DOUGLAS CARVALHO AMARAL

STRATEGIES TO DECREASE ARSENIC AND CADMIUM IN RICE GRAINS BY RICE RESIDUE MANAGEMENT AND FLOODING

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Recursos Ambientais e Uso da Terra, para a obtenção do título de Doutor.

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RESUMO GERAL

O arroz (*Oryza sativa* L.), alimento básico para a metade da população mundial, é uma fonte potencial de arsênio (As) e cádmio (Cd). Elevados teores de As e Cd no solo e/ou água de irrigação podem afetar o crescimento e desenvolvimento, assim como decrescer a produção e qualidade do arroz. Em adição, o arroz contaminado com As e Cd pode afetar diretamente a saúde humana. Portanto se faz necessário desenvolver diferentes estratégias para minimizar o risco de acúmulo de As e Cd em grãos de arroz. Diferentes técnicas e manejos de irrigação tem sido introduzidas para decrescer o acúmulo de As e Cd em grãos de arroz. No entanto, As e Cd tem respostas opostas aos diferentes manejos de irrigação e podem ainda se acumular nos grãos. Em contraste, técnicas que visam o manejo do silício (Si) podem ser altamente efetivas em combater o problema de acúmulo excessivo de As e Cd nos grãos de arroz. Assim, investigações adicionais são necessárias para desenvolver técnicas simples e economicamente viáveis para decrescer o acúmulo de As e Cd em grãos de arroz. No presente estudo, dois experimentos foram conduzidos onde nós (1) desenvolvemos uma técnica para amostrar e concentrar minerais presentes nas placas de ferro formadas nas raízes de arroz para entender o impacto da aplicação de Si na quantidade e composição mineralógica destas placas e seu efeito na tranferência de As para a parte aérea das plantas de arroz, e (2) investigamos os efeitos de diferentes estratégias de irrigação na absorção de As e Cd por plantas de arroz em solo altamente intemperizado utilizando como amenizantes resíduos de arroz ricos em Si. No primeiro experimento, a técnica desenvolvida foi efetiva em concentrar placas de ferro das raízes de arroz. Os resultados mostram que estratégias que promovem mais Fe e As nas placas ainda podem transferir As para a parte aérea e acumular nos grãos de arroz, mas estratégias que promovam mais Si nas placas podem decrescer o acúmulo de As tanto na parte aérea quanto em grãos de arroz. No segundo experimento, nossos resultados indicam que a utilização de resíduos ricos em Si é uma técnica promissora para decreascer o acúmulo de As e Cd em grãos de arroz sob diferentes manejos de irrigação sem afetar negativamente a produção e os teores de Zn e Fe nos grãos de arroz. Nós sugerimos que a incorporação de casca de arroz (FH) e cinzas da casca de arroz (RHA) podem ser estratégias promissoras dependendo da escolha do amenizante sendo que os resíduos tiveram diferentes efeitos em difentes condições de manejo. Pesquisas adicionais são necessárias para avaliar o efeito residual dos amenizantes assim como confirmar ambos os riscos e benefícios de diferentes técnicas de manejo na produção de arroz.

Palavras-chave: *Oryza sativa* L. Manejo de irrigação. Resíduos ricos em Si. Elementos-traço. Saúde humana.

GENERAL ABSTRACT

Rice (*Oryza sativa* L.), a staple food for half of the world's human population, is a potential source of arsenic (As) and cadmium (Cd). Elevated As and Cd levels in soil and/or irrigation water can affect growth and development of plants, decreasing rice yield and quality. Moreover, contaminated rice grain may threaten human health worldwide. It is therefore imperative to investigate the effects of different strategies to develop new techniques for minimizing the risk for As and Cd transfer into rice grains. Water management has been introduced to decrease As and Cd accumulation in rice grains. However, As and Cd have opposite responses to water management and may still accumulate in rice grains. In contrast, Si management techniques may prove to be highly effective in combating the problem of excessive accumulation of As and Cd in rice grains. Therefore, additional investigations are needed to develop simple and cost-effective methods to decrease As and Cd in rice grains. In this study two experiments were conducted where we (1) developed a technique to sample and concentrate intact Fe plaque minerals to understand the impact of increasing solution-phase Si on the quantity and mineral composition of Fe plaque and controls on shoot transfer of As, and (2) investigated the effects of different water management strategies on As and Cd uptake by rice in well-weathered soil amended with Si-rich rice residues compared to non-amended controls. In the first study, the technique reported was effective at concentrating As-bearing Fe plaque from root systems. Our data indicate that strategies that promote more Fe and As in plaque may still transfer As to shoots but strategies that promote more Si in plaque decrease both shoot As and grain As. In the second experiment, our data indicate that utilizing Si-rich rice amendments is a promising strategy to decrease heavy metals contamination in rice under different water managements without negatively affecting rice yield, and grain Zn or Fe. We suggest that soil-incorporation of fresh husk (FH) and rice husk ash (RHA) may be a promising strategy depending on the choice of amendment in view of the fact that the amendments had different behavior over different conditions. Further research is needed to evaluate the residual effects of the amendments as well to confirm both risks and benefits for different management strategies.

Keywords: *Oryza sativa* L. Water management. Si-rich residues. Trace elements. Human health.

SUMMARY

CHAPTER I

CHAPTER II: ARTICLES

CHAPTER I

1 INTRODUCTION

Environmental pollution is considered major threat to arable lands, water reserves and ultimate to food chain (COMMISSION OF THE EUROPEAN COMMUNITIES - CEC, 2002). This contamination is originated from various sources including combusting, industries, mining, weathering as well as human activities (NAGAJYOTI; LEE; SREEKANTH, 2010). Among the toxic substances, trace elements arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb), may enter the food chain and pose health risks to humans and animals (MCLAUGHLIN; PARKER; CLARKE, 1999). In this respect, there is a growing need to manage toxic elements to protect the agricultural production and human health.

Rice (*Oryza sativa* L.) is the second most extensively cultivated cereal crop throughout the world and it provides food for more than half of the world's population (ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT; FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - OECD; FAO, 2011). Rice is also a major source of As and Cd, particularly in China, Japan, Taiwan and many other countries (ARAO et al., 2009; CHEN, 1991; WILLIAMS et al., 2009). Elevated As and Cd levels in soil and/or irrigation water can affect growth and development of plants, and reduce yield and could add considerable amounts of these elements to the diet intake through plant-based item for consumption (BRAMMER; RAVENSCROFT, 2009). Arsenic and Cd contents in rice grains varies widely, with most reported concentrations found in the range 0.03 to 1.83 mg kg^{-1} for As and 0.01 to 0.1 mg kg^{-1} for Cd (CORGUINHA et al., 2015; MEHARG et al., 2009, 2013; MEHARG; RAHMAN, 2003). At high concentrations, long-term consumption of As-contaminated rice is involved in various health problems including cancers typically involving the bladder, skin and lungs (AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY - ATSDR, 2007); and Cd-contaminated grains is involved in kidney diseases, cancer and osteoporosis (ATSDR, 2008).

Changes in soil pH and redox potential (Eh) state affect the solid-phase speciation and solubility of As and Cd and their availability for uptake by rice. Decrease in Cd solubility or extractability during paddy soil reduction was observed in numerous laboratory studies and was attributed to the precipitation of Cd-sulfide under sulfate reducing conditions (CORNU et al., 2007; DE LIVERA et al., 2011; HUANG et al., 2013). The extent of Cd-sulfide formation during paddy soil flooding may also be relevant with respect to Cd remobilization during paddy soil drainage and re-oxidation (FULDA; VOEGELIN; KRETZSCHMAR, 2013). Additionally, when paddy soil is under aerobic conditions, As is strongly sorbed to mineral soil components such as Fe and Al (hydr)oxides as As(V), and less mobile (GOLDBERG, 2002). In contrast, when anaerobic conditions develop in flooded soils, solubilized As is vigorously taken up by rice plants as As(III) and highly accumulates in grains (HONMA et al., 2016). It is therefore imperative to investigate the effects of different strategies on the dissolved As and Cd concentrations in soil, As and Cd uptake by rice, and As speciation in rice grains to develop new techniques for minimizing the risk for As and Cd transfer into rice grains.

Water is an integral component of a rice paddy system. Various water management methods have been introduced to decrease As and Cd accumulation in rice as well to increase rice production, such as shallow water depth with wetting and drying (LIN et al., 2005), alternate wetting and drying throughout the crop period (YE et al., 2013), and intermittent irrigation followed by shallow water management (UPHOFF; KASSAM; HARWOOD, 2011). However, there are significant obstacles to the adoption of different systems, principally limited information on water management at large scales and the risk of yield loss with improper management (LAHUE et al., 2016). Furthermore, As and Cd have opposite responses to water management. Thus, alternative management techniques under natural flooded and non-flooded rice systems are therefore needed to reduce the environmental problem associated with rice cultivation without affecting rice production and global food security.

Silicon (Si) is an agronomically important element that can have beneficial effects on crop production (IMTIAZ et al., 2016). For example, the accumulation of Si in rice results in positive impacts on rice yield and quality by protecting against abiotic and biotic stresses including heavy metals toxicity (LI et al., 2009; SEYFFERTH; FENDORF, 2012). Rice husk residues, an agricultural waste, is abundantly available in rice producing countries like China, India, Bangladesh, Brazil, United States, Cambodia, Vietnam, Myanmar, and South East Asia (PODE, 2016). However, only a small quantity of rice husk is utilized in agriculture for the purpose of soil-incorporation of Si-rich rice husk at present.

Soil-incorporation of Si-rich rice husk residues such as fresh husk (FH) and rice husk ash (RHA) has positive impacts increasing dissolved and plant-available Si (PENIDO et al., 2016). Silicon addition into soil has shown to decrease both inorganic As and Cd in rice without negatively affecting grain nutrients and improving rice yield and quality (SEYFFERTH et al., 2016). Recent studies have shown that soil-incorporation of Si alleviated the toxicity of Cd in rice, which partly overcame the reduction in growth due to Cd exposure (SHI et al., 2005). In addition, Si is also reported to decrease As in rice plants. The main pathway identified to uptake As in rice plants include silicate transport pathways since As(III) is an analogue of silicic acid and enter into the root by aquaporin channels (MA et al., 2008). Thus, increasing dissolved Si through soil-incorporation of Si-rich rice residues may decrease As and Cd toxicity in rice plants. Consequently, the FH and RHA amendments could become a potential resource of low cost to improve rice yield and quality decreasing heavy metals accumulation in rice grains.

Here, we (1) developed a technique to sample and concentrate intact Fe plaque minerals from aquatic plant root systems to understand the impact of increasing solution-phase Si on the quantity and mineral composition of Fe plaque and controls on shoot transfer of As, and (2) investigated the effects of different water management strategies on As and Cd uptake by rice in well-weathered soil amended with Si-rich rice residues compared to non-amended controls.

2 LITERATURE REVIEW

2.1 Human health exposure to trace elements through rice

Rice (*Oryza sativa* L.), a staple food for half of the world's human population, is a potential source of arsenic (As) and cadmium (Cd) (HONMA et al., 2016; TSUKAHARA et al., 2003). These elements are known to have many toxic effects in humans and are ranked first and seventh, respectively, in the priority list of hazardous substances compiled by the US Environmental Protection Agency (USEPA) (ATSDR, 2011). Arsenic exposed humans may develop skin lesions, neuropathy, gastrointestinal diseases, cardiovascular diseases, and cancer (ATSDR, 2007). Cadmium contamination also causes serious damage to human health, such as stomach irritation, kidney disease, cancer and osteoporosis (ATSDR, 2008).

