily light-dark cycle being  
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316 set at 16 h : 8 h. The light density during the photoperiod was fixed at 2500 lx. Temperature during  
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318 the dark and light periods was set at 20  $^\circ\text{C}$  and 28  $^\circ\text{C}$ , respectively. Relative humidity was  
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320 maintained at a range of 80-85 %. All the experiments were performed in 4 replicates.  
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324 For the 30-day experiment, the culture solution in each hydroponic container was replenished  
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326 every 3 days. This included addition of arsenite-As for the control and addition of arsenite-As plus  
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328 Fenton reagent for the treatment.  
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332 At the end of the 1-day experiment, samples of the spent culture solution were taken to  
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334 determine various As species. For the 30-day experiment, only the first (3 days or 72 hours) spent  
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336 culture solution was used for analysis of As species. These spent solution samples were labelled as  
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338 CS1d and TS1d for the control and treatment of the 1-day experiment, respectively, and CS3d and  
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340 TS3d for the control and the treatment of the first spent solution of the 30-day experiment,  
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342 respectively.  
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346 At the end of each experiment, the plants were harvested for determinations of biomass, various  
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348 As species in the plant tissues, and Fe and various As species in the root plaques. Since all the six  
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350 plants growing in each hydroponic container had very similar growth performance, only three of the  
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357 six plants were randomly selected: (a) the first one was used for determination of the biomass; (b) the  
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359 second one was used for measurement of As in various plant organs; and (c) the third one was used  
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361 to extract iron plaque.  
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### 364 **2.2.2 Pot Experiment**

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367 A greenhouse experiment was conducted to observe the growth performance of the rice plants  
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369 and uptake of As by the rice plants. The experiment lasted for more than 9 months, including two  
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371 continuous crops with a fallow period of about 3 months. The first crop commenced on September 8,  
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373 2013 and the rice plants were harvested on January 7, 2014; the second crop commenced on April 3,  
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375 2014 and the rice plants were harvested on July 22, 2014.  
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379 The soil without added As was used as the control (Ck); Treatments 1 and 2 (T1 and T2,  
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381 respectively) were the artificially contaminated soils without and with added Fenton reagent (100  
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383  $\mu\text{M H}_2\text{O}_2$ :100  $\mu\text{M Fe}^{2+}$ ), respectively. The dose of added arsenite-As in the contaminated soils was  
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385 set at 50 mg/kg. The thickness of the overlying water layer was maintained at approximately 2 cm.  
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387 For T2, an appropriate amount of standardized  $\text{H}_2\text{O}_2$  and  $\text{FeSO}_4$  solution was added to the overlying  
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389 water to maintain a theoretical concentration of  $\text{H}_2\text{O}_2$  and  $\text{Fe}^{2+}$  at 100  $\mu\text{M}$  each at the beginning of  
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391 Fenton reagent addition for each 3-day cycle.  
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395 Two seedlings were transplanted to a soil column consisting of alternating layers (1 cm thick) of  
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397 quartz sand and a relevant soil material. This design was to allow easy separation of the root  
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399 materials from the soils upon harvest. The soil column was contained in a nylon mesh bag (#400  
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401 mesh; diameter: 8 cm; depth: 12 cm). Four soil columns were placed in a plastic bucket (Diameter:  
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403 22 cm; Height: 15 cm) that was filled with the same soil material. This design allowed the separation  
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405 of rhizospheric soil from the bulk soil by confining the rice plant roots within the nylon mesh bag or  
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407 so-called rhizo-bag.  
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416 Compound fertilizer (N:P:K=15:15:15) was applied at a rate of 19 g per pot at the 7<sup>th</sup> day of the  
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418 experiment. Additional fertilizers were added at a rate of 6.8 g/pot for compound fertilizer and 9.6  
419  
420 g/pot for urea in the early tillering stage of the first crop. In the second crop, 6.8 g/pot and 7 g/pot  
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422 were added 7 days after transplanting of the rice seedlings and in the heading stage, respectively.  
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426 In the first crop, one of the four rhizo-bags (together with the above-ground portion) was  
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428 randomly removed from each bucket in the heading stage. A second rhizo-bag was removed in the  
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430 maturity stage. For the second crop, sampling was carried out in the tillering, heading and maturity  
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432 stages. After collection, the soil materials in each rhizo-bag were recovered by separation from the  
433  
434 quartz sands. One of the two rice plants from each rhizo-bag was used for measurement of biomass  
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436 and another one was used for determination of various As species in the plant tissues.  
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### 439 **2.3 Sample Preparation and Analytical Methods**

