

## Attenuation of inorganic arsenic and cadmium in rice grains using by-product iron materials from the casting industry combined with different water management practices

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1 Original article / Full-length paper

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3 Attenuation of inorganic arsenic and cadmium in rice grains using by-  
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5 water management practices  
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38 **Abstract**

39 We examined the effect of two types of iron (Fe) material produced by the casting industry (spent steel  
40 shot and residual iron material from steel shot production) on the mobility of arsenic (As) and cadmium  
41 (Cd) in soils. We also examined the uptake of these elements by rice plants (*Oryza Sativa* L.) under  
42 continuously flooded (CF) and water-saving (WS) cultivation. The application of both Fe materials (at  
43 10 and 30 t ha<sup>-1</sup>) strictly limited As mobilization in soils under CF cultivation. As a result, As uptake  
44 by rice plants declined, along with the total and inorganic As (iAs) concentration in rice grains. In  
45 comparison, As immobilization caused by the application of Fe material was less clear under WS  
46 cultivation. The rate of Fe material application was negatively correlated with As uptake by rice plants.  
47 It was also negatively correlated with total and inorganic As concentration in rice grains under both  
48 water management practices. The combination of applying Fe materials and WS cultivation decreased  
49 inorganic As concentration in rice grains to approximately one-fifth of that in rice grains produced  
50 from plants grown on soils without Fe material application under CF cultivation. CF cultivation strictly  
51 decreased dissolved Cd in soils, as well as Cd in rice grains with and without Fe material application.  
52 The application of Fe materials decreased Cd mobility and, hence, Cd uptake in rice plants, ultimately  
53 reducing the accumulation of Cd in rice grains under WS cultivation. Residual Fe material had a  
54 statistically greater effect at attenuating Cd accumulation in rice grains than spent steel shot. The  
55 present study demonstrated the potential of combining by-product Fe material application and water  
56 management practices to attenuate iAs and/or Cd concentrations in rice grains. Practical  
57 countermeasures should be carefully adopted that consider the existing risks of iAs and Cd on each  
58 paddy field, and the combined effect of Fe material application and water management practices.

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60 Key words: arsenic, cadmium, by-product iron, water management

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## 74 1. Introduction

75 Arsenic (As) and cadmium (Cd) are well-known hazardous elements that are ubiquitously present in  
76 soil environments. Long-term exposure to inorganic As (iAs) causes some kinds of cancers; therefore,  
77 the International Agency for Research on Cancer has classified iAs compounds as carcinogenic to  
78 humans (WHO 2016). Cadmium, which causes Itai-Itai disease, induces kidney failure and cancer  
79 (Huff et al. 2007). Rice consumption is one of the major sources of both iAs and Cd in Asian countries  
80 (Shimbo et al. 2001, Li et al. 2011, Oguri et al. 2014). In recent years, the Codex Alimentarius  
81 Commission had adopted maximum levels (maximum permitted concentrations) for iAs in polished  
82 rice and husked rice as  $0.2 \text{ mg kg}^{-1}$  and  $0.35 \text{ mg kg}^{-1}$ , respectively (Codex 2014, 2016), whereas the  
83 maximum level for Cd in polished rice was adopted as  $0.4 \text{ mg kg}^{-1}$  in 2006 (Codex 2006). The  
84 concentrations of iAs and Cd in rice grains should be carefully controlled to decrease the risk to human  
85 health and the agricultural economy.

86 Flooded cultivations attenuate Cd concentration in rice grains, whereas As concentration in rice  
87 grains is markedly increased by As dissolution from soils under such anaerobic soil conditions (Arao  
88 et al. 2009). Thus, there is an obvious trade-off relationship between mitigation measures for As and  
89 Cd in rice grains, in terms of water management for cultivation. Ishikawa et al. (2016) reported that  
90 the application of a low-Cd rice cultivar ('Koshihikari Kan No.1') with a water-saving cultivation  
91 keeps both As and Cd in rice grains at low levels, even though water-saving conditions had an adverse  
92 effect on grain yield and quality. Furthermore, we demonstrated that the combined application of  
93 commercial iron (Fe) materials and a flooded cultivation simultaneously limited iAs and Cd in rice  
94 grains, without any significant decrease in grain yield and quality (Makino et al. 2016). However, the  
95 high cost of the commercial Fe materials might limit their future use in agricultural practices.

96 To decrease the cost of Fe materials, we previously examined an Fe-type water treatment  
97 residue, and showed its ability to attenuate As in rice grains under flooded conditions (Suda et al.  
98 2016). In addition, large quantities of inexpensive Fe materials could be obtained from the casting  
99 industry. Spent steel shot (SSS) is generated after the daily use of steel shots for removing casting sand.  
100 Furthermore, large amount of residual Fe material (RIM) is also produced in the process of steel shot  
101 making. Our previous work demonstrated that these by-product Fe materials are mainly composed of  
102 zero-valent Fe [Fe(0)], wüstite, and magnetite/maghemite, and they significantly immobilize soil As  
103 during long-term flooding (Suda et al. in press). These major components of Fe materials might also  
104 immobilize Cd via sorption and insoluble Cd sulfide formation (Chowdhury et al. 2013, Hashimoto &  
105 Yamaguchi 2013). For example, Ok et al. (2011) demonstrated that Cd concentration in rice grains  
106 grown on polluted soils in Korea are reduced by 42% following the application of Fe(0) at  
107 approximately 2wt%. Thus, the application of SSS and RIM, which are composed of Fe(0) and Fe  
108 oxides, are expected to alleviate the uptake of both As and Cd by rice plants at low cost.