Rice is the second most extensively cultivated cereal crop throughout the world and has multiple uses for humans particularly in most developing countries in Latin America, sub-Saharan Africa, South and South-east Asia (AZAM; SARKER; NAZ, 2016). According to FAO, rice ingestion contributes a large intake of energy in various countries such as Bangladesh 438 g day^{-1}, Vietnam 452 g day^{-1}, India, 195 g day^{-1}, Thailand 278 g day^{-1}, China 212 g day^{-1}, Japan 165 g day^{-1}, Ivory Coast 153 g day^{-1}, 158 Brazil 108 g day^{-1}, and the United States 25 g day^{-1} (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO, 2010). Therefore, the evaluation of As and Cd exposure pathways through rice and rice-based products in the Cd- and As-contaminated zones with high rice consumption will form a basis for risk assessment to develop agro-environmental sustainability of world rice supplies.

Food crops have highly variable percentages of As compounds. In most dietary As models, the food component is dominated by organo-As compounds

(MCLAUGHLIN; PARKER; CLARKE, 1999). However, concentration of As in rice followed the trend: As(III) > MMA > As(V) > DMA (AZAM; SARKER; NAZ, 2016). Moreover, rice has been identified as an important source of inorganic As, which may vary from 10% to 90% of total As (WILLIAMS et al., 2005, 2007). Inorganic As(III) and As(V) are considered the far greater health hazards than organic As compounds (CHAPPEL; ABERNATHY; COTHERN, 1994). Arsenic contents in rice varies widely, with most reported concentrations found in the range 0.03 to 1.83 mg kg^{-1} in different countries such as United States, Bangladesh, India, and China (BHATTACHARYA et al., 2010; MEHARG et al., 2009; MEHARG; RAHMAN, 2003; NORTON et al., 2012). Recently, the Codex Alimentarius Commission adopted a maximum level of inorganic As of 0.2 mg kg^{-1} in polished rice grain (FAO, 2014). However, grain samples from supposedly uncontaminated paddy fields have been shown to contain over 0.2 mg kg^{-1} of inorganic As in polished rice grain, exceeding the maximum permissible concentration established by the Codex Alimentarius Commission (HONMA et al., 2016). Since rice is a potential source and exposure pathway of As, further studies understanding how As is taken up by plants and subsequently transformed is essential for estimating the risks posed by arsenic-contaminated soils to human.

In addition to As, Cd poisoning has largely occurred and has been detected in the majority of food samples analyzed (CLEMENS et al., 2013). Cadmium toxicity especially affects humans rather than animals, because of their longevity and the accumulation of Cd in their organs by eating Cd-contaminated food (TUDOREANU; PHILLIPS, 2004). Moderate Cd contamination of arable soils can result in considerable Cd accumulation in edible parts of crops. Such levels of Cd in plants are not toxic to crops but can contribute to substantial Cd dietary intake by humans (WAGNER, 1993). It was reported that Cd can be readily taken up by rice and translocated to shoot and then to grains (WANG et al., 2014). Thus, increased consumption of white and especially brown rice contributes to the recent increase of the dietary Cd intake. In the case of “Itai-itai disease”, Cd-polluted rice was the major source of Cd intake in the patients (YAMAGATA; SHIGEMATSU, 1970). This is the early case of chronic Cd toxicity in general populations without specific industrial exposure (URAGUCHI; FUJIWARA, 2012). In japan, even though Cd concentrations in rice grains are usually much lower than the limit established by the Codex Alimentarius Commission of FAO/WHO (0.4 mg kg^{-1}), recent general populations have shown internal Cd level higher than those of other countries (SHIMBO et al., 2001). It is due to daily consumption of rice which contains relatively high Cd (TSUKAHARA et al., 2003). Many reports suggest importance to consider chronic effects of Cd exposure through foods and suggest the importance of reducing grain Cd accumulation in rice and other cereals for better human health (GAO et al., 2016; URAGUCHI; FUJIWARA, 2012). In short, paddy rice contaminated by Cd in the soil challenges the food safety as a whole.

Contaminated food particularly rice grain may threaten human health worldwide, which speaks for the necessity of evaluating food safety with respect to the presence of non-essential trace elements such as Cd and As. Thus, research on As and Cd bioavailability is necessary for detecting As and Cd intake of human from rice for the use in accurate risk assessments to develop a mitigation strategy to minimize As and Cd accumulation in rice grains.

2.2 Trace elements accumulation in rice

Trace elements, for example arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb), may enter the food chain and pose health risks to humans and animals (MCLAUGHLIN; PARKER; CLARKE, 1999).

The sources of these elements vary, and the propensity for plants to accumulate and translocate them to edible and harvested parts depends on soil and climatic factors, plant genotype and agronomic management. Bioavailability of the contaminants to humans also depends on the speciation of the contaminant in the food, dietary composition and the nutritional status of the individual.

Of the elements listed above, the elements that have most commonly given rise to health concerns about food safety are the heavy metals Cd and As. These elements are two of the most challenging environmental problems in food crops especially in rice, affecting millions of people worldwide (HONMA et al., 2016). Understanding how As and Cd are taken up by plants and subsequently transformed is essential for estimating the risks posed by As- and Cd-contaminated soils to human population.

The principal causes of elevated soil As are the historically widespread use of As compounds as insecticides and herbicides for agricultural production (MANDAL; SUZUKI, 2002). The toxicity of As compounds depends on a number of factors, including the chemical form present: inorganic As forms are more toxic than organic, and arsenite (As(III)) is more toxic than arsenate (As(V)) (WORLD HEALTH ORGANIZATION - WHO, 1981). The availability of As in soil depends on Eh and pH. Arsenic (V) dominates in aerated soils and typically has limited mobility at circumneutral pH due to its strong affinity for Al and Fe (hydr)oxides (FENDORF; KOCAR, 2009); thus, the concentrations of arsenate in soil solutions are usually low. Under anaerobic conditions, the propensity for As migration increases due to the reductive dissolution of Fe (hydr)oxides and the reduction of As(V) to As(III), which has a limited affinity for most solids other than Fe (hydr)oxides (HERBEL; FENDORF, 2006).

Two main pathways identified to uptake As in rice plants include phosphate transport pathways since As(V) is an analogue of phosphate and new evidence reveals that As(III) (silicic acid analogue) enter into the root by aquaporin channels, which are used as silicate transport (MA et al., 2008; WU et al., 2011). It is therefore established that As(V) and phosphate share the similar transport pathway in rice roots. Thus, under aerobic condition, the phosphorus availability may strongly affect the As bioaccumulation in plants with the transporters having a higher affinity for phosphate than for As(V) (MEHARG; NAYLOR; MACNAIR, 1994). In contrast, As(III) uptake shares the highly efficient silicon (Si) pathway of entry to root cells and efflux towards the xylem (ZHAO et al., 2009). Thus, As(III) uptake is of particular importance for rice and other aquatic plants with their roots growing in anaerobic or semi-anaerobic environments. Rice is a strong accumulator of Si, with Si concentration in the shoots typically varying from 5 to 10% (HODSON et al., 2005); thus, the efficient Si uptake pathway in rice allow inadvertent passage of As(III), which explain why rice is efficient in accumulation of As (ZHAO et al., 2009).

Arsenic content of rice plant parts generally follow the pattern: root > straw > husk > grain (LEI et al., 2013). Moreover, the concentration of As in all plant parts increased with an increase in soil As, and As concentrations in rice grains is approximately 10 times higher than those in other cereals such as wheat and barley (WILLIAMS et al., 2007). Since the predominant As species in rice grain is inorganic As, minimizing the intake of As from rice in the diet is an important health issue.

In addition to As contamination, it has been reported that human activities contribute a major share of annual Cd addition to the environment (CLEMENS et al., 2013). In agricultural soils, atmospheric deposition is known as a major source of Cd input (KELLER; SCHULIN, 2003). In rice fields, application of phosphate fertilizers and water irrigation is the major anthropogenic Cd polluting sources (DU et al., 2013). Cadmium accumulation in plant-derived food is mostly due to the uptake of soil Cd. Although reported mean values for topsoils around the world are usually in the range of 0.2–1.0 mg

kg^{-1} dry soil, there is enormous variation, with maximum levels near 50 mg kg^{-1} in some agricultural soils (UNITED NATIONS ENVIRONMENT PROGRAMME - UNEP, 2008).

Cadmium solubility and bioavailability in soil mainly depend on soil redox potential (Eh) and pH (HONMA et al., 2016). In general, the enrichment of Cd in paddy rice grain occurs during soil oxidation, which accompanies pre-harvest drainage of the flooded paddy fields as well in upland rice cultivation. The predominant form of Cd taken up by rice plants is Cd^{2+}, and the uptake is suppressed under reduced conditions (URAGUCHI; FUJIWARA, 2012). Drainage of soils results in aerobic conditions that enhance the availability of Cd for plant uptake through oxidation of CdS to Cd^{2+} and SO_4^{2-}, which has a much higher solubility than CdS formed under flooding conditions (BINGHAM et al., 1976). After absorption by the roots, Cd is transported to the stele by passing through endodermis and Casparian strips and then Cd is translocated to shoot via xylem and finally accumulates in grains (SONG et al., 2013). It has been extensively reported that Cd stress caused a reduction in rice growth and biomass. Furthermore, Cd may affect the uptake and translocation of essential nutrients by rice (LI et al., 2012).

Cadmium and As are highly toxic heavy metals that can readily accumulate in rice grains. Moreover, the rice grain contaminated with Cd and As represents a major risk to human health. Therefore, more detailed studies are still required to understand the mechanisms of different strategies in reducing these elements toxicity in rice.

2.3 Management practices to control trace elements in rice

As described above, rice can accumulate Cd and As in grains and could transfer them to humans via the food chain. Thus, reduction of Cd and As in rice plants is among the major issues for sustainable agriculture and human health. During the past decades, several practices have been employed in the management of Cd and As toxicity in rice plants to minimize the health impacts of As and Cd from rice consumption.

Water management is a promising method that affects Cd and As bioavailability in soils and their subsequent uptake by rice (ARAO et al., 2009). For example, alternate wetting and drying (AWD) of rice paddies, in which fields are drained and re-flooded one or more times during the growing season, has been promoted as a strategy to decrease irrigation water use and reduce trace elements without affecting rice production (LAHUE et al., 2016; RICHARDS; SANDER, 2014). However, despite these potential benefits, there are significant obstacles to the adoption of AWD across the world; principally limited information on AWD management at large scales and the risk of yield loss with improper management (LAHUE et al., 2016). In addition, to make it more difficult, As and Cd have opposite responses to water management. When a paddy field is flooded and the soil has a low redox potential, any Cd present in the soil combines with sulfur (S) to form CdS, which has low solubility in water. Thus, flooding of paddy fields during the growing season, especially during later stages of plant growth, can effectively reduce Cd concentrations in rice grains (ARAO et al., 2009). In contrast, anaerobic conditions in paddy soils usually lead to the reduction of As(V) to As(III), which enhances the bioavailability of As to the rice plants (HONMA et al., 2016). It is therefore necessary to investigate the feasibility of simultaneously mitigating both As and Cd accumulation in rice grains through appropriate management technique.

