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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5   water management practices

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8   Aomi Suda* and Tomoyuki Makino

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10   *Institute for Agro-Environmental Sciences, NARO, 3-1-3, Kannondai, Tsukuba, Ibaraki 305-8604, Japan*

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13   *Correspondence*:

14

15   A. Suda, National Institute for Agro-Environmental Sciences, Kannondai 3-1-3, Tsukuba, Ibaraki 305-
16   8604, Japan

17   **E-mail:** suda_aomi@affrc.go.jp

18   **Tel:** +81-29-838-8314

19   **Fax**: +81-29-838-8314

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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^{-1}) 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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1. Introduction

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

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

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

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

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

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

2. Materials and Methods

Pot experiment

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

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

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

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

Sampling and analysis of the soil solution

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

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

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^{-1} hydrofluoric
192 acid and 0.6 mol L^{-1} 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**
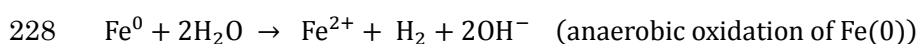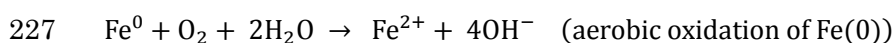211 *3.1 Grain yield and biomass production*
212 For both water management treatments, simple linear regression analyses showed no significant
213 negative relationships between grain yield or shoot biomass and the SSS application rate up to 30 t ha^{-}
214 ^{1} (Table 1). In comparison, the increase 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

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:

227 $Fe^0 + O_2 + 2H_2O \rightarrow Fe^{2+} + 4OH^-$ (aerobic oxidation of Fe(0))

228 $Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^-$ (anaerobic oxidation of Fe(0))

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:

235 $4Fe^{2+} + O_2 + 10H_2O \rightarrow 4Fe(OH)_3 + 8H^+$
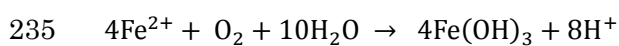
236 The Eh of soils applied with Fe materials remained at lower levels than that of control soils in the

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

3.3 As in the soil solution and rice plants

Soil solution

244 Dissolved As concentration in control soils under CF cultivation gradually increased, peaking at 120

245 μg 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 μg 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^{-1} 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^{-1}).

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 flesh 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^{-1}, 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 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 (2.20×10^{-2} 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 (2.20×10^{-2} 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^{-1}) 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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Table 1 Simple linear regression analyses for grain yield and shoot biomass in relation to iron material application rates.

X: Application rate (t ha^{-1})	Y: Grain yield (g pot^{-1})		Y: Shoot biomass (g pot^{-1})	
	CF	WS	CF	WS
SSS				
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
Regression				
Slope	0.0125	-0.0065	0.0899	0.0496
Intercept	12.5	10.5	38.6	37.4
R^2	0.054	0.009	0.471	0.295
Significance	*ns*	*ns*	*p < 0.05*	*ns*
RIM				
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
Regression				
Slope	-0.0833	-0.0343	0.0365	0.0038
Intercept	13.4	11.0	39.7	37.8
R^2	0.812	0.512	0.260	0.007
Significance	*p < 0.001*	*p< 0.01*	*ns*	*ns*