109 In most studies focusing on the attenuation of As uptake by rice plants using Fe material

110 application, rice plants have been grown on flooded soils over most of the cultivation period (e.g.,  
111 Ultra et al. 2009, Farrow et al. 2015, Matsumoto et al. 2015), with a few exceptions (Honma et al.  
112 2016a). Because Cd mobility in flooded soils is strictly limited, evaluating the effect of Fe material  
113 application on Cd uptake is difficult under such conditions. In addition, the stability and ability of Fe  
114 minerals in Fe materials to immobilize As and Cd probably changes in relation to the surrounding  
115 environment, such as pH and dissolved oxygen concentration (Mishra & Farrell 2005, Chowdhury et  
116 al. 2013).

117 Therefore, the main objective of the present study was to determine how by-product Fe materials  
118 from the casting industry combined with different water management practices affect the dissolution  
119 of As and Cd in soils, the uptake of As and Cd, and concentrations of As (especially iAs) and Cd in  
120 rice grains. Our results are expected to contribute novel information towards improving the  
121 management of hazardous materials in agricultural soils.

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## 124 **2. Materials and Methods**

### 125 ***Pot experiment***

126 Soil was collected from the plow layer of a paddy field (Fluvisols) in Japan for a pot experiment. The  
127 soil had a pH of 5.88, cation exchange capacity of 16.0 cmol kg<sup>-1</sup>, and contained 21 g kg<sup>-1</sup> total  
128 carbon, 1.74 g kg<sup>-1</sup> total nitrogen, 9.78 g kg<sup>-1</sup> oxalate-extractable Fe, 14.4 g kg<sup>-1</sup> dithionite-citrate-  
129 extractable Fe, 277 g kg<sup>-1</sup> clay, 2.48 mg kg<sup>-1</sup> 1 mol L<sup>-1</sup> hydrochloric-acid-extractable As, and 0.306  
130 mg kg<sup>-1</sup> of 0.1 mol L<sup>-1</sup> hydrochloric acid-extractable Cd. According to the Agricultural Land-Soil  
131 Pollution Prevention Law in Japan, the soil is not defined as As-polluted soil (1 mol L<sup>-1</sup> hydrochloric  
132 acid-extractable As > 15 mg kg<sup>-1</sup>). Also, the sampling field is located in a non-Cd-polluted area,  
133 which is determined by Cd concentration in rice grains.

134 Two types of by-product Fe materials were tested for their ability to attenuate the uptake of As  
135 and Cd by rice plants, and their concentration in rice grains. One of the Fe materials was spent steel  
136 shot (SSS), which was collected from some foundries and was screened magnetically. The other Fe  
137 material was residual Fe material (RIM), which is generated through steel shot making. Details on  
138 the properties of the Fe materials (<1 mm) were reported previously (Suda et al, 2018). In brief, SSS  
139 was composed of Fe(0) and a small amount of quartz, whereas RIM contained magnetite and/or  
140 maghemite (magnetite/maghemite), wüstite, and a small amount of Fe(0) (steel shot-like spherical  
141 particles). RIM showed a finer particle size distribution compared to SSS. Unlike RIM, SSS  
142 contained minor amounts of particles larger than 1 mm (21.9 wt%), therefore the concentrations of  
143 elements in SSS were slightly different from those reported in the literature. SSS and RIM contained  
144 876 and 642 g kg<sup>-1</sup> Fe, 49.7 and 57.4 g kg<sup>-1</sup> of silicon (Si), and 6.61 and 49.3 g kg<sup>-1</sup> of manganese  
145 (Mn), respectively.

146 A pot experiment was conducted in a greenhouse under natural sunlight from May to September  
147 2016. Two-point-two kilograms (oven-dry basis) of raw soil (<8 mm) was thoroughly mixed together  
148 with a compound fertilizer equivalent to 0.2 g nitrogen, 0.087 g phosphorus (P), and 0.17 g potassium,  
149 and 0, 20, or 60 g of either SSS or RIM. The added amounts of Fe materials were equivalent to the  
150 application rates of 0, 10, and 30 t ha<sup>-1</sup>, respectively. Tap water was added to each pot with soil (May  
151 3, 2016). Three rice plant seedlings (*Oryza sativa* L. cv. Koshihikari) were transplanted to each pot  
152 (May 13, 2016). The rice plants were grown under each water management practice described below  
153 during the cultivation period. The rice plant was cut at the base of the stem after its seeds matured  
154 (September 6, 2016). The harvested rice plant was air-dried, and then separated into straws (stems and  
155 leaves), husks, and grains. Unfilled rice grains were screened with a 1.85-mm- mesh sieve.

156 We carried out two water management practices; namely, continuously flooded (CF) and water  
157 saving (WS) cultivation. For CF cultivation (conducted in triplicate), the soil in a pot was flooded by  
158 water throughout the growing period (until September 2, 2016). For WS cultivation (conducted in  
159 quadruplicate), the soil in a pot was kept flooded during the early part of cultivation, and was then  
160 intermittently irrigated from approximately two weeks before heading to the end of the growing period  
161 (until September 2, 2016). During the period of flooded cultivation, the irrigation depth was kept  
162 between 2 and 5 cm. During the period of intermittent irrigation, irrigation water was not supplied  
163 until the soil surface had visually dried out.

164

#### 165 ***Sampling and analysis of the soil solution***

166 On days 25, 40, 52, 69, 83, and 97 after transplanting, approximately 10-mL of the soil solution was  
167 collected two times at a depth of 5–10 cm with a soil solution sampler (DIK-305A; Daiki Rika Kogyo,  
168 Saitama, Japan) connected with a syringe. Sampling was carried out approximately 24 h after the pot  
169 was last irrigated. The pH of the first soil solution was measured as soon as possible. The second soil  
170 solution was mixed with 1-mL of 10% nitric acid to dissolve the Fe oxides that were generated when  
171 the solution was sampled and to prevent the Fe precipitating during storage. The acidified soil solution  
172 was left until the precipitated Fe oxides completely disappeared, and was then filtrated with a 0.2 µm  
173 filter and diluted with ultrapure water. Dissolved As and Cd in the diluted solution were measured by  
174 inductively coupled plasma mass spectrometry (ICP-MS: Elan DRCe; PerkinElmer, Waltham, MA,  
175 USA). The concentrations of Fe, Si, and Mn were determined by inductively coupled plasma optical  
176 emission spectrometry (ICP-OES: Agilent 700 Series; Agilent Technologies, Santa Clara, CA, USA).