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443 For biomass measurements, the straw and root portions of the rice plant were separated. The  
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445 roots were rinsed with water and the excess moisture on the root surfaces was removed using  
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447 absorbent paper towels. Fresh biomass of the two portions was obtained before they were oven-dried  
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449 at 60 °C until constant weight was attained.  
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452 For measurements of various As species in plant tissues, different organs of the rice plant (leaf,  
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454 stem, root and grain) were deep-frozen at -40 °C immediately after collection. The samples were then  
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456 freeze-dried using a VirTis freeze dryer. The dried plant tissue samples were pulverized (For the rice  
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458 grains, the hulls were removed but no polish was applied prior to pulverization; for the roots, iron  
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460 plaque was not removed) and then stored at -20 °C before being analyzed. Four As species were  
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462 determined. These include arsenate-As, arsenite-As, monomethylarsonic acid-As (MMA-As) and  
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464 dimethylarsinic acid-As (DMA-As). Measurements of various As species were performed using a  
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466 HPLC-ICP-MS system. For HPLC (Agilent1260) separation, Athena C18-WP column and guard  
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475 column were used. The mobile phase was a mixed solution of citric acid and sodium sulfonate. The  
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477 flow rate was set at 1.0 mL/min with an injection volume of 20  $\mu$ L. For ICP-MS (Agilent 7700),  
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479 argon was used as carrier gas and make-up gas. Details on the instrumental operating conditions are  
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481 given in Supplementary Table S1.  
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485 Iron plaque attached on the root surface was extracted by dithionite-citrate-bicarbonate (DCB,  
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487 [Liu et al., 2005](#)). Briefly, fresh roots were rinsed with deionized water and then dried with adsorbent  
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489 paper towels. For each rhizo-bag, an appropriate amount of root materials were randomly taken and  
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491 placed in a beaker containing 30 mL of mixed solution of 0.03 M  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  and 0.125 M  
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493  $\text{NaHCO}_3$ . 1 g of  $\text{Na}_2\text{S}_2\text{O}_4$  was then added into the beaker. After mixing, the beaker with its content  
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495 was allowed to stand for 30 min. The root materials were removed from the beaker and washed with  
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497 deionized water three times. The extract, together with the spent washing water, was transferred into  
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499 a 100 mL volumetric flask, followed by adding an appropriate amount of water to the mark. The  
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501 washed roots were then oven-dried at 70  $^\circ\text{C}$  to constant weight.  
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505 The iron in the DCB extract was determined by atomic absorption spectrometry (ZEEnit 700 P).  
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507 Measurements of various As species in the DCB extract were performed using a HPLC-ICP-MS  
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509 system. The total As in the root plaque was estimated by the sum of various As species.  
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## 512 **2.4 QC/QA and statistical analysis**

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516 The hydroponic culture experiment was performed in 4 replicates and the pot experiment was  
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518 performed in 3 replicates. The recovery rates of matrix spike for plant tissue samples in the  
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520 hydroponic experiment were  $80.7 \pm 3.51$  for arsenate-As,  $92.5 \pm 2.41$  for arsenite-As,  $82.2 \pm 2.72$  for  
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522 MMA-As and  $123 \pm 5.98$  for DMA-As. The recovery rates of matrix spike for plant tissue samples in  
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524 the pot experiment were  $89.6 \pm 3.43$  for arsenate-As,  $105 \pm 3.44$  for arsenite-As,  $106 \pm 3.18$  for MMA-  
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526 As and  $122 \pm 6.96$  for DMA-As. Statistical difference analysis was performed using One-way  
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534 ANOVA (SPSS17.0).  
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### 537 **3 RESULTS** 538

#### 539 **3.1 Hydroponic Culture Experiment** 540 541 542

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544 For both CS1d and CS3d, the concentration of the originally added arsenite-As more or less  
545 remained unchanged (Fig. 1). However, for TS1d and TS3d, all the originally added arsenite-As  
546 disappeared and arsenate-As was the only As species detected. The concentration of As<sup>5+</sup> in the  
547 solution was lower than that of arsenite-As originally added into the system. In particular, TS3d only  
548 contained about 3.8% of the originally added As. Iron precipitates were observed to occur on the  
549 bottom and wall of the hydroponic containers.  
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558 The fresh biomass (either total, shoot or root) of the rice plant was significantly ( $p<0.05$ )  
559 higher in the treatment than in the control for both the 1-day and the 30-day experiments though for  
560 the dry biomass, the difference between the control and the treatment was statistically insignificant  
561 for the 1-day experiment (Table 1).  
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568 Arsenate-As dominated the As species, followed by arsenite-As. Very small amounts of  
569 methylated As species were also detected (Table 1). There was a consistent trend showing that the  
570 arsenate-As and DMA-As in the root portion was significantly ( $p<0.05$ ) higher in the control than in  
571 the treatment for both the 1-day experiment and the 30-day experiment while there was no  
572 significant difference ( $p>0.05$ ) in arsenite-As and MMA-As between the control and the treatment  
573 for both the 1-day experiment and the 30-day experiment. Overall, the sum of various As species was  
574 higher in the control than in the treatment, especially for the leaf portion.  
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584 For the stem portion, mixed results were observed. The 1-day experiment showed a higher sum  
585 of As species in the control than in the treatment (Table 1). However, the opposite was observed for  
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593 the 30-day experiment. Unlike root portion, arsenite-As dominated As species in the stem portion  
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595 and methylated As species was detected only in the 1-day experiment. There was no significant  
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597 ( $p>0.05$ ) difference in any As specie between the control and the treatment for both the 1-day  
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599 experiment and the 30-day experiment.  
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603 For the leaf portion, there was no significant ( $p>0.05$ ) difference in any As species between the  
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605 control and the treatment except for arsenite-As in the 30-day experiment, which showed a  
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607 significantly ( $p<0.05$ ) higher value of arsenite-As in the control than in the treatment (Table 1).  
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609 Similar to the stem portion, arsenite-As dominated As species and no methylated As species were  
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611 detected in the treatment for the 30-day experiment.  
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615 For both the 1-day and 30-day experiments, the total Fe in the root plaque was greater in the  
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617 treatment than in the control (Table 1). Total As in the root plaque was significantly greater in the  
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619 treatment than in the control for the 30-day experiment. However, the same was not observed for the  
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621 1-day experiment; there was no significant difference in root plaque-borne As between the control  
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623 and the treatment.  
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### 626 **3.2 Pot Experiment**