Silicon (Si) is the second most abundant element in the earth’s crust and plays a number of important roles in the mineral nutrition of plants (DUAN et al., 2013). The scientific documentation on the benefits of Si to crops has helped establish Si fertilization as an agronomic practice in many agricultural lands worldwide (TUBANA; BABU; DATNOFF, 2016). For example, Si fertilizers have been widely used in Japan to increase rice yield. This can be attributed to the fact that Si fertilization leads to better structural support and enhanced biomass and, consequently, higher yield bearing capacity, as well as increased resistance against various biotic and abiotic stresses that would otherwise cause yield decline (LI et al., 2009). It was estimated that off-take of Si by a rice crop is 270 kg/ha/year and if this plant-incorporated Si is not recycled back into the paddy ecosystem, it is a serious loss with major implications for rice fertility (MEHARG; MEHARG, 2015). Studies illustrate that continuous input of Si, either through straw recycling or as mineral fertilizer, is essential to ensure long-term productivity of paddy soils.

In addition to the role of Si in alleviating various stresses, Si appears to be fundamental to regulating grain nutrition by playing an important role with respect to assimilation of the problematic toxins such as As and Cd in rice. Arsenite (As(III)), the prevalent form of As under anaerobic conditions in paddy soils, is a silicic acid analogue and rice is efficient at assimilating As(III). It was observed that higher Si in soil solution outcompeted As assimilation by rice. In addition, Si fertilization is also known to reduce cadmium uptake and transport by rice. However, the mechanisms are still not well understood.

It is shown that soil incorporation of Si leads to higher rice yields, as well improves the quality of rice grain through decreasing Cd and As contents. Therefore, finding sustainable approaches to improve Si availability is of extreme importance. Si-rich residues are abundantly available in rice producing countries like China, India, Bangladesh, Brazil, United States, Cambodia, Vietnam, Myanmar, and South East Asia, which are continually available to farmers and could be incorporated into paddy soils with minimal change and cost to current practices (PODE, 2016). For example, rice husk (e.g. fresh, and ash) is an underutilized Si-rich material with high potential agronomic benefit if reincorporated to paddy soil to increase plant-available Si (SEYFFERTH et al., 2016). Rice husk residues are advantageous over straw because contains less labile carbon, an order of magnitude less As, and upon incorporation to plant-less soil leads to 50% more porewater Si (PENIDO et al., 2016). Thus, rice husk may be an economically viable resource to improve rice nutrition and decrease rice As and Cd due its trivial increase in soil-dissolved Si. However, the biogeochemical impact of incorporating these residues into soils under typical flooded and non-flooded water managements is unknown.

Perhaps a more sustainable strategy with respect to Si fertilization is to increase the once common practice of reincorporating rice residues back into paddy fields. Furthermore, optimization of biogeochemical cycling of Si in paddy environments is, therefore, a good long-term agricultural policy with great potential benefit to the global economy and human health. Overall, continued research in these areas may contribute to the effort of reducing toxic As and Cd in rice, and can greatly help to reduce overall intake in regions prone to As and Cd problems.

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Chapter II: Articles

Article 1: *A new approach to sampling intact Fe plaque reveals Si-induced changes in Fe mineral composition and shoot As in rice*

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A new approach to sampling intact Fe plaque reveals Si-induced changes in Fe mineral composition and shoot As in rice

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ABSTRACT

The Fe (oxyhydr)oxide rind, or Fe plaque, that forms on aquatic plant roots is an important sorbent of metal(loid)s and plays a role in the attenuation of metal(loid) uptake into higher plants. However, the mineral composition of Fe plaque and thus its potential to sorb metal(loid)s is affected by solution chemistry. The predominant strategy to characterize Fe plaque using dithionite-citrate-bicarbonate (DCB) extraction and elemental analysis reveal total Fe quantity but misses the mineral structure of the Fe (oxyhydr)oxide. Here, we developed a new technique using gentle sonication to sample intact Fe plaque from the root system and concentrate it for subsequent mineralogical characterization using synchrotron-based X-ray diffraction and X-ray absorption spectroscopy. We then coupled that data with conventional DCB extraction. The sample preparation method was effective at concentrating As-bound Fe plaque minerals in a uniform coating onto membranes that could easily be analyzed with X-ray techniques. Using these methods, we show that the percentage of poorly-ordered Fe minerals in Fe plaque increases with increasing pore-water Si in flooded rice paddy soils. These findings have implications for understanding mineral controls on As cycling in the soil-rice nexus, and the sampling approach can be adopted for other aquatic plant systems.

INTRODUCTION

Iron (Fe) plaque is a heterogeneous mixture of Fe oxides, hydroxides, and oxyhydroxides that form on aquatic plant roots and plays a role in the cycling of metal(loid)s at the soil-water-plant nexus.[1-3] These neoformed Fe plaques are precipitated at the oxic/anoxic interface of aquatic rhizospheres where Fe(II) from reductive dissolution of Fe (oxyhydr)oxides and O2 from radial oxygen loss intercept.[3, 4] This process may be biotic or abiotic,[5, 6] and the type of Fe phase that forms is dependent upon the solution chemistry and rate of oxidation.[6] For example, dissolved organic C and Si are known to slow or inhibit the transformation of poorly-crystalline Fe (oxyhydr)oxides to higher ordered phases.[7] The mineral composition, or crystallinity, of Fe plaque is important for understanding the sorption capacity of plaque for metal(loid) retention[8, 9] and thus rhizospheric cycling.[10, 11] However, the characterization of Fe plaque phases and how they may change with treatments is currently challenging due to limited strategies for collecting and concentrating intact Fe plaque. Several techniques to investigate Fe plaque on aquatic plant roots from the natural environment[2, 12, 13] and under experimental conditions[14-17] have been employed. The conventional characterization of Fe plaque by dithionite-citrate-bicarbonate (DCB) extraction and elemental analysis to quantify the total Fe and other elements is certainly useful[18], but does not provide information about the mineral phase(s) of the Fe plaque. Much of what we know about the mineral composition of Fe plaque was gleaned from a relatively small number of studies that utilized micro-X-ray fluorescence imaging (μXRF), micro-X-ray absorption spectroscopy (μXAS), and micro-X-ray diffraction (μXRD).[1, 6, 11, 13, 19-21] These studies revealed that the composition of Fe plaque is heterogeneous and consists of a mix of minerals including ferrihydrite, lepidocrocite, goethite, and siderite depending on local conditions.[22-25] However, these techniques focus on a microscopic portion of one root, and even with multiple "spots" investigated they do not necessarily capture the mineral composition of the root system and how the mineral composition, and thus metal(loid) retention, might change due to changes in solution chemistry. Bulk XRD coupled to bulk XAS can provide information about the type (XRD) and quantity (XAS with linear combination) of Fe mineral phases on the root system,[6, 26] but the preparation of homogenous Fe plaque samples for these techniques have limitations. For example, XAS spectra of freeze-dried and powdered rice roots with intact plaque will have spectral interferences between Fe (and As) in the plaque and inside of the root. Here, we describe a technique to sample and concentrate the Fe plaque associated metal(loid)s from aquatic roots using the Si-treated rice rhizosphere as a model system. Seyfferth et al.[27] recently showed that the addition of Si-rich rice husk residues (fresh and ashed husk) to flooded paddy soil increased solution-phase Si without affecting pH and decreased inorganic grain As by 25-50%. While several possible mechanisms were likely at play that attenuated As uptake in rice including differences in the expression of Si transporters in roots,[28, 29] microbial activity,[30, 31] and competition for uptake,[10, 28] another possible mechanism is that increased solution-phase Si due to husk amendments[27] affected the quantity and mineral composition of As-scavenging Fe (oxyhydr)oxides that comprise Fe plaque.[32] Here, we 1) developed a technique to sample and concentrate intact Fe plaque minerals from aquatic plant root systems for subsequent mineralogical characterization using bulk XRD and XAS, and 2) used this technique and conventional techniques (DCB extraction and elemental analysis) to understand the impact of increasing solution-phase Si on the quantity and mineral composition of Fe plaque and controls on shoot transfer of As. We hypothesized that Si addition to rice paddy soils leads to Fe plaques on roots comprised of more poorly-ordered minerals. We developed a systematic approach to obtain DCB-extractable elements in root plaques and intact samples of Fe plaque for XRD and XAS using sonication. This technique uses low-powered sound waves where the ultra-sound energy emitted by the frequency generator is transmitted through water and removes Fe plaques without the use of chemical reagents, which preserves the integrity of the Fe plaque samples. The sonicating solution is then filtered through nitro cellulosic membranes and Fe plaques on the filter membranes can be directly analyzed at the bulk XRD and XAS beamlines.

MATERIALS AND METHODS

Plant Growth and Root Samples

A full description of the study from which root and root plaque samples were obtained can be found in Seyfferth et al.[27] Briefly, three cultivars of rice (Oryza sativa L. cv. M206, IR66, and Nipponbare) were grown in well-weathered, flooded soil amended with silica-rich fresh husk (FH) or rice husk ash (RHA) at 1% (w/w) and compared to a non-amended control in a replicated pot study utilizing 3.5 kg of soil. Solution-phase Si decreased in the order FH- amended > RHA-amended > nonamended control, and pH was not affected by Si treatment.[27] Total As in the soil was 16 mg kg^{-1} and plants were kept flooded with deionized water until grain filling. At maturity, shoots were separated from roots, and the entire root system was carefully removed from soil and thoroughly washed by a series of submergence in DDI water and gentle massaging. Washed roots were placed onto paper towels and allowed to air dry. Once dry, roots were hand shaken to remove any residual soil particles, and washed again. This process was repeated until no visible soil particles remained on the root system, at least four times. Once clean and air-dried, the root system was bisected longitudinally in half creating two mirror images of the root system: one half was utilized for DCB extraction and total elemental analysis in sequence,[27] and the other half was utilized for intact Fe plaque sampling via sonication and characterization with bulk XRD and XAS. Plaque-free roots, straw, husk, and unpolished grain were digested and analyzed as reported previously.[27]

Dithionite-citrate-bicarbonate (DCB) extraction and total elemental analysis

Fe plaque on root surfaces was extracted using a cold DCB solution, modified from Taylor and Crowder.[33] Briefly, one half of the entire root system, bisected longitudinally, was placed into a 50 mL falcon tube to which 40 mL of 0.3 M sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 5 mL of 1.0 M sodium bicarbonate ($NaHCO_3$), and 3 g sodium dithionite ($Na_2S_2O_4$) was added. After 3 h of agitation at room temperature, roots were removed from the solution and rinsed thoroughly with 18 MΩ●cm (DDI) water, with the rinse solution added to the extract solution. The DCB extract solution was brought to 100 mL volume and then analyzed for total Fe, As, Si, P, and other elements using ICP-OES with matrix-matched standards after 10- to 100-fold dilution. Plaque-free roots were dried at 65°C, ground to a fine powder, digested with hot nitric acid, and the digestates were subject to elemental analysis with ICP-OES.[27]

Intact Fe plaque sampling for mineral and speciation characterization

The remaining half root system with intact Fe plaque was subject to gentle sonication to obtain samples of Fe plaque minerals for characterization using bulk XAS and XRD techniques. Roots with Fe plaque were placed into 400-mL glass beakers partially filled with DDI water, and the beakers were placed into a sonicating bath. Sonics at 20 kHz were applied for 2 hours at 25°C, utilizing ice packs in the water bath to ensure standard temperature. This method physically removed the Fe plaques from the roots without the use of chemical reagents. After sonication, roots were lifted above the sonication solution and gently rinsed with DDI water, carefully ensuring the rinses were collected into the sonication solution. The sonication solution containing the dislodged Fe plaques was then vacuum filtered through an acid-washed glass filtration device equipped with a 0.2 µm nitro cellulosic filter membrane (47 mm disk). The Fe plaques were collected onto the nitro cellulosic filter (Fig. 1), which was carefully placed into a petri dish and loosely covered. Once dry, dishes were capped and transported to Stanford Synchrotron Radiation Lightsource (SSRL) for bulk XRD and XAS analyses.