177 Soil Eh was measured at a soil depth of 7 cm on days 11, 17, 24, 31, 39, 52, 60, 69, 78, 83, 89,  
178 and 97 after transplanting. A platinum electrode and a silver-silver chloride reference electrode (Type  
179 4400, DKK-TOA Corporation, Tokyo, Japan) were used for the measurement.

180

#### 181 ***Analysis of harvested rice plants***

182 The harvested rice straws, husks, and grains (not polished) were dried at 75 °C. Only the straw samples  
183 were crushed coarsely in advance. All of the different types of plant samples were ground in a ball  
184 mill to a fine powder. The powdered samples were used for various chemical analyses. Rice powder  
185 was digested with nitric acid on a heating block, and the digested solution was used for As speciation  
186 with a high-performance liquid chromatography/ICP-MS (HPLC: Flexar, PerkinElmer; ICP-MS:  
187 Nexion 300X, PerkinElmer). The rice powder was also digested with nitric acid and hydrogen peroxide  
188 to quantify total As (tAs) and other elements. For the same purpose, straw and husk powders were  
189 digested with nitric acid, hydrogen peroxide, and hydrogen fluoride. Although straw and husk powders  
190 were incompletely digested, the obtained value was referred to as the total amount of each element for  
191 simplicity. Silicon in straw and husk powder was extracted using a mixture of 1.5 mol L<sup>-1</sup> hydrofluoric  
192 acid and 0.6 mol L<sup>-1</sup> hydrogen chloride, as described in Saito et al. (2005). The element concentrations  
193 in the digested solution were measured with ICP-MS (Elan DRCe or Nexion) and ICP-OES (Agilent  
194 700 Series). Details on the procedures and conditions for the chemical analyses of the plant samples  
195 are described in our previous report (Suda et al. 2016).

196

### 197 *Statistical analysis*

198 Simple linear regression and Pearson's correlation coefficient analyses were carried out to examine  
199 the effect of Fe material application rate under each water management strategy, and, if necessary, the  
200 differences between the slopes of the regression lines were assessed by testing the *F*-value. The effect  
201 of water management alone was evaluated by analysis of variance (ANOVA), using data obtained  
202 from the cultivations without Fe material application (control). The concentrations of iAs, tAs, and Cd  
203 in rice grains were expressed as ordinary water content (15%); however, the data of other plant samples  
204 were shown as an oven-dried (135 °C) basis. When a dataset contained values below the limit of  
205 determination (LOD) or limit of quantitation (LOQ) of measurements, only its average was calculated  
206 using half the value of LOD or LOQ, and further statistical analysis was not performed. The statistical  
207 analyses were carried out using Microsoft Excel and R software.

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## 210 **3. Results and Discussion**

### 211 *3.1 Grain yield and biomass production*

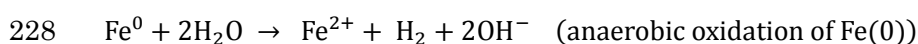
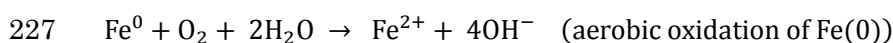
212 For both water management treatments, simple linear regression analyses showed no significant  
213 negative relationships between grain yield or shoot biomass and the SSS application rate up to 30 t ha<sup>-1</sup>  
214 (Table 1). In comparison, the increase in RIM application rate statistically lowered grain yield. Water-  
215 saving cultivation tended to decreased grain yield and shoot biomass (Fig. S1). The uptake of P and Si  
216 by rice plants was not negatively affected by increasing the application rate of SSS and RIM (Table  
217 S1). Further studies at the field scale are needed to clarify how Fe material applications affect rice

218 growth and grain yield under paddy field conditions.

219

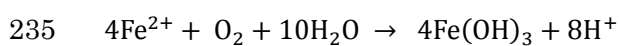
### 220 **3.2 Soil solution pH and soil Eh**

221 The soil solution pH of Fe material-applied soils tended to be higher than that of soils without Fe  
222 material application (control soils; Fig. 1a–b). However, the differences among treatments decreased  
223 with time, and converged at a pH of approximately 6.5. The soil solution pH remained around 6.5 until  
224 the final sampling event under CF cultivation. Nozoe et al. (2001) demonstrated a similar trend of pH  
225 change during the 45-days flooded incubation of soils mixed with powdered Fe(0). The increases of  
226 pH by Fe(0) application can be explained by the following equations:



229 Additionally, surface hydroxyl groups of Fe oxides in RIM consumed proton from soil solution,  
230 because they would have higher point of zero charges (e.g., around 6.5 for maghemite; Kosmulski  
231 2011) than pH of the control soils.

232 In comparison, under WS cultivation, the soil solution pH drastically dropped as the soil dried  
233 out during the intermittent irrigation period. The decrease of pH can be explained by the oxidation of  
234 Fe(II), which is expressed by the following equation:



236 The Eh of soils applied with Fe materials remained at lower levels than that of control soils in the  
237 early period of cultivation (Fig. 1c–d). The Eh of all soils gradually decreased and converged at around  
238 –230 mV. Then, the soil Eh value was maintained until the final measuring event under CF cultivation.  
239 In comparison, under WS cultivation, soil Eh drastically rose to more than +80 mV as the soil dried  
240 out during the intermittent irrigation period.