F; continuously flooded, WS water-saving, SSS spent steel shot, RIM residual iron material, *ns* not significant
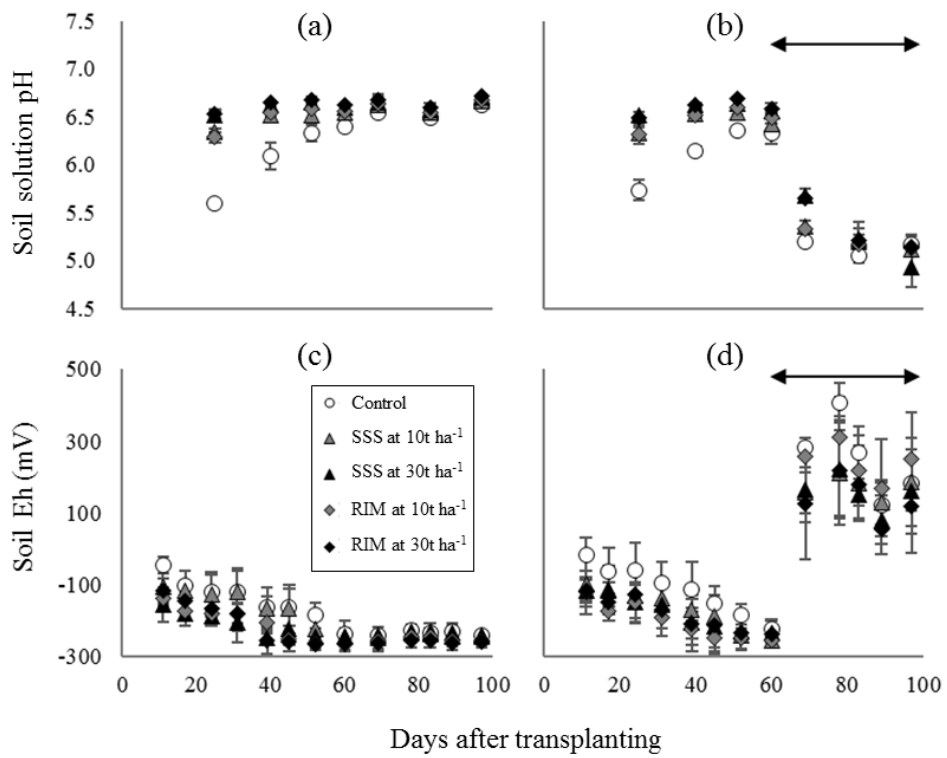
Fig. 1 Time courses of soil solution pH and soil Eh during the cultivation period. (a) and (c) are under continuously flooded cultivation and (b) and (d) are under water-saving cultivation. The plot and error bar represent the average and standard deviation, respectively. SSS and RIM denote spent steel shot and residual iron material, respectively. The double-headed arrow indicates intermittent irrigation period.
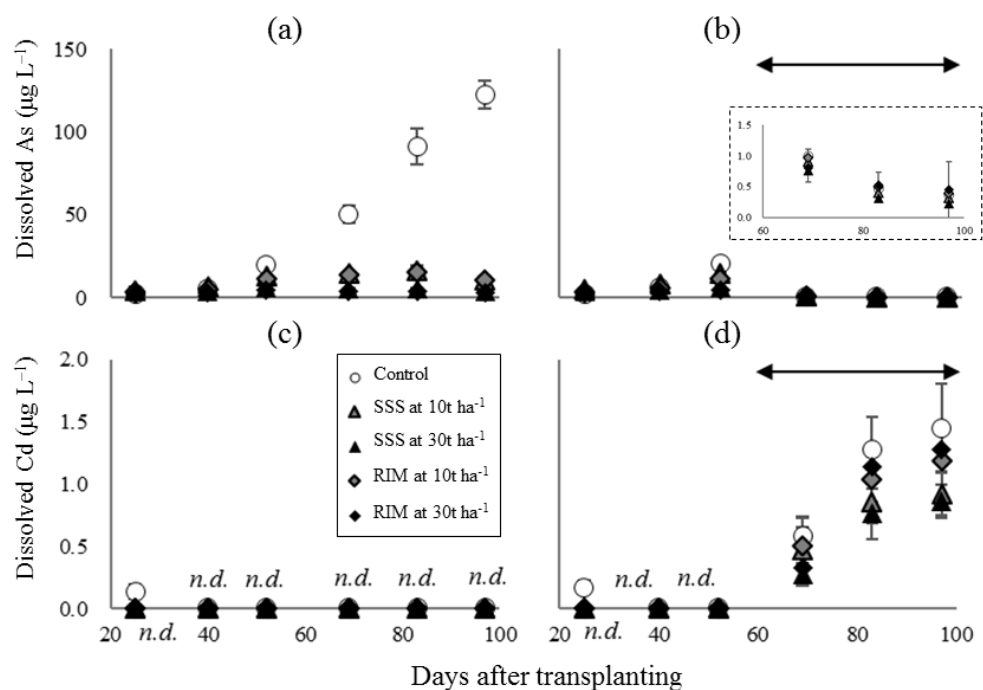
Fig. 2 Time courses of the concentrations of dissolved arsenic (As) and cadmium (Cd) in soil solution during the cultivation period. (a) and (c) are under