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630 As expected, biomass tended to be smaller in the contaminated soils (T1 and T2) than in the  
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632 control (Ck, non-contaminated soil) due to As toxicity (Table 2). Comparison shows that treatment  
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634 of the contaminated soil with Fenton reagent (T2) resulted in a significant ( $p<0.05$ ) increase in  
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636 biomass, as compared to T1 for the first crop and the tillering stage of the second crop. For the  
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638 maturity stage of the first crop, the growth performance was even better in T2 than in Ck. However,  
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640 it is interesting to note that there was no significant difference in dry biomass of the shoot portion  
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642 between T1 and T2 for the heading stage of the second crop and the dry biomass of the shoot portion  
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644 was even greater in T1 than in T2 for the maturity stage of the second crop. For the root portion,  
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652 there was no significant difference in the dry biomass among Ck, T1 and T2. In consistent with the  
653 biomass, grain yield also tended to be in the following decreasing order: Ck (10.6 g) > T2 (8.0 g) >  
654 T1 (5.7 g).  
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660 The sum of various As species in the root portion was greater in T1 than in Ck, particularly in  
661 the first crop and the tillering stage of the second crop. By comparison, the root-borne As was  
662 significantly ( $p < 0.05$ ) less in T2 than in T1 for the heading stage of the first crop and the tillering  
663 stage of the second crop. However, no significant ( $p > 0.05$ ) difference in root-borne As between T1  
664 and T2 was observed for the other sampling occasions (Table 3).  
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672 Methylated As species only accounted for a small proportion of the root-borne As (Table 3).  
673 In most of situations, arsenite-As dominated As species except in T1 for the heading stage of the first  
674 crop and in T1 and T2 for the tillering stage of the second crop when the amount of arsenate-As was  
675 close to that of arsenite-As or even slightly greater. One thing in common was that root-borne As  
676 tended to be lower in the maturity stage than in the respective earlier growing stages for either  
677 arsenite-As or arsenate-As. By comparison, root-borne As at the same growth stage tended to be  
678 higher in the first crop than in the second crop for the control and the treatments.  
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688 In comparison with the root-borne As, the concentration of As in the stem portion was  
689 relatively smaller (Table 4). Like root-borne As, stem-borne As (sum of various As species) also  
690 showed a significantly higher value in T1 than in Ck for any of the growth stages for the two crops.  
691 Unlike the root-borne As, stem-borne As was smaller in T1 than in T2 for the heading stage of the  
692 first crop and the tillering stage of the second crop while the opposite was observed for the other  
693 three sampling occasions.  
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702 The proportion of methylated As species in the sum of As species was generally small except  
703 for those in the maturity stage of the first crop (Table 4). For the first crop and the tillering stage of  
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711 the second crop, arsenate-As was greater than did arsenate-As while the opposite was observed for  
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713 the heading stage and maturity stage of the second crop.  
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717 Like the root and stem portions, leaf-borne As (sum of the As species) was consistently  
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719 greater (significantly at  $p<0.05$ ) in T1 than in Ck though the difference was not statistically  
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721 significant in the maturity stage of the second crop (Table 5). For all of the five sampling occasions,  
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723 there was no significant difference in leaf-borne As between T1 and T2. Like the root and stem  
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725 portions, methylated As species only took up a small proportion in the sum of various As species.  
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727 There was a clear trend showing that arsenite-As dominated As species in the heading and maturity  
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729 stages of the second crop. However, mixed results were observed for other sampling occasions.  
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733 The abundance of grain-borne As (sum of various As species) in both the first and second  
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735 crops had the same pattern:  $T1 > T2 > Ck$ . This was consistent with the pattern observed for the stem  
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737 portion in the maturity stage (Fig. 2). By comparison, the concentration of As in the grain portion  
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739 was consistently higher in the second crop than in the first crop. This was accompanied by the same  
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741 trend for the stem-borne As. Arsenite-As and DMA-As were the two dominant species. Depending  
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743 on individual treatments, Ck had more DMA-As; T2 contained more arsenite-As; and T1 tended to  
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745 have equal amounts of arsenite-As and DMA-As.  
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749 There were orange-colored coating materials (root plaque) on the surfaces of plant roots.  
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751 However, root coatings did not cover the entire root surface with T1 tending to have a lower  
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753 coverage of root plaque, as compared to T2. The abundance of root plaque-borne Fe, as measured by  
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755 the amount of Fe attached to the surface of per unit of root biomass (g/kg) in the different stages of  
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757 rice plant growth for the control and the two treatments is shown in Table 6. The root plaque-borne  
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759 Fe tended to be higher in T2 than in either T1 or Ck (significant at  $p<0.05$ ). There was a clear trend  