241

### 242 **3.3 As in the soil solution and rice plants**

#### 243 **Soil solution**

244 Dissolved As concentration in control soils under CF cultivation gradually increased, peaking at 120  
245  $\mu\text{g L}^{-1}$  on the final day of sampling (day 97) (Fig. 2a). As dissolution from soils is caused by the  
246 reductive dissolution of As-bearing Fe oxides and the transformation of As(V) to As(III), which  
247 progresses under flooded conditions. In contrast, dissolved As concentration in control soils under WS  
248 cultivation sharply dropped with soil-drying, and became less than 2  $\mu\text{g L}^{-1}$  in the later sampling days  
249 (Fig. 2b). The decrease of dissolved As by soil-drying is explained by co-precipitation (including  
250 sorption) of As with newly formed Fe oxides and the oxidative transformation of As(III) to As(V).  
251 This reaction is accelerated when air penetrates soils when soil-drying occurs during the intermittent  
252 irrigation period.

253 The application of Fe materials strictly limited the mobilization of As in soils under CF cultivation



254 (Fig. 2a). The maximum dissolved As concentration in control soils was 50 times greater than that on  
255 the first sampling day. In comparison, the maximum dissolved As concentration in soils with Fe  
256 materials was less than 5 times greater than that of the control soils on the first sampling day. On the  
257 final sampling day, the dissolved As concentration in soils with Fe materials at the rate of 10 and 30 t  
258 ha<sup>-1</sup> was less than 10% and 4%, respectively, than that of control soils under CF cultivation. In  
259 comparison, the effect of Fe material application was not clear on the sampling days during the  
260 intermittent irrigation period under WS cultivation. This result was probably obtained because the  
261 flooded duration before sampling (24 h) followed by soil-drying was not long enough for the  
262 considerable re-mobilization of As in the soil solution (dissolved As levels were less than 2 µg L<sup>-1</sup>).  
263 Therefore, the data obtained on the final three sampling days might not exactly reflect actual As  
264 availability to rice plants during the intermittent irrigation period.

265 Evaluating the extent to which potential mechanisms contribute towards immobilizing As by  
266 applying Fe contribute seems to be very difficult. Fe oxides have high capabilities for removing As  
267 from solution. The application of Fe materials increases Fe oxides in the applied soils. In particular,  
268 RIM directly supplies Fe oxides to the soils, because certain types of Fe oxides (magnetite/maghemite  
269 and wüstite) are its major components. Furthermore, the oxidation of Fe(II) and Fe(0) in Fe materials  
270 could provide an indirect pathway for supplying Fe oxide from Fe materials. The increase of dissolved  
271 Fe by Fe material application (Fig. S2a–b) probably occurs because of the dissolution of Fe oxides  
272 and Fe(0). Because dissolved Fe, mainly Fe(II), is oxidized under aerobic (i.e., enough dissolved  
273 oxygen) conditions, soil-drying during the intermittent period should be an inevitable trigger for the  
274 formation of fresh Fe oxides and consequent As immobilization. The Fe(II) oxidation should also occur  
275 at locally oxidized areas, even in flooded soils, such as around the root surface of rice plants and the  
276 oxidized layer of the soil surface (Kirk & Bajita 1995; Ratering & Schnell 2000). Furthermore, Fe(0),  
277 which is a major and minor component of SSS and RIM, respectively, might immobilize As via another  
278 mechanism. Our previous work showed that Fe(0) strictly limits As mobilization in anaerobic soils  
279 and enhances the formation of orpiment and/or arsenopyrite-like compounds (Suda et al. in press).  
280 This phenomenon might occur and contribute towards immobilizing As by SSS and RIM, which  
281 contain Fe(0), under flooded conditions (i.e., entire period of CF cultivation and the early part of WS  
282 cultivation).

283

#### 284 ***Rice plants***

285 Figure 3a–f shows the relationship between the concentrations of each element (iAs, tAs, and Cd)  
286 and the application rate of Fe materials. The maximum iAs concentration of rice grains under CF and  
287 WS cultivation (0.354 and 0.159 mg kg<sup>-1</sup>, respectively) was obtained from rice grains grown on  
288 control soils. The ANOVA test demonstrated that the two values were significantly different ( $p <$   
289 0.001), indicating that WS cultivation decreases iAs concentration in rice grains on control soils.

290 A regression analysis clearly demonstrated that Fe materials attenuated iAs in rice grains in  
291 response to their application rate ( $R^2 = 0.881$  for SSS under CF cultivation, 0.873 for RIM under CF  
292 cultivation, 0.937 for SSS under WS cultivation, 0.961 for RIM under WS cultivation;  $p < 0.001$ ). The  
293 slope of the regression line for each Fe material under CF cultivation was statistically greater than that  
294 under WS cultivation ( $p < 0.001$ ), indicating that Fe materials work more effectively under CF  
295 cultivation than under WS cultivation. This difference might arise from the difference in soil physical-  
296 chemical conditions between CF and WS cultivation, and/or from the difference in As levels in rice  
297 grains, as pointed out by Makino et al. (2016). In comparison, the slope of the regression line of two  
298 Fe materials under the same water management practice was not statistically different ( $p > 0.05$ ). Thus,  
299 the two Fe materials might have comparable ability for decreasing iAs in grains, even though they  
300 have different Fe contents and mineralogical properties. The comparable ability of RIM to that of SSS  
301 was probably attributable to the finer particle size of RIM (Suda et al. 2018).

302 The results of the statistical analyses for tAs in grains and As uptake in the shoot were similar to  
303 those obtained for iAs in grains (Table S2). In brief, tAs in rice grains and As uptake in the shoot  
304 linearly decreased with increasing Fe material application rates. The effect of Fe material application  
305 under CF cultivation was stronger than that under WS cultivation. The two Fe materials had  
306 comparable ability at attenuating As under both water management practices.