Fe plaque mineral characterization

The mineral composition of Fe plaque deposited on the filters was analyzed with bulk XRD at SSRL on beamline 11-3 utilizing an incident energy of 12724 eV (λ = 0.9744 Å) with MAR 345 Image Plate Detector. Lanthanum hexaboride was used to calibrate the MAR 345 detector parameters upon integration of the diffractograms in Q space between 0 and 6 $Å^{-1}$ at 0.006 steps and total Q of 1000 counts per 500 s with the Area Diffraction Machine (v2 Beta 248, SSRL, 2007). An integrated diffractogram of a blank nitro cellulosic filter membrane was utilized as a blank and its signal was subtracted from the signal of each sample using XRD-BS. The integrated peaks were analyzed using Match! Software (v3, Crystal Impact, 2015). These data were used to constrain the fitting parameters for X-ray absorption spectroscopy. In order to quantify the mineral composition of Fe plaques and to detect short-range ordered Fe plaque minerals, the intact Fe plaques were further characterized with extended X-ray absorption fine structure (EXAFS) spectroscopy on beam line 11-2 at SSRL. This beam line is equipped with a liquid nitrogen-cooled double crystal monochromator (Si (2 2 0) phi 90) and Kirkpatrick-Baez mirrors. The incident energy was calibrated to the first Fe inflection point at 7112 eV obtained from a standard Fe foil placed in between I1 and I2. Iron K-edge spectra were collected with a Lytle detector from 150 eV below the absorption edge to k values of 12.5 $Å^{-1}$. For each sample, four spectra were obtained and averaged. These averaged spectra were background subtracted, normalized, fitted with a spline function (*k*-weight = 3) and fitted by linear combination (*k* range of 3 – 12 $Å^{-1}$) with Athena software[34] utilizing both ferrihydrite and the results of the XRD analyses to constrain the fits, which were 2-line ferrihydrite ($Fe(OH)_3 \cdot nH_2O$), lepidocrocite (γ-FeOOH), and goethite (α-FeOOH); see Seyfferth et al.[6] and Hansel et al.[1] for a detailed explanation of the preparation of these standards.

Arsenic speciation on Fe plaques

The oxidation state of As in the Fe plaque samples were determined by X-ray absorption near edge structure (XANES) at SSRL on beamline 11-2. The incident energy was calibrated using a prepared sodium arsenate standard, which was assigned its first inflection point at 11874.0 eV. The As fluorescence was monitored using a 100-element Germanium detector. Three spectra per sample were averaged, background-subtracted, normalized, and fitted by linear combination in the region 11850–11900 eV using Athena software.[34] The following materials were included as As standards: As(III)$_i$ as $NaAsO_2$, and As(V)$_i$ as Na_3AsO_4.

RESULTS

Fe plaque sampling method. The sonication and deposition method was effective at removing As-bound Fe plaque minerals from rice roots and evenly depositing them onto nitro cellulosic membranes (Fig. 1). The technique concentrated Fe and As onto the membranes by two orders of magnitude. The method removed 47 (± 9.2)% of the DCB-extractable Fe and 57 (± 18)% of the DCB-extractable As from roots with minimal loss of Fe (0.02% of DCB-extractable) or As (bdl) through the filter. Deposited Fe plaques were clearly visible on the filter membrane disks and varied in color depending on the amendment-induced differences in solution-phase Si (Fig. 1). Independently of the cultivars, the color of the Fe plaque deposited onto nitro cellulosic membranes was similar between treatments showing a light orange color for the FH amended (~500 μM Si) soils, a dark orange color for the RHA amended (~ 200 μM Si) soils, and a medium orange color for the nonamended (~ 70 μM Si) soils. These membrane filters were easily placed directly in front of the X-ray beam for bulk XRD and XAS analyses, and could be divided for simultaneous analyses on multiple beamlines due to the large (47 mm) size of the filters and homogenous deposition of the Fe plaques.

XRD and EXAFS characterization of Fe plaque minerals. The integrated diffractograms of Fe plaques deposited onto filter membranes reveal differences in dominant Fe (oxyhydr)oxides due to Si amendment and cultivar (Fig. 2). Peaks of α-FeOOH and γ-FeOOH were observed in all samples, but the peak sharpness and magnitude differed among cultivar and amendment (Fig. 2). Secondary XRD peaks of scorodite ($FeAsO_4 \cdot nH_2O$), pyrite (FeS_2), and magnetite (Fe_3O_4) were also observed in some samples, but were minor components (Fig. 2). Due to weak diffraction intensity relative to other more crystalline minerals, Fe(OH)3·nH2O was not detected with XRD. The *k3*-weighted EXAFS spectra and linear combination fits (LCF) of the Fe plaque samples show that $Fe(OH)_3 \cdot nH_2O$, α-FeOOH, and γ-FeOOH were the dominant

Fe minerals but the magnitude of each varied among cultivars and soil amendments (Fig. 3 and Table 1). Soil amendments that led to the highest average pore water Si (FH, ~ 500 μM Si) led to a higher percentage of $Fe(OH)_3{\cdot}nH_2O$ and a lower percentage of γ-FeOOH in plaque (Table 1). While the change was subtle for Nipponbare, M206 and IR66 plants grown in soil amended with FH had 30 and 19% more $Fe(OH)_3{\cdot}nH_2O$, respectively, in plaque compared to nonamended control plants (Table 1). RHA amendment, which resulted in a "medium" level of average pore water Si (~ 200 μM Si) also increased the percentage of $Fe(OH)_3{\cdot}nH_2O$ in plaque relative to nonamended control by 12% for IR66 and 2% for M206, but not Nipponbare. Plaque from M206 roots was dominated by $Fe(OH)_3{\cdot}nH_2O$, with lesser amounts of α-FeOOH and γ-FeOOH. Plaque from Nipponbare roots was dominated by α-FeOOH followed closely by $Fe(OH)_3{\cdot}nH_2O$ with minor amounts of γ-FeOOH. IR66 plaques were comprised of equal proportions of $Fe(OH)_3{\cdot}nH_2O$ and α-FeOOH as dominant phases and minor amounts of γ-FeOOH. The addition of other Fe phases including scorodite ($FeAsO_4{\cdot}nH_2O$), pyrite (FeS_2), or magnetite (Fe_3O_4) in the LCF that were indicated as possible phases by XRD did not improve fits; these phases are thus possibly present only in trace amounts.

Speciation of Arsenic in Fe plaques. LCF fits of the As-XANES spectra (Fig. 4) show clear differences in oxidation states of As due to amendment and cultivar. For all samples, sorbed As on plaques was mainly in the As(V) oxidation state (60-80%) with lesser amounts of As(III) (20-40%), but the ranges varied with amendment and cultivar (Table 2). In general, the percent arsenite sorbed decreased in the order RHA > FH > non-amended control for all cultivars.

Role of Fe plaque mineral composition on transfer of As to rice shoots. In general, the amount of As in shoots increased and the mass ratio of shoot/root As decreased as the amount of total Fe in plaque from DCB extraction increased (Fig. 5A, D); however, this relation differed when considering the role of each mineral and among cultivars (Fig. 5). When considering the proportion of DCB-extractable Fe in plaque as $Fe(OH)_3 \cdot nH_2O$, α-FeOOH, or γ-FeOOH, differences in their impact on shoot As were observed (Fig. 5 B-D). While an increase in shoot As was observed as $Fe(OH)_3 \cdot nH_2O$ or α-FeOOH increased, the increase in shoot As per unit increase in DCB-extractable Fe was greater for γ-FeOOH (Fig. 5 B-D). Similarly, the decrease in the mass ratio of shoot/root As per unit increase in DCB-extractable Fe was greater for γ-FeOOH than for $Fe(OH)_3 \cdot nH_2O$ or α-FeOOH (Fig. 5 F-H).

Relationship between shoot As and plaque Fe, As and Si and P. We observed strong and significant relationships between grain and shoot As and DCB-extractable Si in plaque, and weak or no relations between shoot As and DCB-extractable Fe or As (Fig. 5A, Fig. 6). A significant power regression was found between inorganic grain As and plaque Si for Nipponbare ($r^2 = 0.41$, $p = 0.031$), and significant linear regressions were found between inorganic grain As and plaque Si for M206 ($r^2 = 0.672$, $p = 0.007$) and IR66 ($r^2 = 0.608$, $p = 0.013$) (Fig. 6A). Significant linear regressions between shoot As and DCB-extractable Si were observed with negative slopes for M206 ($r^2 = 0.776$, $p = 0.002$), IR66 ($r^2 = 0.57$, $p = 0.019$), and Nipponbare ($r^2 = 0.648$, $P = 0.009$) (Fig. 6C). In contrast, relations between inorganic grain or shoot As and DCB-extractable Fe or As were less evident and not significant, but generally trended toward a positive relationship when all cultivars were considered together (Fig. 5A and Fig. 6B and D). There was also no relation between shoot As or grain As and plaque P (data not shown).

DISCUSSION

The sample preparation technique was effective at concentrating and depositing As-bound Fe plaque minerals in a uniform coating onto nitro cellulosic membranes that could easily be analyzed with synchrotron X-ray spectroscopic methods. This technique has several advantages over existing methods aimed to characterize Fe plaque on aquatic plant roots. In contrast to other techniques where a microscopic portion of a single root is exposed to the

X-ray beam,[2, 3, 6, 11] the method reported here extracts Fe plaque from a root system, which does not bias the Fe mineral characterization to a few “hot spots”. By characterizing the roots as a system, changes in the proportion of different Fe minerals that comprise plaque can be attributed to differences in solution chemistry, rather than the inherent heterogeneity of the Fe plaque microenvironment.[1] Moreover, the method concentrates Fe and As by two orders of magnitude compared to DCB-extractable totals, resulting in higher signal to noise ratios6 and thus less repeat scans needed per sample (Figs. 3 and 4). This method can be applied not only to rice, but to any aquatic plant root system on which Fe plaques form.