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761 showing that root plaque-borne As increased from Ck to T1 to T2 for all the five sampling occasions.  
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774 **4 DISCUSSION**  
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777 The results obtained from the hydroponic experiment suggest that, under the set experimental  
778 conditions,  $\text{As}^{3+}$  was resistant to oxidation in the presence of molecular oxygen only. However,  
781 addition of Fenton reagent markedly accelerated the oxidation of  $\text{As}^{3+}$ , resulting in formation of  $\text{As}^{5+}$ .  
782 The decrease in As concentration in the culture solutions suggests that immobilization of As took  
783 place. The presence of iron precipitates on the bottom and wall of the hydroponic containers suggests  
784 that the  $\text{Fe}^{3+}$  formed from Fenton reaction acted as a scavenger to sequester As from the hydroponic  
785 solution, resulting in a decrease in solution-borne As. Since the hydroponic nutrient solution  
786 contained FeII-EDTA, which is not stable in the presence of oxygen, the Fe from this source could  
787 be oxidized to  $\text{Fe}^{3+}$ , resulting in the formation of iron oxyhydroxide that might add to the plaque  
788 (Seibig and van Eldik, 1997). This was also likely to enhance the local oxidation of  $\text{As}^{3+}$  to  $\text{As}^{5+}$   
789 (Hug and Leupin, 2003). In addition, the As(III)-oxidizing microbes could also play an important  
790 role in oxidizing As on the root iron plaque (Hu et al. 2015).  
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803 The generally lower concentration of As in the rice plant tissue in the treatment, relative to the  
804 control, can be attributed to the reduced availability of As in the hydroponic solution. The  
805 predominant presence of arsenate-As in the root portion appears to suggest that while both arsenate  
806 and arsenite might be taken up by the rice seedlings, the uptake of As by root took place more  
807 favourably through an arsenate pathway. The change in the predominant As species from arsenate-  
808 As to arsenite-As in the above-ground portion reflects the in-plant reduction of arsenate-As (Kramar  
809 et al., 2015).  
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819 The significantly greater root plaque-borne Fe concentration in the treatment than in the control  
820 suggests that addition of Fenton reagent significantly enhanced the formation of iron compounds on  
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829 the root surfaces of the rice plants. Liu et al. (2006) suggested that root plaque-Fe was in mineral  
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831 forms of iron oxyhydroxides. This work demonstrates that addition of Fenton reagent enhanced the  
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833 formation of Fe<sup>3+</sup>-containing chemical compounds on the root surface.  
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837 Although no significant difference between the control and the treatment was observed for the 1-  
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839 day experiment, the root plaque-borne As was significantly ( $p<0.05$ ) greater in the treatment than in  
840  
841 the control for the 30-day experiment. This suggests that the addition of Fenton reagent could  
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843 enhance retention of As by the root plaque. However it took time to incorporate solution-borne As  
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845 into root plaque and a duration of 24 hours was not sufficient to allow this to take place even when  
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847 Fenton reagent was added into the system. For the control, transformation of arsenite-As to arsenate-  
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849 As did not take place and arsenite was the only form of arsenic in the nutrient solution (Fig. 1).  
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851 Therefore, any arsenate contained in the root plaque was likely to be formed as a result of arsenite  
852  
853 oxidation driven by root-released oxygen. For the treatment, production of arsenate was markedly  
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855 enhanced due to Fenton reaction. From Fig. 1, it is clear that conversion of all arsenite-As into  
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857 arsenate-As was completed within 1 day after addition of Fenton reagent. The arsenate formed was  
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859 then gradually removed from the nutrient solution by deposition as iron precipitates and plant uptake.  
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863 In the pot experiment, the poorer growth performance, as indicated by smaller biomass in T1 than in  
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865 Ck during the first crop and the tillering stage of the second crop suggests that an initial dose of As at  
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867 50 mg/kg was sufficient to cause phytotoxicity to the rice plants under the set experimental  
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869 conditions. Das et al. (2013) observed phytotoxicity to paddy rice at a dose of 40 mg As/kg, which is  
870  
871 very similar to 50 mg As/kg in this experiment. The toxic effects of As on rice plant growth became  
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873 less significant during the heading and maturity stages of the second crop. This may be attributed to  
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875 reduced bioavailability of the added As due to As immobilization through formation of practically  
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877 insoluble minerals such as scorodite or adsorption by soil colloids such as Fe oxyhydroxides (Lin  
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879 and Puls, 2000; Campbell and Nordstrom, 2014; Serrano et al., 2015). Contamination of the soils by  
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889 As led to increased uptake of As by the plants, which impedes the physiological functions of the  
890 plants (Hughes, 2002; Islam et al., 2015). However, the application of Fenton reagent effectively  
891 reduced this harmful effect and significantly enhanced the growth of the rice plants grown in the As-  
892 contaminated soils. It is interesting to note that the significant increase in biomass in the first crop  
893 and the tillering stage of the second crop in T2, as compared to T1, was accompanied by a significant  
894 reduction in root-borne As in T2, relative to T1 while the insignificant difference in biomass between  
895 T1 and T2 in the heading and maturity stages of the second crop was consistent with the insignificant  
896 difference in root-borne As between T1 and T2. It is noted that the biomass tended to be greater in  