307 Under CF cultivation, strong positive correlations were obtained between the logarithmic  
308 concentration of dissolved As in soil solution (average of three sampling days around heading) and  
309 the concentrations of iAs and tAs in rice grains (Fig. 4a–b;  $r = 0.977$  for iAs;  $r = 0.971$  for tAs;  $p <$   
310  $0.001$ ). Thus, the immobilization of soil As by Fe material application attenuated the As load in rice  
311 grains grown on soils under CF cultivation. For WS cultivation, this relationship less clear than under  
312 CF cultivation (Fig. 4 a–b;  $r = 0.430$ ,  $p = 0.058$  for iAs;  $r = 0.455$ ,  $p < 0.05$  for tAs). These relatively  
313 low correlation coefficients probably reflect the fact that the measured dissolved As does not represent  
314 actual dissolved As levels during the intermittent irrigation period, rather than dissolved As being a  
315 weak factor for determining As uptake under WS cultivation. Alternatively, the inhibition of As(III)  
316 absorption by dissolved Si is a known mechanism for the attenuation of As uptake by rice plants (Guo  
317 et al. 2005, Li et al. 2009). More or less, the competition of absorption between As and Si contributes  
318 towards alleviating As uptake by rice plants, because the application of Fe materials tends to increase  
319 dissolved Si in the soil (Fig. S2c–d).

320

### 321 ***3.4 Cd in the soil solution and rice plants***

#### 322 ***Soil solution***

323 The level of dissolved Cd in control soils under CF cultivation remained very low and was not  
324 detectable, except on the first sampling day (Fig. 2c). In contrast, dissolved Cd in control soils under  
325 WS cultivation rose sharply following soil-drying, which enhanced the aerobic conditions of the soil

326 (Fig. 2d). These observations were consistent with well-established relationships between soil redox  
327 potential and Cd mobility in soils (e.g., Huang et al. 2012). Because pH is a prime factor to determine  
328 Cd solubility in soils (Chuan et al. 1996), the decrease in soil pH causes Cd mobilization when soil  
329 dries. The oxidative dissolution of Cd sulfide, which would occur during the CF period, might also  
330 explain the increase of dissolved Cd after soil-drying (Furuya et al. 2016).

331 The application of Fe materials tended to decrease dissolved Cd in soil solution after the  
332 start of intermittent irrigation period of WS cultivation. For the flooded period during WS cultivation  
333 and whole period during CF cultivation, Cd solubilization was highly limited, making discussion  
334 difficult. For the intermittent irrigation period during WS cultivation, the higher soil solution pH (Fig.  
335 1b) might cause the lower dissolved Cd in soils with Fe materials than that in control soils. Furthermore,  
336 the direct and indirect increase of Fe oxides along with Fe material application might immobilize Cd,  
337 even though the affinity of Cd to Fe oxides is relatively low when compared to other heavy metals  
338 (Kinniburgh et al. 1976). Hashimoto & Yamaguchi (2013) reported that Fe(0) enhances the formation  
339 of Cd sulfide, which is an insoluble compound, and decreases Cd in soil solution. Furuya et al. (2016)  
340 showed that Cd sulfide forms under anaerobic conditions, and remains, to some extent, even under  
341 subsequent aerobic conditions, for more than 20 days. Therefore, the acceleration of Cd sulfide  
342 formation, which is promoted by Fe(0) in Fe materials, might be a possible mechanism for the  
343 immobilization of Cd in soils (Fig. 2d).

344

### 345 *Rice plants*

346 Figure 3e–f shows the effect of Fe material application on Cd concentration in rice grains. The highest  
347 Cd concentration of  $0.127 \text{ mg kg}^{-1}$  was obtained from rice grains grown on control soils under WS  
348 cultivation. In comparison, rice grains grown on control soils under CF cultivation had Cd less than  
349 LOQ ( $2.20 \times 10^{-2} \text{ mg kg}^{-1}$ ).

350 The effect of Fe material application on Cd in rice grains grown under CF cultivation was unclear,  
351 because the concentration of Cd in rice grains was less than LOQ ( $2.20 \times 10^{-2} \text{ mg kg}^{-1}$ ), regardless of  
352 Fe material input under CF cultivation (Fig. 3e). In comparison, under WS cultivation, an increase in  
353 the application rate of Fe materials caused a linear decrease of Cd in rice grains (Fig. 3c;  $R^2 = 0.775$   
354 for SSS and 0.953 for RIM;  $p < 0.001$ ). The slope of the regression line for RIM under WS cultivation  
355 was statistically greater than that for SSS ( $p < 0.01$ ). Thus, the application of RIM more efficiently  
356 decreased Cd in rice grains than SSS.

357 Table S2 shows that Fe material application caused a linear decrease in total Cd uptake in shoots  
358 grown on soils under WS cultivation in response to their application rate ( $p < 0.001$ ). The slope of the  
359 regression line for RIM was distinctly greater than that of SSS under WS cultivation, but was not  
360 significant at the 0.05 significance level ( $p = 0.059$ ). For CF cultivation, the total uptake of Cd in the  
361 shoot could not be determined because Cd concentration in rice grains was too low to quantify.

362 However, total Cd uptake seemed to decrease in response to Fe material application rates, judging  
363 from the sum of straw Cd, husk Cd, and possible range of Cd in grains (Table S2).

364 Although Honma et al. (2016b) reported a linear relationship between dissolved Cd in soil  
365 solution and Cd in grains, we found no linear or logarithmic relationship between these two parameters  
366 in the present study (Fig. 4c). Of note, at the equivalent application levels, RIM attenuated Cd in rice  
367 grains more than SSS (Fig. 3f). This result was unexpected because SSS lowered dissolved Cd in soils  
368 more than RIM under WS cultivation (Fig. 2d). Differences in the ability of the two Fe materials to  
369 supply Mn to the soil solution (Fig. S2f) might explain this inconsistency, because dissolved Mn  
370 inhibits Cd absorption by rice roots (Yoshikawa et al. 1986).