Limitations to the technique also exist, which represent a trade-off between complete plaque removal and undesired As(III) oxidation. The technique removes close to 50% of the DCB-extractable Fe and nearly 60% of the DCB-extractable As from the plaque-coated roots, and we observed patchy pigmented areas remaining on the roots after sonication. While the technique does not remove 100% of the DCB-extractable amounts, it is difficult to conclude exactly how much of the Fe plaque was removed because DCB extraction is also known to strip metal(loid)s from root cell membranes.[35] Thus, at least 50%, and perhaps more, As-bound Fe plaque was removed with gentle sonication (i.e. 20 kHz for 2 hours). This compromise in duration and power of sonics was an advantage for minimizing As(III) oxidation, which can occur up to

10% when sonicated at over 180 kHz.[36] It should be noted that the intent of the present approach was not to remove all plaques, but assess whether soil treatments (i.e. Si amendments) would cause global changes in Fe plaque phases, as current methods to spot-check micron-scale areas on root-bound plaque is simply not feasible to answer that question due to the heterogeneity of root plaque and low signal to noise. Because the plaque removed represented a large fraction of the outer plaque that As-bearing rhizosphere solution would encounter during solute delivery to the root, the present technique is an advance over existing methods. Furthermore, there were large fractions of $Fe(OH)_3 \cdot nH_2O$ and α-FeOOH present, indicating that no selection for poorly-crystalline materials had occurred during sampling.

The dominant method of Fe plaque characterization with DCB extraction and elemental analysis can also capture information about the entire root system; however, mineral information is lost in the DCB extraction method because the Fe minerals reductively dissolve. Our data show that solution chemistry impacts the proportion of minerals that comprise plaque, and each mineral affects plant concentrations of As differently (Fig. 5, Table 1). These observations corroborate previous work that shows the amount of DCB-extractable Fe differs with solution chemistry due to the formation of different minerals that comprise Fe plaque.[37] Moreover, the reactive surface area for As sorption differs among Fe plaque minerals, which affects availability of As to roots for uptake and translocation to shoots.[38, 39] Thus, it is important to not only know how much Fe is present in plaque, but also the proportion of different minerals that may impact As retention (Fig. 5).

Our data corroborate previous findings that used μXAS and μXRD on whole root mounts and revealed $Fe(OH)_3 \cdot nH_2O$, γ-FeOOH, and α-FeOOH, as dominant Fe phases in plaque.[3, 6, 11] In addition, our data also show that the proportion of these minerals differs as solution-phase Si changes and also among rice cultivar, differences that may not have been revealed with μXAS and μXRD on whole root mounts. As hypothesized, the proportion of $Fe(OH)_3 \cdot nH_2O$ in Fe plaque increased with increasing solution-phase Si, which was achieved with the FH treatment. We also observed a decrease in γ-FeOOH with increasing solution-phase Si, and γ-FeOOH seems to have a greater proportional impact on the shoot As concentrations than $Fe(OH)_3 \cdot nH_2O$ or α-FeOOH (Fig. 5). Thus, the alteration of the proportion of Fe minerals that comprise plaque (Table 1) due to amendment-induced changes in solution-phase Si is at least partially responsible for the differences in As accumulation in rice shoots and grains reported previously.[27]

The present study shows that in addition to amendment-induced changes in solution-phase Si, the mineral composition of Fe plaque and its relation to shoot As levels also differed among rice cultivars (Table 1 and Fig. 5). Whereas $Fe(OH)_3 \cdot nH_2O$ was dominant in M206 plaque, α-FeOOH was dominant in

Nipponbare plaque and dominant phases in IR66 plaque were roughly equal proportions of $Fe(OH)_3 \cdot nH_2O$ and α-FeOOH. The formation of Fe coatings on aquatic plant roots is a function of several abiotic and biotic factors,6 including solution chemistry (e.g., dissolved Fe^{2+}, Si, pH) as well as biologic factors such as the rate of oxygen loss (ROL) from roots.[4, 32, 40, 41] Since all cultivars were grown in the same soil with the same treatments that did not affect pH, the differences observed were likely due to different extents or rates of ROL between cultivars, and illustrate the need for assessing multiple cultivars in rice studies involving Fe plaque and As cycling.[26, 32, 40, 41]

It is commonly observed that inorganic As – both As(III) and As(V) – are the predominant forms of As encountered by rice roots in most paddy soils;[3, 6, 11, 42] however, some evidence suggests that Si addition may increase the methylated As forms in soil solution. Here, we observed only inorganic As associated with Fe plaque (Table 2), but in the previously reported study from where the roots were obtained, Seyfferth et al. [27] observed an increase of methylated As in pore water and in grain with increasing solution-phase Si.[27] A mixture of As(III) and As(V) with no detectable methylated As on Fe plaque of rice roots has been previously reported.[3, 6, 11, 21, 41-43] These findings indicate that as As is methylated, it has a lower affinity for sorption onto Fe plaque compared to inorganic As species, and is thus more plant available.[44]

Our data show that the type of Fe mineral and the amount of DCB-extractable Si were important factors that govern shoot As concentrations, whereas the role of total DCB-extractable Fe or As was less clear (Figs. 5-6). A higher proportion of γ-FeOOH in plaque and lower proportion of $Fe(OH)_3 \cdot nH_2O$ in plaque increases accumulation of As in rice shoots. As solution-phase Si increases, both the amount of $Fe(OH)_3 \cdot nH_2O$ and the ability of Si to precipitate and encrust As-bearing $Fe(OH)_3 \cdot nH_2O$ and decrease inorganic grain As increases (Table 1; Figure 6).[7] This Si coprecipitation with As-bearing $Fe(OH)_3 \cdot nH_2O$, and more $Fe(OH)_3 \cdot nH_2O$ in plaque, renders As less mobile and thus less plant available. These mechanisms thus seem likely for explaining the decrease in shoot As as solution-phase Si increases (Fig. 6).[27]

Implications. The technique reported here was effective at concentrating As-bearing Fe plaque from a root systems for subsequent mineral characterization synchrotron X-ray characterization with improved signal to noise ratios. When coupled to traditional DCB extraction and elemental analysis, the proportion of Fe phases that comprise plaque and how they change with solution chemistry can be evaluated free from heterogeneity between individual "hot spots" of plant roots. This technique can be applied to any aquatic plant that forms Fe plaque. Our data indicate that strategies that promote more Fe and As in plaque may still transfer As to shoots, thus potentially leading to higher levels of As in shoots and grain. However, strategies that promote more Si in plaque decrease both shoot As and grain As. Incorporation of Si-rich rice husk residue to flooded soils increases pore-water Si, promotes more $Fe(OH)_3{\cdot}nH_2O$ formation, likely leads to coprecipitation of opaline Si and As-bearing $Fe(OH)_3{\cdot}nH_2O$, more As methylation, and less methylated As retention onto Fe plaque, which all play a role in decreasing shoot As and inorganic As in grain. However, cultivar differences, especially with respect to ROL should be considered when devising management strategies aimed to lower shoot As.

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FIGURES

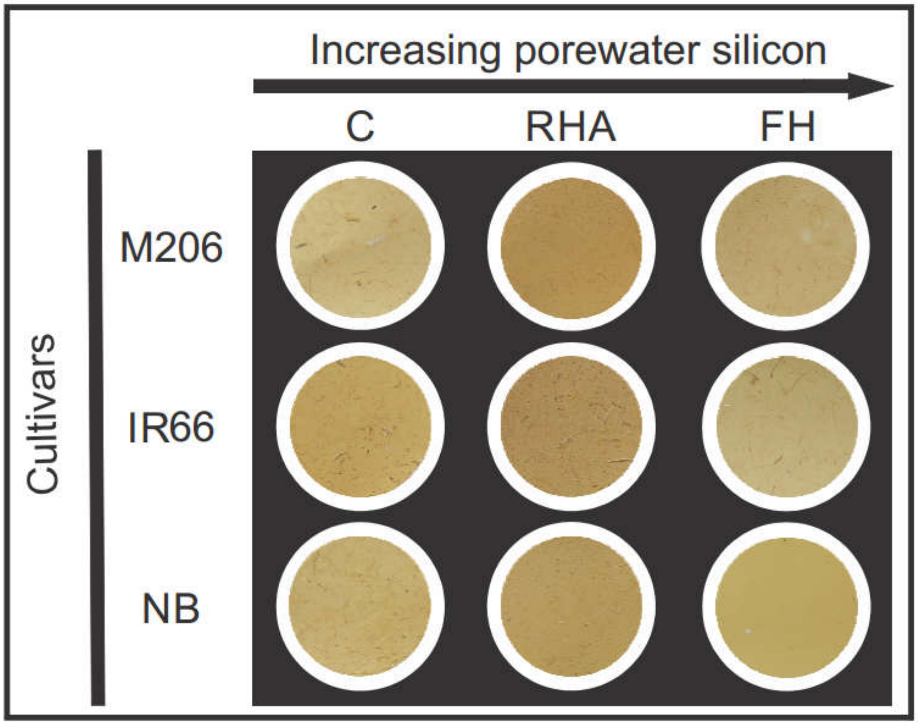


Figure 1. Fe plaque minerals sampled from rice root systems of M206, IR66, or Nipponbare (NB) cultivars grown in well-weathered soil and amended with either fresh rice husk (FH, ~500 µM Si), rice husk ash (RHA, ~ 200 µM Si) (1% w/w) or a nonamended control (C, ~ 70 µM Si). Fe minerals were deposited onto 47 mm nitro cellulosic filter membranes after sonication.

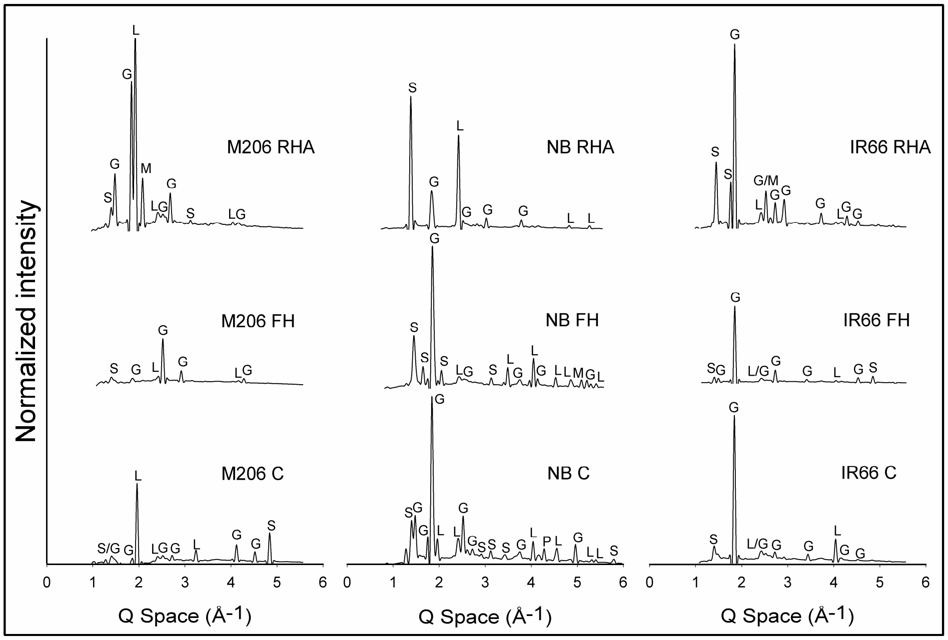


Figure 2. Integrated XRD patterns in Q space at an X-ray photon energy of 12724 eV (λ = 0.9744 Å) of intact Fe plaque sampled from rice roots of M206, IR66, or Nipponbare (NB) cultivars grown in well-weathered soil and amended with either fresh rice husk (FH, ~500 μM Si), rice husk ash (RHA, ~ 200 μM Si) (1% w/w) or a nonamended control (C, ~ 70 μM Si). Letters above peaks indicate Fe minerals: L = lepidocrocite, G = goethite, S = scorodite, M = magnetite, P = pyrite.