897 the first crop than in the second crop (Table 2). The rice cultivar (TY122) used for the experiment  
898 was the one that is more suitable for being grown during the period from autumn to early winter (the  
899 first crop) than during the period from late spring to summer (the second crop). In addition, the  
900 application rate of chemical fertilizers was relatively lower in the second crop than in the first crop,  
901 and this might also affect the growth performance of the rice plants in the second crop.  
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917 The relatively low level of root plaque-borne As in Ck reflected the limited availability of As in  
918 the non-contaminated soil. A significantly higher level of root plaque-borne As in T2, as compared  
919 to T1 is attributable to the enhanced formation of iron plaques on the root surfaces of the rice plants  
920 due to application of Fenton reagent, which in turn allowed more As being intercepted when As in  
921 the soil solution moved towards the surfaces of the plant roots, and consequently reduced the  
922 amounts of As being taken by the plant roots.  
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931 The trend that  $As_{Stem}/As_{Root}$  and  $As_{leaf}/As_{Root}$  increased over time (Table 7 and Table 8) suggests  
932 that the root-to-shoot translocation of As was enhanced as the rice plants became more mature,  
933 possibly due to intensified transpiration. The much higher  $As_{Stem}/As_{Root}$  at the maturity stage in the  
934 second crop than in the first crop indicates that the efficiency of root-to-stem As translocation was  
935 improved due to the reduced As phytotoxicity, which allowed better growth performance of the rice  
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947 plants being achieved. This explains the much higher rice grain-borne As in the second crop than in  
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949 the first crop.  
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952 The capacity of root plaque to impede As flux towards the root surfaces in T1 were limited,  
953 leading to substantial uptake of As by the roots. This could also be due to that younger roots and the  
954 younger parts of the old roots that play key role in plant uptake of nutrients and metals were hardly  
955 coated by iron plaque, as also pointed out by other workers (Seyfferth et al., 2010; Yamaguchi et al.,  
956 2014). The addition of Fenton reagent led to production of  $\text{Fe}^{3+}$  and hydroxyl radical that enhanced  
957 formation of iron precipitates and  $\text{As}^{3+}$ - $\text{As}^{5+}$  conversion. This effect was not limited to rhizosphere  
958 but also the bulk soils. As demonstrated in the hydroponic experiment, solution-borne arsenite can be  
959 oxidized and removed from the culture solution within a relatively short period of time after addition  
960 of Fenton reagent. It is therefore likely that arsenite in the soil pore water could experience the same  
961 process for the pot experiment. The immobilization of As in the bulk soil could markedly reduce the  
962 supply of dissolved As for the plant root, leading to reduced uptake of As by the rice plants. The  
963 effect of  $\text{FeSO}_4$  addition on enhancing formation of iron plaque on rice root surfaces was previously  
964 observed by Hossain et al. (2009)  
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981 The concentration of As (0.26 mg/kg for the 1<sup>st</sup> crop and 0.55 mg/kg for the 2<sup>nd</sup> crop) in the  
982 grain of rice plants grown in the contaminated soils (T1) far exceeded the maximum limit of 0.1  
983 mg/kg set by the European Union for the rice destined for the production of foods for infants and  
984 young children (Signes-Pastor et al. 2017) though the level of As could be lower than these values if  
985 the rice grains are polished (Meharg et al., 2008). The significant reduction in rice grain-borne As in  
986 both the first and second crops due to addition of Fenton reagent sheds some light on the possible  
987 role of rainwater-borne  $\text{H}_2\text{O}_2$  in alleviating As contamination in rice grain. In our recent experiment  
988 examining the paddy soils receiving natural rainwater containing hydrogen peroxide, a similar effect  
989 like what was showed in this microcosm experiment was observed, suggesting that rainwater-borne  
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1006 hydrogen peroxide does affect arsenic chemistry in paddy soils (unpublished data). This raises a  
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1008 question on whether rice produced from areas receiving abundant rainfall tends to contain less  
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1010 arsenic. It will be interesting to establish whether there is a relationship between annual rainfall and  
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1012 rice grain-borne As on a global scale.  
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1016 From a mitigation perspective, the research findings have implications for developing cost-  
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1018 effective management strategies and remediation techniques to reduce As uptake by rice plants and  
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1020 accumulation in the rice grain. The uses of industrial grade H<sub>2</sub>O<sub>2</sub> (US\$500/t, source: Zhengzhou  
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1022 Huize Biochemical Technology Co., Ltd) and FeSO<sub>4</sub> (US\$100/t, source: Dalian Future International  
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1024 Co., Ltd.) are not economically prohibitive. A rough calculation based on the experimental design in  
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1026 this study gives an estimated cost of US\$89 per hectare for the purchase of the required chemicals. If  
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1028 appropriate procedure for mixing the Fenton reagent into the irrigation water can be developed,  
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1030 significant reduction of As level in rice grain may be achieved cost-effectively in rice-producing  
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1032 areas where the soils contain high level of As or where As-bearing groundwater is used for irrigation  
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1034 purpose.  
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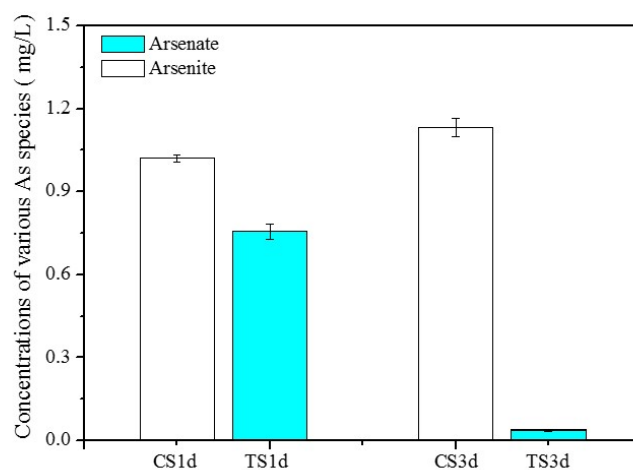
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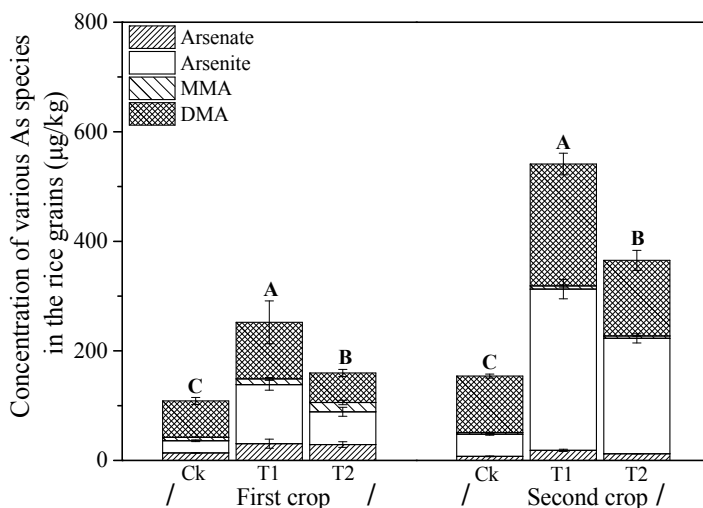
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**Fig 1 Concentration of various arsenic species in the culture solution in the control (CS) and the treatment (TS) at the end of the 1-day experiment (24 h) and at the end of the first nutrient replenishment cycle (72 h) of the 30-day experiment. All values are presented as mean  $\pm$  standard error (n=4).**