continuously flooded cultivation and (b) and (d) are under water-saving cultivation. SSS and RIM denote spent steel shot and residual iron material, 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 ($< 4.05 \times 10^{-3}$ μg L^{-1}).
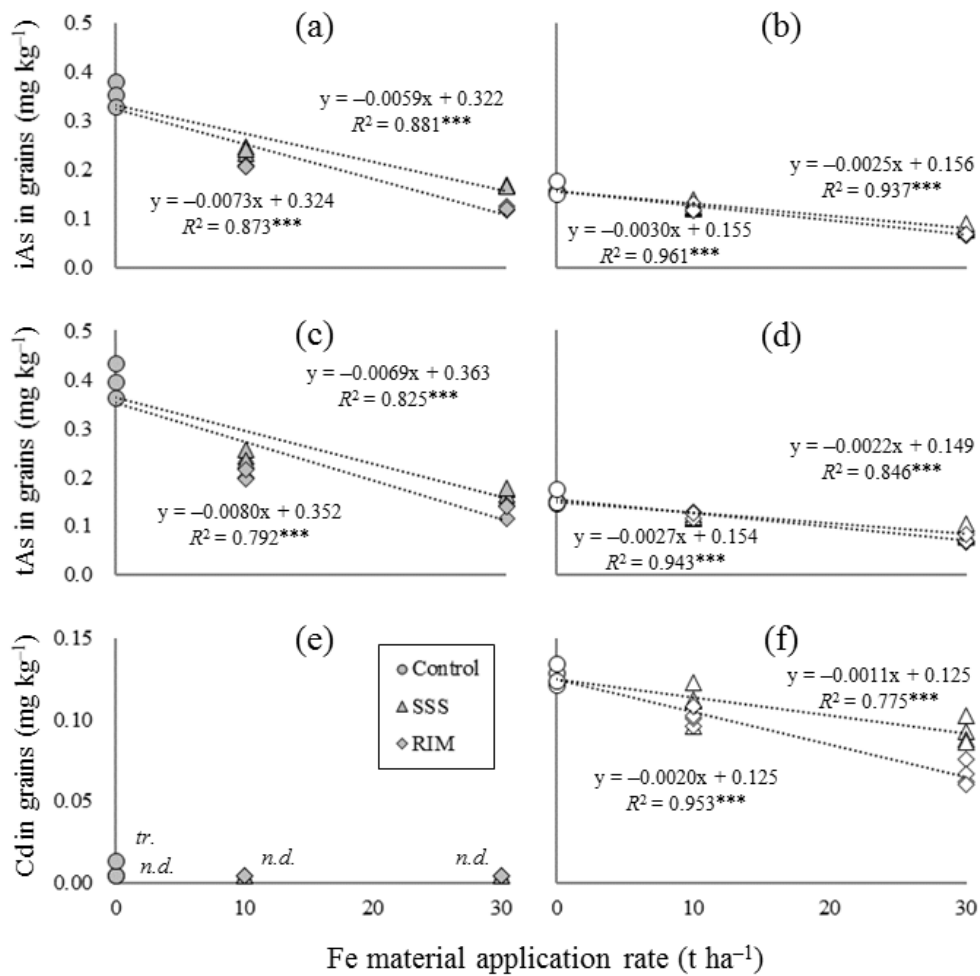
Fig 3. Simple linear regressions of the concentration of inorganic arsenic (iAs), total arsenic (tAs) and cadmium (Cd) in grains of rice grown under continuously flooded [(a), (c), (e)] and water-saving [(b), (d), (f)] cultivation in relation to iron (Fe) material application rates. Plot colors of gray 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 7.57×10^{-3} and 7.57×10^{-3} to 2.53×10^{-2} mg kg^{-1}, respectively.
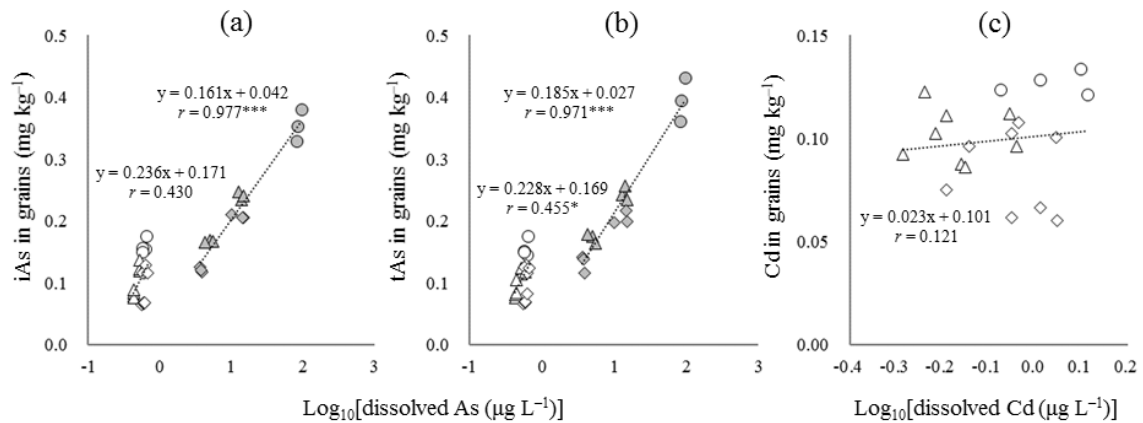