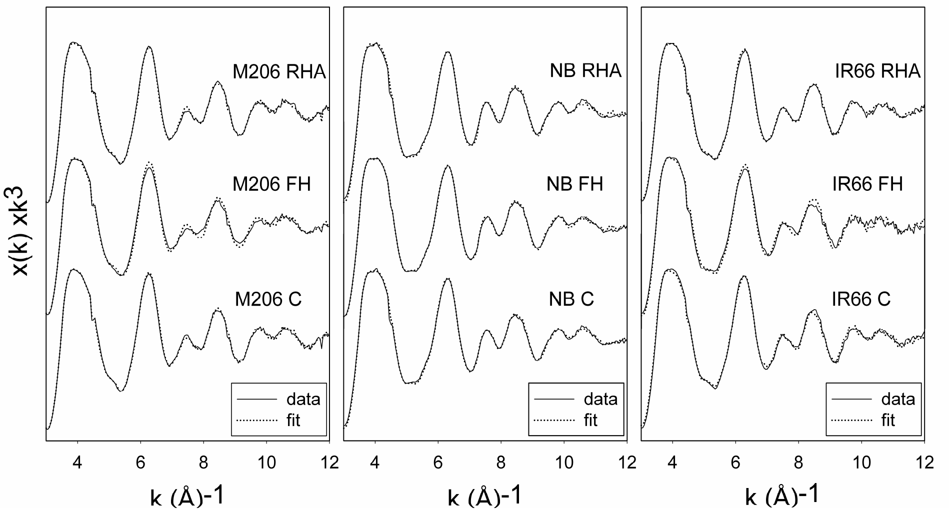


Figure 3. Linear combination fits of k^3-weighted Fe-EXAFS spectra of intact Fe plaque sampled from rice roots of M206, IR66, or Nipponbare (NB) cultivars grown in well-weathered soil and amended with either fresh rice husk (FH, high Si), rice husk ash (RHA, medium Si) (1% w/w) or a nonamended control (C, Low Si).

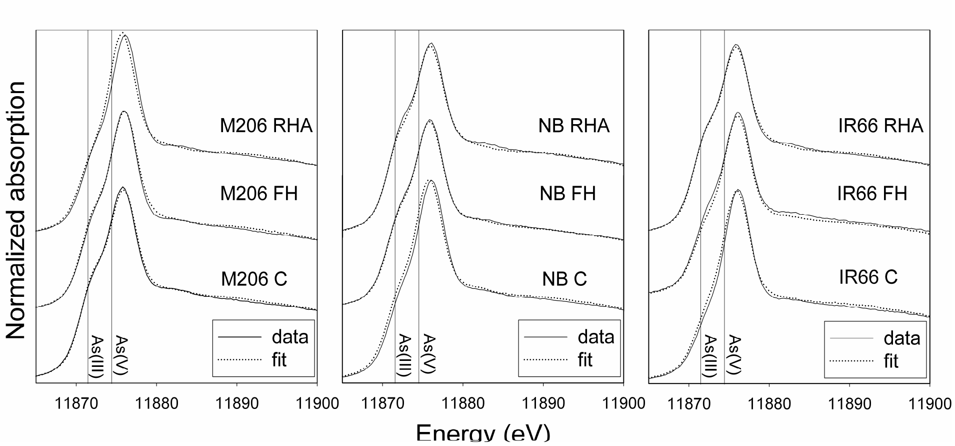


Figure 4. Normalized As K-edge XANES spectra of Fe plaques from rice roots of M206, Nipponbare (NB), and IR66 cultivars grown in soil amended with fresh rice husk (FH) or rice husk ash (RHA) (1% w/w) compared to those grown in a nonamended control soil (C).

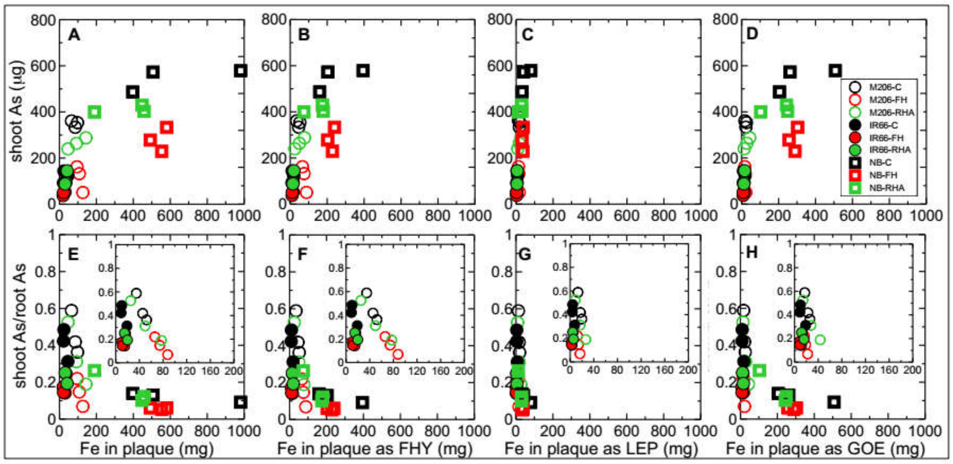


Figure 5. Relationships between shoot As (A-D) or shoot/root As (E-H) and DCB-extractable Fe in plaque as total Fe (A, E), ferrihydrite-Fe (B, F), goethite-Fe (C, G) or lepidocrocite-Fe (D, H) from M206, IR66, or Nipponbare (NB) rice cultivars grown in well-weathered soil and amended with either fresh rice husk (FH) or rice husk ash (RHA) compared to a nonamended control soil. Insets in E-H are zoomed in versions without Nipponbare to make M206 and IR66 more evident.

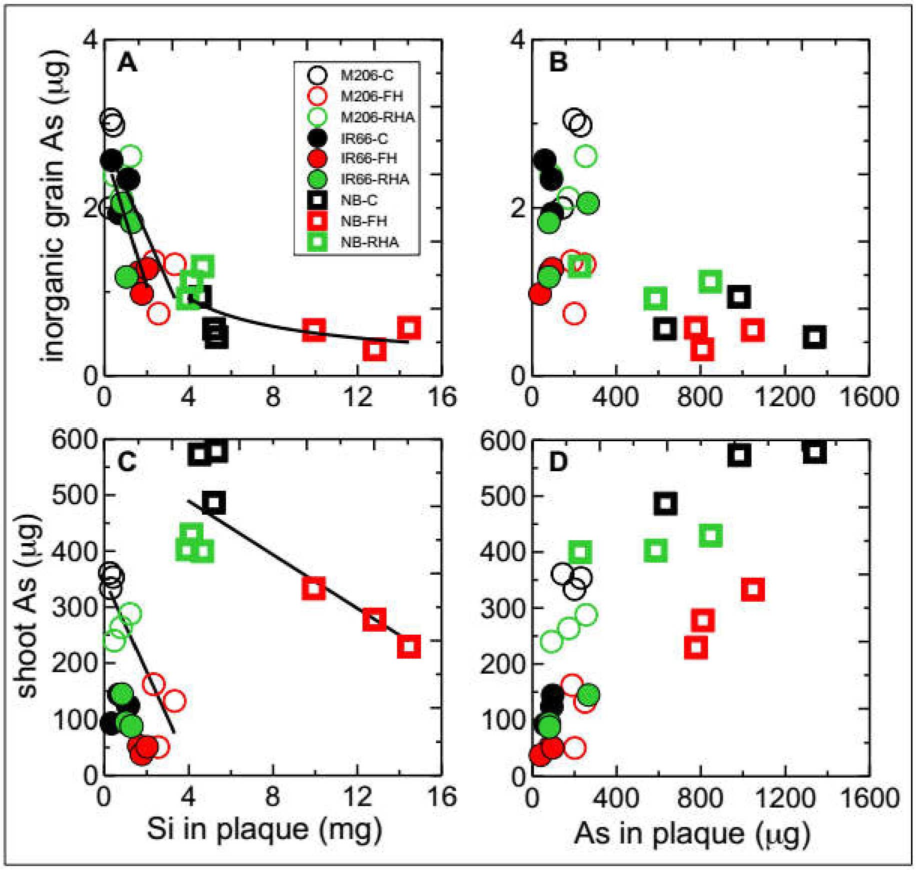


Figure 6. Relationships between inorganic grain As and plaque Si (A) or As (B) and shoot As and plaque Si (C) or As (D) from M206, IR66, or Nipponbare (NB) rice cultivars grown in well-weathered soil and amended with either fresh rice husk (FH) or rice husk ash (RHA) compared to a nonamended control soil. A significant power regression was found between inorganic grain As and plaque Si for Nipponbare (r^2 = 0.41, p = 0.031). Significant linear regressions were found between inorganic grain As and plaque Si for M206 (r^2 = 0.672, p = 0.007) and IR66 (r^2 = 0.608, p = 0.013), and for shoot As and plaque Si for M206 (r^2 = 0.776, p = 0.002), IR66 (r^2 = 0.57, p = 0.019), and Nipponbare (r^2 = 0.648, P = 0.009).

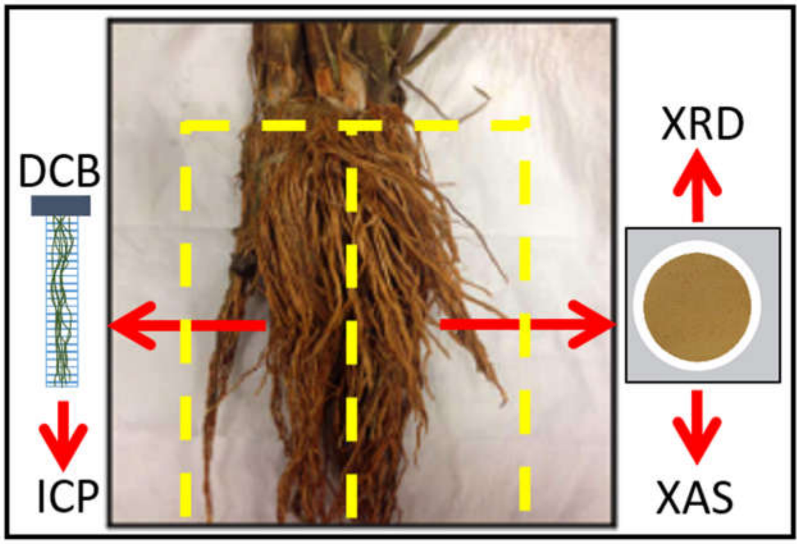
TABLES

Table 1. Linear combination fitting results of first-shell Fe EXAFS spectra of sonicated and deposited Fe plaque from rice roots of M206, Nipponbare (NB), and IR66 cultivars grown in soil amended with fresh rice husk (FH, ~500 µM Si), rice husk ash (RHA, ~ 200 µM Si) (1% w/w) or a nonamended control (C, ~ 70 µM Si).

Treatments	Percent of fitted species			R value
	$Fe(OH)_3 \cdot nH2O$	γ-FeOOH	α-FeOOH	
M206 C	53	20	27	0.024
M206 FH	69	13	18	0.035
M206 RHA	54	18	30	0.025
NB C	40	8	52	0.033
NB FH	41	7	52	0.034
NB RHA	39	7	54	0.033
IR66 C	42	17	41	0.031
IR66 FH	50	10	40	0.040
IR66 RHA	47	14	40	0.028

Table 2. Linear combination fitting of normalized As K-edge XANES spectra of sonicated and deposited Fe plaque from rice roots of M206, Nipponbare (NB), and IR66 cultivars grown in soil amended with fresh rice husk (FH, ~500 μM Si), rice husk ash (RHA, ~ 200 μM Si) (1% w/w) or a nonamended control (C, ~ 70 μM Si).