**Fig 2 Concentration of various arsenic species in the rice grain harvested in the first crop and second crop for the control (Ck) and the two treatments (T1 and T2) in the pot experiment. All values are presented as mean ± standard error (n=3) and bars with different letters indicate significantly ( $P < 0.05$ ) different means for the sum of various arsenic species (arsenate-As, arsenite-As, MMA-As and DMA-As).**



**Table 1 Dry biomass, arsenic species in plant tissues, and iron and arsenic species in root plaque for the 1-day and 30-day hydroponic experiments**

		C1d	T1d	C30d	T30d
Dry biomass (g)	Straw	0.20±0.01	0.26±0.05	0.56±0.05	0.78±0.06
	Root	0.07±0.01	0.06±0.00	0.23±0.06	0.30±0.02
As (root portion, mg/kg)	Arsenate-As	98.5±3.04*	70.0±1.85	315±9.74*	238±6.30
	Arsenite-As	18.4±0.63	18.7±3.02	59.0±2.03	59.9±9.67
	MMA-As	2.08±0.44	0.82±0.10	4.99±1.06	1.96±0.24
	DMA-As	3.40±0.78*	1.65±0.15	8.84±2.04*	4.29±0.38
	Total As	122	91.1	387	304
As (stem portion, mg/kg)	Arsenate-As	5.14±0.61	4.42±0.68	15.9±0.21	19.5±1.49
	Arsenite-As	11.4±0.73	8.86±0.63	22.5±1.70	25.7±3.29
	MMA-As	ND	ND	ND	ND
	DMA-As	1.1±0.12	0.64±0.31	ND	ND
	Total As	17.6	13.9	38.4	45.2
As (leaf portion, mg/kg)	Arsenate-As	1.85±0.38	2.04±0.26	20.6±2.56	11.3±1.47
	Arsenite-As	3.13±0.37	3.48±0.12	45.7±6.11*	27.6±2.69
	MMA-As	ND	ND	ND	ND
	DMA-As	0.14±0.02	0.06±0.02	ND	ND
	Total As	5.12	5.58	66.3	38.9
Root plaque-Fe (g/kg)	Total Fe	5.86±1.38*	8.29±0.46	2.00±0.18*	8.45±0.58
Root plaque-As (mg/kg)	Total As	60.9±1.26	56.2±1.12	76.6±0.51*	115±5.59

All values are presented as mean ± standard error (n=4). Independent sample t-test was used to determine whether the two mean values obtained for the control and the treatment differ significantly. Pairs marked with an asterisk indicate significant (P <0.05) difference between the control and the treatment for each harvest time. ND: not detectable.

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**Table 2 Fresh and dry weight (g) of the shoot and root in the first crop and second crop for the control and the two treatments for the pot experiment**

Growth stage	Treatments	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
Heading (1 <sup>st</sup> crop)	Ck	47.4±1.11a	8.88±0.59a	20.5±1.35a	4.48±0.74a
	T1	14.5±1.32c	2.88±0.3c	6.04±0.97c	0.94±0.21c
	T2	28.8±1.84b	6.14±0.27b	13.9±0.68b	2.76±0.24b
Maturity (1 <sup>st</sup> crop)	Ck	29.1±4.20ab	14.7±1.45a	24.0±0.91b	8.52±0.91b
	T1	21.1±0.45c	8.24±1.05c	4.11±0.29c	1.64±0.32c
	T2	31.0±1.82a	13.2±0.60ab	31.0±4.21a	14.6±1.32a
Tillering (2 <sup>nd</sup> crop)	Ck	5.40±0.58a	0.71±0.11a	0.52±0.05a	0.12±0.02a
	T1	1.57±0.13c	0.23±0.02c	0.14±0.00c	0.07±0.02ab
	T2	2.75±0.53bc	0.41±0.09bc	0.30±0.05bc	0.08±0.01ab
Heading (2 <sup>nd</sup> crop)	Ck	24.7±2.72a	8.07±1.23a	13.3±1.70ab	1.73±0.17a
	T1	18.5±1.29ab	6.52±0.31ab	17.8±0.80a	2.12±0.11a
	T2	16.8±2.17b	6.42±0.76ab	18.4±2.01a	2.25±0.64a
Maturity (2 <sup>nd</sup> crop)	Ck	15.9±1.46a	6.63±0.68ab	14.0±1.50a	1.77±0.24a
	T1	16.7±1.87a	7.27±0.78a	11.8±2.87a	2.43±0.67a
	T2	12.8±1.68ab	4.74±0.76b	9.36±0.58ab	1.87±0.12a

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p<0.05$ ).

**Table 3 Concentration (mg/kg) of various As species in the root portion of the rice plant during different growth stages for the pot experiment**