371

### 372 ***3.5 Practical application***

373 In the present study, we clarified the combined effect of Fe material application and water management  
374 practices on iAs, tAs, and Cd concentrations in rice grains. Figure 5 summarizes effect of water  
375 management practices with and without applying Fe materials. The application of Fe materials  
376 decreased both Cd and, especially, iAs accumulation in rice grains in relation to their application rate.  
377 As expected from previous studies (Arao et al. 2009, Hu et al. 2013), CF cultivation kept Cd in rice  
378 grains at very low levels, whereas WS cultivation attenuated the iAs load in rice grains. Focusing on  
379 iAs only, the combination of Fe material application and WS cultivation seemed to be the most  
380 powerful countermeasure. In the present study, iAs concentration in rice grains grown on Fe material-  
381 applied soils (at 30 t ha<sup>-1</sup>) under WS cultivation was approximately only 20% of that grown on control  
382 soils under CF cultivation. However, Cd in rice grains under WS cultivation was substantially greater  
383 than that under CF cultivation. Moreover, grain yield under WS cultivation tended to be less than that  
384 under CF cultivation (Fig. S1). Therefore, the application of this combination might be limited because  
385 of these adverse effects. Alternatively, the combination of Fe material application and CF cultivation  
386 might be a better choice in many cases, because it simultaneously achieves relatively low iAs and  
387 extremely low Cd in rice grains, and probably maintains grain yields. However, for all the  
388 countermeasures involving Fe material application, cost-cutting of Fe material is essential. Therefore,  
389 further studies focusing on inexpensive by-product Fe materials, such as SSS and RIM, are needed to  
390 establish practical countermeasures for alleviating iAs and Cd concentrations in rice grains.

391

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403

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407

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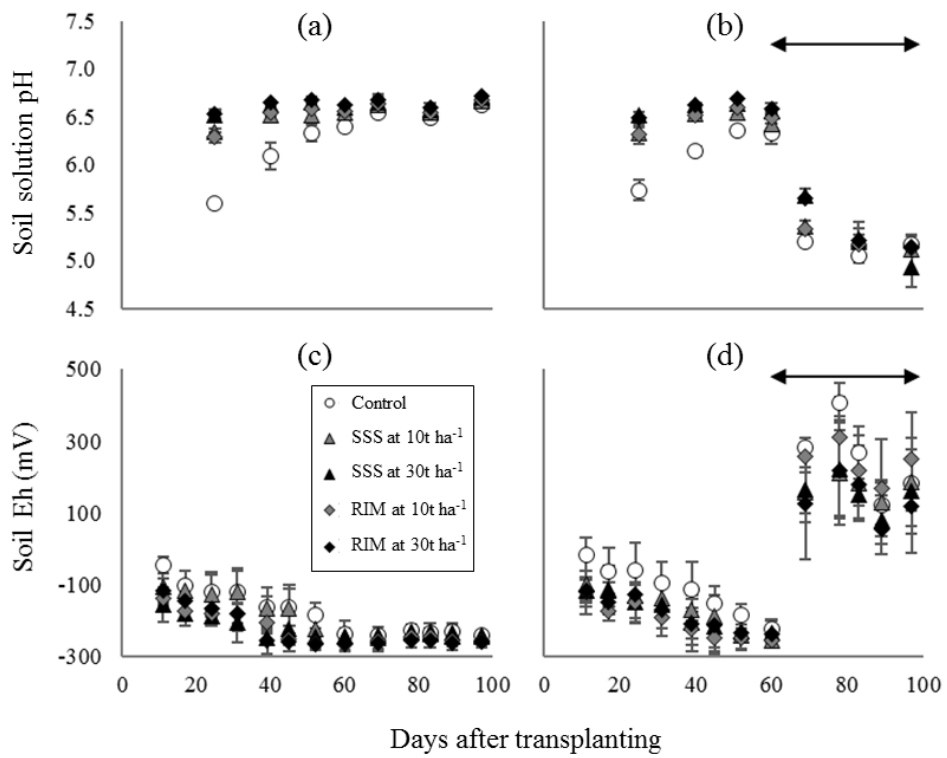
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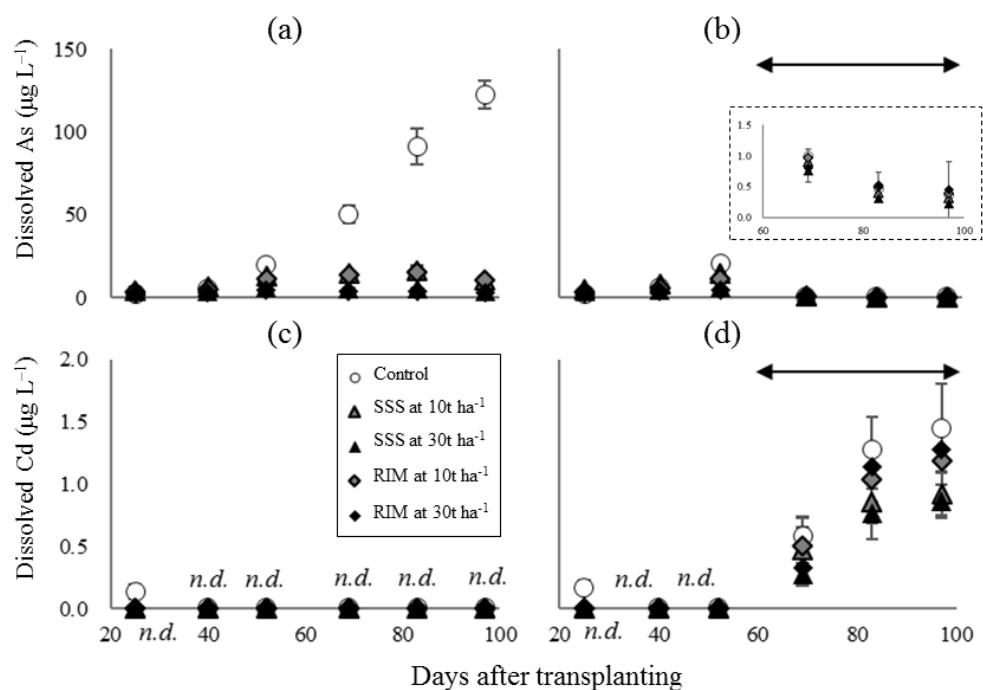
**Table 1** Simple linear regression analyses for grain yield and shoot biomass in relation to iron material application rates.