Fig. 4 Relationships between the concentrations of dissolved arsenic (As) and cadmium (Cd) and the logarithmic concentrations of inorganic As (a), total As (b), and Cd (c) in rice grains. Only for Cd, data under continuously flooded cultivation was not shown because most of the concentration of dissolved 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.
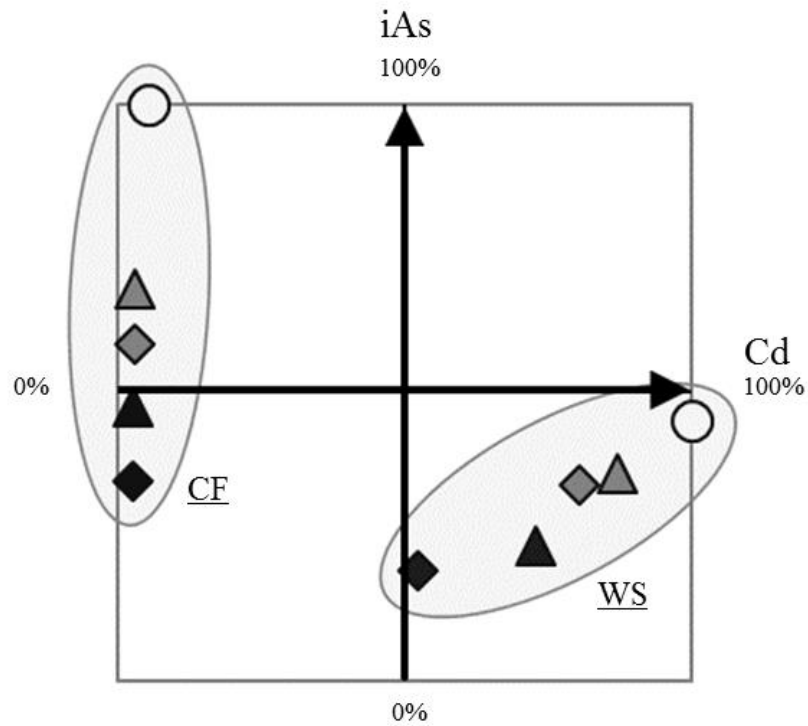
Fig. 5 Relationship between the relative concentrations of inorganic arsenic (iAs) and cadmium (Cd) in grains grown under each culture condition. CF and WS mean continuously flooded and water-saving, respectively. The concentrations of iAs and Cd in grains grown on soils under CF and WS cultivation, 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^{-1}, respectively.