Growth stage	Treatment	Arsenate-As	Arsenite-As	MMA-As	DMA-As	Sum
Heading (1 <sup>st</sup> crop)	Ck	12.7±2.10bc	19.1±1.94c	1.20±0.14a	0.10±0.01c	33.1±3.74b
	T1	73.2±0.78a	60.1±1.57a	1.21±0.12a	1.41±0.22a	136±2.16a
	T2	11.8±0.37c	32.8±5.95b	0.65±0.04b	0.79±0.11b	46.1±6.16b
Maturity (1 <sup>st</sup> crop)	Ck	1.21±0.05c	7.04±1.08c	0.79±0.02c	0.89±0.09ab	9.93±1.10b
	T1	2.03±0.31b	30.0±1.56a	1.19±0.15b	0.60±0.03b	33.8±1.11a
	T2	3.82±0.24a	22.1±2.96b	1.52±0.10a	1.28±0.23a	28.7±3.38a
Tillering (2 <sup>nd</sup> crop)	Ck	8.56±0.68c	11.1±1.15b	0.03±0.01b	0.59±0.03b	20.3±1.45c
	T1	82.6±4.15a	85.9±2.15a	2.90±0.37a	2.51±0.29a	174±6.72a
	T2	27.8±1.27b	18.2±1.26b	0.17±0.02b	0.66±0.04b	46.9±0.38b
Heading (2 <sup>nd</sup> crop)	Ck	3.04±0.39b	14.6±0.64b	0.14±0.00a	1.33±0.23a	19.1±0.53b
	T1	4.91±0.64ab	49.7±5.64a	0.35±0.05a	0.77±0.17a	55.7±6.11a
	T2	6.52±0.94a	52.4±2.92a	0.22±0.01a	1.27±0.23a	60.4±4.01a
Maturity (2 <sup>nd</sup> crop)	Ck	1.60±0.21a	4.94±0.32c	0.34±0.04b	0.50±0.08b	7.38±0.57b
	T1	1.89±0.55a	9.30±0.69b	1.42±0.20a	0.73±0.13ab	13.3±1.05a
	T2	1.61±0.39a	12.4±1.06a	0.82±0.04ab	0.88±0.05a	15.7±1.43a

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p < 0.05$ ).

**Table 4 Concentration (mg/kg) of various As species in the stem portion of the rice plant during different growth stages for the pot experiment**

Growth stage	Treatment	Arsenate	Arsenite	MMA	DMA	Sum
Heading (1 <sup>st</sup> crop)	Ck	1.40±0.09c	1.02±0.13c	0.19±0.01b	0.13±0.01c	2.74±0.15c
	T1	6.47±0.82ab	4.03±0.29ab	0.28±0.01ab	0.44±0.07b	11.2±0.49b
	T2	7.70±0.48a	5.12±0.16a	0.34±0.00a	0.60±0.01a	13.7±0.35a
Maturity (1 <sup>st</sup> crop)	Ck	2.16±0.21b	1.58±0.20ab	0.36±0.06c	0.47±0.03c	4.57±0.22c
	T1	3.75±0.30a	2.28±0.13a	1.30±0.21a	1.54±0.16a	8.87±0.34a
	T2	3.01±0.21ab	1.84±0.06ab	0.94±0.07ab	1.26±0.1ab	7.06±0.08b
Tillering (2 <sup>nd</sup> crop)	Ck	0.43±0.07c	0.34±0.03b	0.00±0.00ab	0.04±0.00a	0.80±0.05c
	T1	1.33±0.30b	0.55±0.01a	0.03±0.00a	0.04±0.00a	1.94±0.30b
	T2	2.45±0.13a	0.64±0.08a	0.02±0.00ab	0.07±0.00a	3.17±0.06a
Heading (2 <sup>nd</sup> crop)	Ck	0.56±0.13ab	7.03±0.78c	0.03±0.00b	0.06±0.00b	7.68±0.81c
	T1	0.88±0.19a	18.21±1.77a	0.08±0.02a	0.23±0.03a	19.3±1.84a
	T2	0.57±0.03ab	12.58±1.02b	0.05±0.02ab	0.24±0.05a	13.4±0.93b
Maturity (2 <sup>nd</sup> crop)	Ck	0.57±0.22a	8.78±0.55b	0.03±0.00a	0.08±0.02ab	9.46±0.60b
	T1	0.67±0.06a	20.46±2.65a	0.05±0.02a	0.23±0.06a	21.4±2.78a
	T2	0.91±0.37a	10.75±2.19b	0.03±0.00a	0.18±0.00a	11.8±2.52b

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p < 0.05$ ).

**Table 5 Concentration (mg/kg) of various As species in the leaf portion of the rice plant during different growth stages for the pot experiment**

Growth stage	Treatment	Arsenate-As	Arsenite-As	MMA-As	DMA-As	Sum
Heading (1 <sup>st</sup> crop)	Ck	1.64±0.16b	1.76±0.22c	0.08±0.01a	0.06±0.01b	3.53±0.33b
	T1	5.16±0.36a	3.48±0.56ab	0.09±0.01a	0.23±0.03b	8.97±0.87a
	T2	4.45±0.38a	3.83±0.33a	0.11±0.02a	0.88±0.07a	9.27±0.36a
Maturity (1 <sup>st</sup> crop)	Ck	1.87±0.18c	1.66±0.07c	0.09±0.02a	0.15±0.01b	3.77±0.13b
	T1	4.10±0.29a	4.81±0.42ab	0.08±0.01a	0.28±0.01a	9.27±0.66a
	T2	2.94±0.31b	5.40±0.42a	0.12±0.02a	0.12±0.02b	8.58±0.64a
Tillering (2 <sup>nd</sup> crop)	Ck	1.70±0.07c	2.40±0.20ab	0.04±0.00a	0.06±0.00ab	4.19±0.18b
	T1	3.73±0.28b	4.47±0.81a	0.05±0.00a	0.15±0.01a	8.41±1.08a
	T2	5.57±0.50a	3.04±0.57a	0.05±0.01a	0.09±0.01a	8.76±1.03a
Heading (2 <sup>nd</sup> crop)	Ck	0.11±0.01b	1.14±0.06b	0.00±0.00b	0.02±0.00b	1.26±0.07b
	T1	0.15±0.01ab	1.88±0.18a	0.02±0.00a	0.05±0.01a	2.10±0.19a
	T2	0.19±0.02a	2.08±0.21a	0.01±0.00ab	0.04±0.01ab	2.32±0.21a
Maturity (2 <sup>nd</sup> crop)	Ck	1.13±0.25a	7.20±0.85ab	0.14±0.03ab	0.25±0.04b	8.72±1.10a
	T1	2.22±1.14a	8.65±1.00a	0.27±0.03a	0.82±0.09a	11.9±0.45a
	T2	2.22±0.49a	7.97±0.51a	0.15±0.02ab	0.80±0.05a	11.1±1.00a

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p < 0.05$ ).