<i>X</i> : Application rate (t ha <sup>-1</sup> )	<i>Y</i> : Grain yield (g pot <sup>-1</sup> )		<i>Y</i> : Shoot biomass (g pot <sup>-1</sup> )	
	CF	WS	CF	WS
<b>SSS</b>				
0	13.0±0.4	11.0±0.7	39.3±0.4	37.6±0.6
10	11.9±0.6	9.65±0.78	38.4±1.4	37.5±1.3
30	13.2±0.3	10.5±0.8	41.7±1.1	39.0±1.2
<i>Regression</i>				
Slope	0.0125	-0.0065	0.0899	0.0496
Intercept	12.5	10.5	38.6	37.4
<i>R</i> <sup>2</sup>	0.054	0.009	0.471	0.295
Significance	<i>ns</i>	<i>ns</i>	<i>p</i> < 0.05	<i>ns</i>
<b>RIM</b>				
0	13.0±0.4	11.0±0.7	39.3±0.4	37.6±0.6
10	13.1±0.2	10.6±0.4	40.6±0.6	38.0±0.7
30	10.7±0.5	9.94±0.30	40.6±1.2	37.8±0.6
<i>Regression</i>				
Slope	-0.0833	-0.0343	0.0365	0.0038
Intercept	13.4	11.0	39.7	37.8
<i>R</i> <sup>2</sup>	0.812	0.512	0.260	0.007
Significance	<i>p</i> < 0.001	<i>p</i> < 0.01	<i>ns</i>	<i>ns</i>

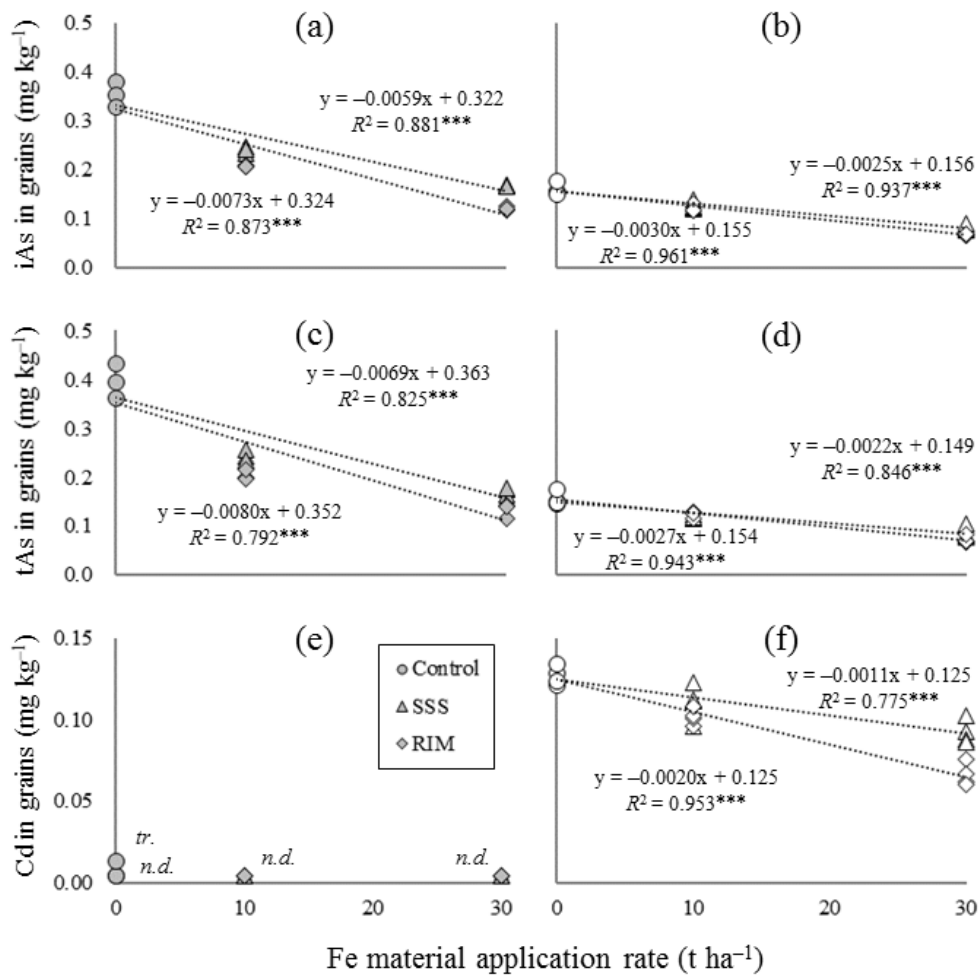
F; continuously flooded, WS water-saving, SSS spent steel shot, RIM residual iron material, *ns* not significant