Treatments	Percent of fitted species		R value
	As(V)	As(III)	
M206 C	60	40	0.006
M206 FH	66	34	0.008
M206 RHA	71	30	0.015
NB C	69	31	0.016
NB FH	62	38	0.005
NB RHA	61	39	0.008
IR66 C	80	20	0.015
IR66 FH	73	27	0.014
IR66 RHA	60	40	0.007



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Article 2: *Impacts of Si-rich rice residues on grain As and Cd under flooded and non-flooded conditions*

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Impacts of Si-rich rice residues on grain As and Cd under flooded and non-flooded conditions

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ABSTRACT

Rice is an important staple food and may be negatively impacted by As and/or Cd in grain. While water management aimed to increase the soil Eh may decrease grain As, it may increase grain Cd. Strategies such as increasing Si through soil amendment of Si-rich rice residue have proven effective at decreasing inorganic without affecting Cd levels in grain under flooded soil conditions. However, the impacts of these amendments on Cd and As uptake by rice under different water managements have not been reported. We investigated the effects of different water management strategies on As and Cd uptake by rice in well-weathered soil amended with fresh rice husk (FH) and rice husk ash (RHA) to assess their effects on As and Cd accumulation in rice grain. Our results show that FH and RHA decreased grain As under both flooded and non-flooded conditions. Under flooded conditions where grain As and yield is higher, FH additionally decreased inorganic grain As. Under non-flooded conditions where grain Cd is highest and yield is lower, RHA decreased grain Cd. These data illustrate that a combined approach of manipulating both Eh and pore water Si may alleviate Cd and As in grain.

INTRODUCTION

Rice is the most widely consumed staple food for 50% of the world's population[1, 2] and is a potential source of arsenic (As) and cadmium (Cd)[3] worldwide.[4-6] There is a growing important health issue underlying the transfer of trace-elements such as Cd and As into rice grains[7-11] in the soil-water-rice nexus.[12-14] Arsenic may be present in soil in different chemical forms, including inorganic As (As(III) and As(V)) and methylated As species (monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Under flooded rice cultivation, inorganic As(III) is the predominant form found in soils and is taken up by plants mediated by Si transporters.[15, 16] However, the availability of As in soil depends on pH and redox potential (Eh).[17] Under different rice management systems, flooding and drainage cycles have a major impact on As dynamics in soil and its bioavailability to plants.[3, 18, 19] In paddy fields under anaerobic conditions, As is released through reductive dissolution of Al and Fe (oxyhydr)oxides into the aqueous phase and the reduction of As(V) to the more soil-mobile As(III).[20] When oxidized conditions develop such as during drainage periods, As(V) is strongly sorbed to Fe and Al (oxyhydr)oxides and is less mobile in soil.[21] Reductive dissolution of Fe (oxyhydr)oxides and reduction of As into soil solution is promoted under decrease of Eh below +100 mV, which is considered to be the point where As(V) reduces to As(III) at neutral pH.[22] Maintaining Eh above this value (e.g., through mid-season drainage) renders As less mobile and thus less plant available, but also increases Cd availability.

Cadmium availability and solubility in soil also depend on soil pH and Eh variations.[3] When Cd is present in soils under flooded conditions and low Eh, plant-available Cd is decreased due to sulfide formation (CdS).[23, 24] However, under typical non-flooded conditions or after drainage of paddy fields the predominant form of Cd taken up by rice plants is available through oxidation of CdS to Cd^{2+} and SO_4^{2-}.[25] It has been clearly shown that increasing soil Eh decreases As plant-availability but increases Cd availability and accumulation in grain.[3, 18] Therefore, water management has important impact on soil Cd and As speciation, availability, and absorption by rice plants as well their accumulation in rice grains.

Studies have shown it may be possible to control Cd and As absorption by rice only through careful water management.[3, 18, 26] For example, alternate wetting and drying (AWD) of rice paddies, in which fields are drained and re-flooded one or more times during the growing season, has been shown to decrease total grain As.[27] Honma et al.[3] show that maintaining soil Eh to -100 mV will keep As and Cd to minimum values. However, according to LaHue et al.[28] there are obstacles to implement this management practice in rice paddies due to lack of information on AWD management at large scales and the risk of yield loss with improper management. For example, rice yield is highest when soils are flooded, despite higher As availability. Therefore, it may be difficult to implement careful redox control at the field scale, particularly in developing countries, due to limited technology and resources, and also due to unpredictable field conditions (e.g., water availability or limited drainage conditions). Thus, it is imperative to develop practical management practices that can decrease both Cd and As in grain without compromising yield.

One promising strategy to combat As in rice is through Si addition to soil, but its simultaneous impacts on Cd has not been thoroughly investigated. Li et al.[19] showed that Si fertilization not only decreased As accumulation in rice shoots and grain but also benefited on rice yield under flooded condition. Recently, soil incorporation of Si-rich rice husk residues (e.g. fresh husk and rice husk ash) has been investigated as a means to improve plant-available Si without some of the negative consequences associated with straw residues (i.e., higher As and CH_4).[29-31] Si-rich rice husk was shown to decrease inorganic As in grain without negatively affecting grain Cd or rice yield under flooded conditions.[31] However, the effects of Si-rich rice amendments incorporation to soil on Cd and As uptake by rice under non-flooded conditions and how they compare to flooded conditions has not been reported. Here, we investigated the effects of fresh rice husk and rice husk ash on As and Cd uptake by rice under flooded and non-flooded conditions. We found that incorporation of fresh rice husk (FH) and rice husk ash (RHA) decreased grain As under flooded and non-flooded conditions. Under flooded conditions where grain As and yield was higher, fresh husk incorporation additionally decreased inorganic grain As. Under non-flooded conditions where grain Cd is highest and yield was lower, RHA decreased grain Cd. These data illustrate that a combined approach of manipulating both Eh and pore water Si may alleviate Cd and As in grain depending on local conditions.

MATERIALS AND METHODS

Soil characterization

A well-weathered soil depleted in plant-available Si was collected from University of Delaware Farm (UD Farm) from 0-20 cm soil depth, after removal of the organic horizon. The soil is classified as a fine-loamy, mixed, semiactive, mesic Typic Hapludult in the Elsinboro series by the U.S. taxonomic system or as an Acrisol according to the Food and Agriculture Organization system. This soil was chosen due to its similar characteristics to well-weathered soils (available Si) worldwide used to produce rice under different managements. Soil collected from several spots on the field was combined into one composite sample and hand-mixed, trying to preserve soil structure as much as possible to be utilized in pot experiments. A detailed explanation of the soil characterization was previously published (Penido et al.[30]) and is briefly given here: pH (1:1) 6.2, the acetic acid-extractable Si was 13 mg kg^{-1}, and the total As (acid-digestible) was 16 mg kg^{-1}. Total Cd (acid-digestible) was < 1 mg kg^{-1}. The site had been used as an orchard prior to 1900's and the elevated As concentration may have been due to the use of As-based herbicides.

Experimental design

To evaluate the effects of Si-rich rice residues on As and Cd uptake by rice, plants were grown in soil incorporated with two Si-rich husk amendments under two different water managements, flooded and non-flooded. The results were compared to non-amended control as well under flooded and non-flooded conditions. Each 4 L pot received 3.5 kg of air-dried soil and based on previous work,[30] powdered fresh rice husk (FH) and rice husk ash (RHA) were added at a rate of 1% (w/w) as Si amendment for each treatment. After hand-mixing each amendment into soil, half of the pots were flooded to 4 cm above the soil surface and another half of the pots were kept under field capacity (40-45%) using deionized water. Rhizon samplers (Soil Moisture Corp., Goleta, CA, USA) were added to each pot in order to collect pore-water for subsequent analysis. Each treatment as well non-amended control was conducted in triplicate.

Plant growth

Sterilized rice seeds (*Oryza sativa* L., cv. IR66) were soaked in deionized water for 24 hours and then germinated in containers filled with non-amended UD Farm soil. The ten-day-old seedlings were transplanted into each treatment pot and transferred to a controlled environment chamber at relative temperature and humidity of 28°C and 70%, respectively. Pots were watered with reverse-osmosis deionized water that did not contain ICP-MS-detectable As, Cd or Si, and no exogenous As or Cd were added. Trace-element availability to rice roots for uptake resulted from their behavior in soil under different water management (i.e., Eh) to simulate conditions where trace-element-contaminated soils are due to anthropogenic activities (e.g. fertilization, pest management) or naturally contaminated.

Pore-water analysis

To understand the impacts of Si-rich amendments on pore-water chemistry under flooded and non-flooded conditions, pore-water was collected and analyzed every two weeks starting at seedling transplantation and continuing to plant harvest. Using previously published methods,[32] pore-water was collected into acid-washed and crimp-sealed vials using Rhizon samplers for pH, redox potential (Eh), and total elemental analysis including As and Cd using ICP-MS, and other plant nutrients using ICP-OES.

Post-harvest plant analysis

At plant maturity, plant parts were collected, dried, weighed, and prepared for elemental analysis. Roots were treated based on previous work[33] to quantify total elements and to characterize Fe-plaque mineralogy. Briefly, roots were removed from the soil, washed with deionized water, air dried, and divided longitudinally: one half was utilized for DCB extraction and total elemental analysis for total As, Cd, Fe, and Si using ICP-OES and the other half was utilized for intact Fe plaque sampling via sonication and characterization with bulk XRD and XAS.[33] DCB-extracted and plaque-free roots, straw, husk, and grain were finely-ground and underwent microwave digestion and elemental analysis using previous methods.[32] Standard reference materials (SRM) rice flour (1568b) and rye grass (ERM-CD281) were used to certificate the results and gave excellent recoveries for As (92% ± 4) and Cd (101% ± 6), respectively. Remaining Si-gel residues from digested roots, straw, and husk were dissolved in 2M NaOH using a modified method from Derry et al.[34] and quantified for Si using a colorimetric method from Kraska and Breitenbeck[35] per previous work.[31]

To quantify inorganic As (As(V) and As(III)) and organic As (MMA and DMA) species in grain, powdered rice grains were extracted in 2% TMG HNO_3 at 100° C and analyzed with HPLC-ICP-MS using a PEEK PRP-X100 anion exchange column using the methods of Maher et al.[36] SRM 1568b rice flour gave good recoveries for inorganic As (As_i) (106%), and DMA (104%).

Fe plaque characterization

A full description of the methodology to characterize plaque-associated Fe minerals and As speciation can be found in Amaral et al.[33] Briefly, the half root systems with intact Fe plaque were subject to gentle sonication to obtain samples of As-bearing Fe plaque minerals. Fe plaque bearing roots were placed into 400-mL glass beakers partially filled with deionized water, and the beakers were placed into a gentle sonicating bath for approximately 2 hours at 25°C. After sonication, the Fe plaques were collected onto a nitrocellulose filter that was placed into a loosely-capped petri dish to air-dry. The filters with deposited Fe plaques were transported to Stanford Synchrotron Radiation Lightsource (SSRL) for analyses.