**Table 6 Concentration of total iron and arsenic in the iron plaque of root surface during different growth stages for the pot experiment**

Growth stage	Treatments	Total Fe (g/kg)	Total As (mg/kg)
Heading (1 <sup>st</sup> crop)	Ck	1.61±0.10c	19.1±4.84c
	T1	2.34±0.15bc	29.7±4.19b
	T2	21.35±1.44a	55.4±12.0a
Maturity (1 <sup>st</sup> crop)	Ck	15.00±0.60a	73.7±18.5c
	T1	11.30±0.36b	330±20.5b
	T2	15.50±0.49a	711±37.1a
Tillering (2 <sup>nd</sup> crop)	Ck	17.79±0.51a	235±3.35c
	T1	9.79±0.57b	526±20.6b
	T2	19.43±1.32a	980±38.2a
Heading (2 <sup>nd</sup> crop)	Ck	28.28±3.73a	175±8.86c
	T1	16.42±0.57b	562±13.5b
	T2	25.62±1.10a	707±38.8a
Maturity (2 <sup>nd</sup> crop)	Ck	17.52±0.98ab	173±4.98c
	T1	15.57±0.43b	297±3.28b
	T2	20.07±1.17a	579±15.6a

All values are presented as mean ± standard error (n=3) and means with different letters in the same column for each of the five sampling occasions are significantly different ( $p<0.05$ ).

**Table 7 The ratios of stem-borne As to root-borne As ( $As_{Stem}/As_{Root}$ ) in the different growth stages of rice plants for the control and the treatments for the pot experiment**

Growth stage	Treatment	Arsenate-As	Arsenite-As	MMA-As	DMA-As	Sum
Heading (1 <sup>st</sup> crop)	Ck	0.110	0.053	0.158	1.300	0.083
	T1	0.088	0.067	0.231	0.312	0.082
	T2	0.651	0.156	0.523	0.759	0.298
Mature (1 <sup>st</sup> crop)	Ck	1.785	0.224	0.456	0.528	0.460
	T1	1.847	0.076	1.092	2.567	0.262
	T2	0.788	0.083	0.618	0.984	0.245
Tillering (2 <sup>nd</sup> crop)	Ck	0.050	0.030	0.000	0.068	0.039
	T1	0.016	0.006	0.010	0.016	0.011
	T2	0.088	0.035	0.118	0.106	0.068
Heading (2 <sup>nd</sup> crop)	Ck	0.184	0.481	0.214	0.045	0.401
	T1	0.179	0.366	0.229	0.299	0.348
	T2	0.087	0.240	0.227	0.189	0.223
Mature (2 <sup>nd</sup> crop)	Ck	0.356	1.777	0.088	0.160	1.282
	T1	0.354	2.200	0.035	0.315	1.606
	T2	0.565	0.866	0.037	0.205	0.753

**Table 8 The ratios of leaf-borne As to root-borne As ( $As_{leaf}/As_{Root}$ ) in the different growth stages of rice plants for the control and the treatments for the pot experiment**

Growth stage	Treatment	Arsenate-As	Arsenite-As	MMA-As	DMA-As	Total
Heading (1 <sup>st</sup> crop)	Ck	0.128	0.092	0.067	0.600	0.106
	T1	0.070	0.058	0.074	0.163	0.066
	T2	0.376	0.116	0.169	1.114	0.201
Mature (1 <sup>st</sup> crop)	Ck	1.545	0.236	0.114	0.169	0.380
	T1	2.020	0.160	0.067	0.467	0.274
	T2	0.770	0.244	0.079	0.094	0.298
Tillering (2 <sup>nd</sup> crop)	Ck	0.199	0.215	1.333	0.102	0.206
	T1	0.045	0.052	0.017	0.060	0.048
	T2	0.200	0.166	0.294	0.136	0.187
Heading (2 <sup>nd</sup> crop)	Ck	0.036	0.078	0.000	0.015	0.066
	T1	0.031	0.038	0.057	0.065	0.038
	T2	0.029	0.040	0.045	0.031	0.038
Mature (2 <sup>nd</sup> crop)	Ck	0.706	1.457	0.412	0.500	1.182
	T1	1.175	0.930	0.190	1.123	0.896
	T2	1.379	0.642	0.183	0.909	0.708



## Supplementary Material

Supplementary Table S1 Instrumental operating conditions for the HPLC-ICP-MS system

Parameter	Detailed information
<b>HPLC</b>	Agilent1260
Column	Athena C18-WP column (4.6*250 mm, 5 $\mu$ m, CNW) CNW guard column (Athena C18-WP, 4.0*20 mm, 5 $\mu$ m)
Mobile phase	2.5 mM Citric acid/2.5 mM Sodium sulfonate (pH 4.5)
Flow rate	1.0 mL/min
Injected volume	20 $\mu$ L
<b>ICP-MS</b>	Agilent 7700
RF (forward and reflected power)	1550 W
Spray chamber	Quartz dual channel type
Carrier gas	0.75 L/min
Make-up gas	0.40 L/min
Sample introduction	Meinhard nebulizer
Channels monitored	75, 77 and 78