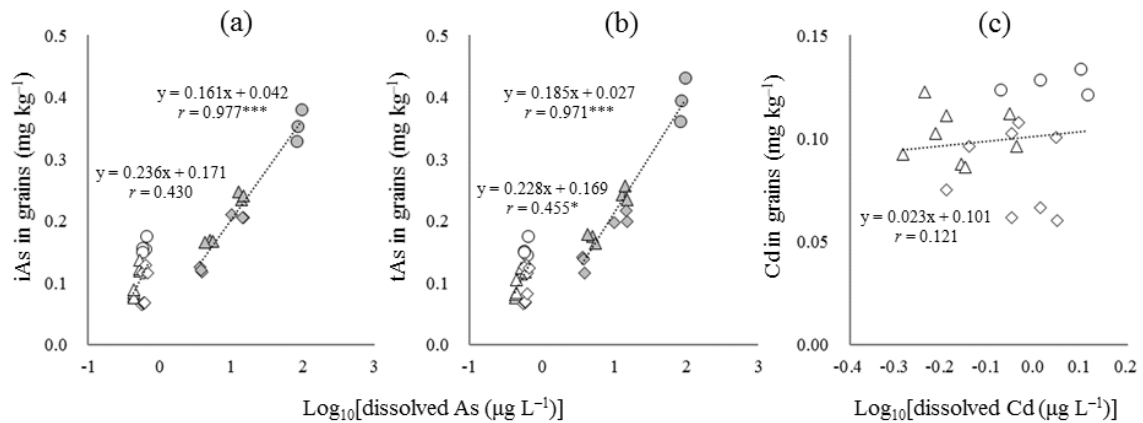
**Fig. 1** Time courses of soil solution pH and soil Eh during the cultivation period. (a) and (c) are under continuously flooded cultivation and (b) and (d) are under water-saving cultivation. The plot and error bar represent the average and standard deviation, respectively. SSS and RIM denote spent steel shot and residual iron material, respectively. The double-headed arrow indicates intermittent irrigation period.



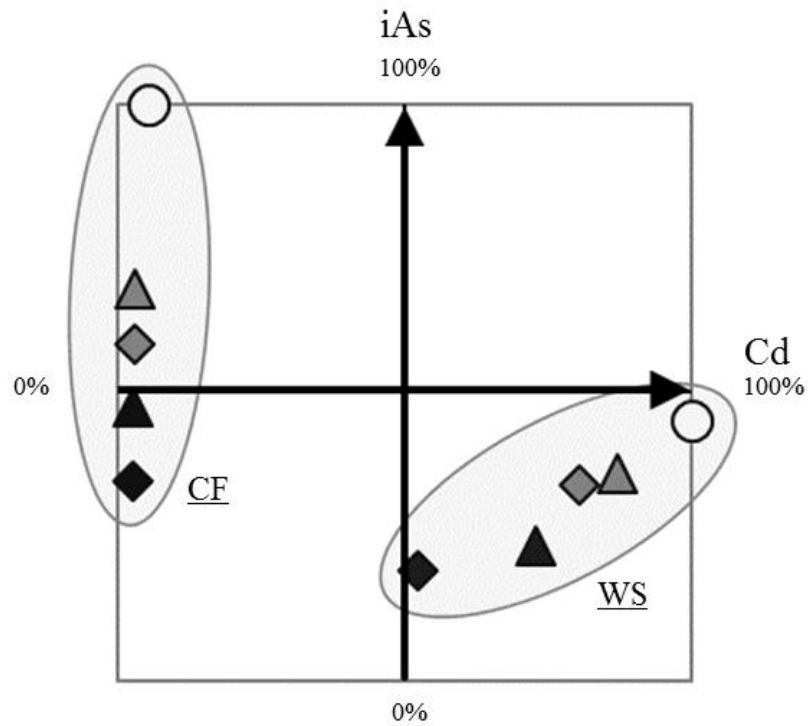
**Fig. 2** Time courses of the concentrations of dissolved arsenic (As) and cadmium (Cd) in soil solution during the cultivation period. (a) and (c) are under continuously flooded cultivation and (b) and (d) are under water-saving cultivation. SSS and RIM denote spent steel shot and residual iron material, respectively. The plot and error bar represent average and standard deviation, respectively. The double-headed arrow indicates intermittent irrigation period. n.d. means dissolved Cd in soil solution was not detected ( $< 4.05 \times 10^{-3} \mu\text{g L}^{-1}$ ).



**Fig 3.** Simple linear regressions of the concentration of inorganic arsenic (iAs), total arsenic (tAs) and cadmium (Cd) in grains of rice grown under continuously flooded [(a), (c), (e)] and water-saving [(b), (d), (f)] cultivation in relation to iron (Fe) material application rates. Plot colors of gray and white indicate continuously flooded and water-saving cultivation, respectively. SSS and RIM denote spent steel shot and residual iron material, respectively. n.d. and tr. indicate that the Cd concentration in grains range from 0 to  $7.57 \times 10^{-3}$  and  $7.57 \times 10^{-3}$  to  $2.53 \times 10^{-2}$  mg kg<sup>-1</sup>, respectively.



**Fig. 4** Relationships between the concentrations of dissolved arsenic (As) and cadmium (Cd) and the logarithmic concentrations of inorganic As (a), total As (b), and Cd (c) in rice grains. Only for Cd, data under continuously flooded cultivation was not shown because most of the concentration of dissolved Cd in the soils and Cd in rice grains were less than LOD or LOQ. Circle, triangle, and square indicate control, application of spent steel shot, and application of residual iron material, respectively. Plot colors of gray and white indicate continuously flooded and water-saving cultivation, respectively.



**Fig. 5** Relationship between the relative concentrations of inorganic arsenic (iAs) and cadmium (Cd) in grains grown under each culture condition. CF and WS mean continuously flooded and water-saving, respectively. The concentrations of iAs and Cd in grains grown on soils under CF and WS cultivation, respectively, were taken as 100%. Circle, triangle, and square indicate control, application of spent steel shot, and application of residual iron material, respectively. Plot colors of white, gray, and black indicate an iron material application rate of 0, 10, and 30 t ha<sup>-1</sup>, respectively.