The Fe plaque deposited on the nitrocellulose filter was characterized with bulk X-Ray Diffraction (XRD) at SSRL on beamline 11-3. The obtained data were analyzed using Match! Software (v3, Crystal Impact, 2015) and the results were used to restrict the Linear Combination Fitting (LCF) for X-ray absorption spectroscopy. To quantify the mineral composition of Fe plaques and the oxidation state of As in the Fe plaques, the filtered Fe plaques were characterized with extended X-ray absorption fine structure (EXAFS) spectroscopy and by X-ray absorption near edge structure (XANES) at SSRL on beamline 11-2 at SSRL, respectively. The obtained spectra were background subtracted, normalized, and fitted by linear combination fitting (LCF) using

Athena software. The following materials were included as standards: 2-line ferrihydrite ($Fe(OH)_3 \cdot nH_2O$), lepidocrocite (γ-FeOOH), hematite (Fe_2O_3), siderite ($FeCO_3$), and goethite (α-FeOOH) as Fe standards; As(III) as $NaAsO_2$, and As(V) as Na_3AsO_4 as As standards.

Statistical analyses

Statistical analyses were performed using SigmaPlot (Scientific Data Analysis and Graphing Software). If significant differences ($P < 0.05$) were identified between treatments, multiple comparisons were made by the Tukey test.

RESULTS AND DISCUSION

Effects of Si amendments and water management on grain As and Cd

Water management and soil incorporation of Si-rich rice residues affected both As and Cd in rice grain (Figure 1). Si-rich rice residues decreased total grain As by 20-45% in both water managements, and inorganic grain As by 30-50% under anaerobic condition (Figure 1A). Prior work studying the effect of Si-rich rice amendments in As uptake in different rice cultivars showed that Si-rich amendments decreased total grain As, and FH reduced inorganic grain As by 25-50%,[31] confirming our findings. In contrast, under non-flooded condition RHA decreased total grain Cd by 45% (Figure 1B). Suksabye et al.[37] showed that soil incorporation of 1% RHA (w/w) in Cd-contaminated soil significantly decreased grain Cd in rice. Our results corroborate these findings indicating that FH and RHA may play an important role in decreasing inorganic grain As or Cd and may be a feasible strategy to decrease heavy metals accumulation in rice under different water managements.

In flooded experiments, inorganic As values in unpolished rice ranged from 0.21 to 0.28 mg kg^{-1}, 0.30 to 0.39 mg kg^{-1}, and 0.41 to 0.47 mg kg^{-1} for FH, RHA, and non-amended control, respectively (Figure 2). In non-flooded experiments, As values ranged from 0.01 to 0.15 mg kg^{-1} (Figure 2). The decreased plant As observed in the amended treatments is due to soil incorporation of Si-rich rice amendments. Rice husk residues enhance dissolved Si in the soil solution, which limits As uptake by increasing Si competition with As(III),[31] which is taken up by rice roots through mainly the Si uptake pathway.[15] Therefore, our results suggest that total grain As considerably decreased due to a noticeable increase of dissolved Si as a result of soil incorporation of Si-rich rice residues when compared to non-amended control.

Under non-flooded treatments we observed grain Cd levels of 0.13 to 0.16 mg kg^{-1} for RHA, 0.19 to 0.39 mg kg^{-1} for FH, and 0.23 to 0.29 mg kg^{-1} for non-amended control, and flooded conditions soil Eh levels of Cd were nearly non-detectable and no statistical difference were observed between treatments (Figure 2). In anaerobic condition Cd is mostly present as CdS, and during soil re-oxidation CdS is readily transformed into more soluble species.[39] In contrast, RHA decreased grain Cd compared to non-amended control in non-flooded experiments. Two main mechanisms may explain the decreased plant Cd due to RHA amendment. We suggest that (1) soil incorporation of RHA may have decreased grain Cd levels by increasing adsorption and immobilization of Cd^{2+} onto the RHA; and (2) increasing soil pH may have increased cation-exchange capacity (CEC) of RHA, enhancing Cd^{2+} retention. The RHA residue utilized was obtained as a by-product from a rice mill in Cambodia. In this process, oxygen is utilized and the combustion temperature is typically 400°C or less leading to an incomplete combustion of rice husks. Even though produced under different conditions, which distinguish RHA from biochar, RHA may present considerable CEC and surface area.[37] It is also noteworthy that CEC of chars is strongly pH-dependent.[40-42] Silber et al. showed that CEC of corn straw biochar was strongly pH-dependent, increasing up to 115 $mmol_c\ kg^{-1}$ for every unit increase of pH.[43] Thus, RHA may play an important role in decreasing grain Cd in rice under non-flooded condition by increasing Cd retention through increase of soil pH. It is known that Cd may be a major health problem,[44-46] which can appreciably accumulate in rice grains even at low soil concentrations.[47] It is therefore imperative to decrease Cd ingestion through rice consumption and RHA may be a promising strategy for decreasing its risk to humans via decreasing Cd in the rice food chain.

Impacts of Si and water management on Fe-plaque formation

The k^3-weighed EXAFS spectra and LCF of the Fe plaque samples revealed significant differences in dominant Fe (hydr)oxides (i.e. $Fe(OH)_3 \cdot nH2O$, α-FeOOH and γ-FeOOH) due to soil incorporation of Si amendment (Figure S2 and Table S1). The results show that increasing Si in the pore-water led to a higher percentage of $Fe(OH)_3 \cdot nH2O$, corroborating our findings from prior work.[33] FH amendment, which resulted in a higher level of dissolved Si, increased $Fe(OH)_3 \cdot nH2O$ under both water managements relative to non-amended control by ~38%. RHA also increased $Fe(OH)_3 \cdot nH2O$ amounts under flooded condition, but no significant difference was observed under non-flooded condition. A higher proportion of $Fe(OH)_3 \cdot nH2O$ in plaque appears to decrease shoot As due to the ability of Si to coprecipitate with As-bearing $Fe(OH)_3 \cdot nH2O$ leading to less mobile and plat available As form.[33] Thus, strategies that promote more $Fe(OH)_3 \cdot nH2O$ formation in plaque are at least partially responsible for decreasing total grain As in rice plants.

The DCB-extractable Cd was under the detection limit, whereas Si, Fe or As was affected by increasing dissolved Si in the pore-water (Figure S1). FH amendment led to the highest average pore-water Si under flooded (~400 μM) and non-flooded (~300 μM) condition and increased Si in plaque, whereas decreased As and Fe in plaque compared to non-amended control under flooded condition. RHA and non-amended control led to highest Fe and As, and lower Si in plaque. Our data corroborate previous findings, which shows that increasing dissolved Si in the soil solution[31] and the type of Fe mineral and the amount of DCB-extractable Si[33] are important factors that govern As concentration in rice shoots. Therefore, our data indicate that management practices that promote more Si in plaque and in the pore-water play an important role in decreasing total grain As.

The linear combination fits (LCF) of the As XANES spectra did not show distinct differences in the oxidation state of As due to amendment, only due to water management (Figure S3 and Table S2). Under both flooded and non-flooded conditions, As sorbed on plaque was mostly in the As(V) oxidation state with lesser amounts of As(III). In aerobic condition the As(V) values ranged from 80-85% with 15-20% As(III) present. Under anaerobic conditions the values ranged from 55-65% and 35-45% for As(V) and As(III), respectively. Our findings are in accordance with previous works that showed those are the most predominant forms of As mainly encountered in rice roots in most paddy fields.[48-51]

Soil Eh, pH, and dissolved As, Cd, Fe, and Si

Both Si and water management affected soil pH under non-flooded and flooded condition, which varied from 4.9 to 6.3, and from 6.2 to 6.9, respectively (Figure 3D). In general, soil incorporation of Si-rich amendments increased soil pH values under both flooded (up to 0.5 units) and non-flooded (up to 1 unit) treatments compared to non-amended control. The application of different Si-rich materials has shown to increase soil pH, which can decrease the phytoavailability of heavy metals (e.g. Cd, Pb) for plant roots to uptake.[52-54] The amendment-induced increased soil pH we observed is noteworthy because heavy metals availability is decreased under higher pH. Thus, Si-rich amendments may attenuate plant toxicity under heavy metals stress (e.g. Cd stress), particularly by increasing soil pH[55, 56] under aerobic conditions. Rice residues did not have any effect in soil Eh and only water management caused different responses of soil Eh, which ranged from 0 to 100 mV under flooded condition, and from 300 to 400 mV under non-flooded condition (Figure S4).

Dissolved As and Fe concentrations quickly increased with the decrease in soil Eh, and the concentrations were lower when the soil Eh was above 300 mV (Figures 3A and 3C). Incorporation of Si-rich amendments slightly affected dissolved As and Fe under anaerobic condition. Even though Cd has had significant effects in the plant, the dissolved Cd was under the detection limit (<10 nM) for both water managements. Prior work investigating soil Eh, pH, and water management showed that water management strongly affected dissolved As, Cd, and Fe concentrations in soil trough changes in Eh,[3] which corroborates our findings. In contrast, Si-rich amendments considerably increased pore-water Si under both flood and non-flooded treatments (Figure

3B). The highest dissolved Si was 2 to 4 times higher in amended treatments than those non-amended control treatments. FH increased dissolved Si up to 600 μM under flooded condition and up to 300 μM under non-flooded condition, and RHA increased dissolved Si up to 300 μM and 200 μM under flooded and non-flooded condition, respectively. The non-amended control had dissolved Si up to 150 μM under anaerobic conditions and up to 100 μM under aerobic condition but, in average, substantially decreased (~20 μM) within time compared to the amended treatments (~100 μM – RHA; ~250 μM – FH). The positive impacts of Si-rich residues in increasing pore-water Si were also shown in recent studies.[30, 31] The noted increase of dissolved Si in soil solution shows that FH and RHA may be two promising choices to increase pore-water Si, a beneficial nutrient for rice, with potential benefits to improve rice quality and yield.

Implications

The use of low cost and environmental safety amendments with the purpose of reducing the mobility and bioavailability of heavy metals in soil, have represented an effective, inexpensive, and environmental compatible remediation way for crop safety regulation and soil remediation. Rice residues such as fresh rice husk (FH) and rice husk ash (RHA) are underutilized Si-rich byproduct that can benefit in improving rice and human health by increasing pore-water Si and decreasing rice As and Cd.[31] In the current study, we utilized

FH and RHA as a Si-rich rice amendments under different water managements to assess their effects on As and Cd accumulation in rice grains. Our data indicate that utilizing Si-rich rice amendments is a promising strategy to decrease heavy metals contamination in rice under different water managements without negatively affecting rice yield, and grain Zn or Fe (Figure S6 and S7). Under aerobic condition RHA decreased grain Cd, whereas under anaerobic condition FH decreased grain As. Our results suggest that soil-incorporation of FH and RHA may be a promising strategy depending on the choice of amendment in view of the fact that the amendments had different behavior over different conditions. Further research is needed to evaluate the residual effects of the amendments under diverse environmental conditions as well to confirm both risks and benefits for various management strategies.

SUPPORTING INFORMATION AVAILABLE

Additional data are provided in the supporting information and includes Figure S1 with DCB-Extractable Si, As, and Fe concentrations, Figure S2 with LCF of first shell Fe EXAFS spectra, Figure S3 with LCF of As K-edge XANES spectra, Figure S4 with soil redox potential, Figure S5 with plant Cd, As, and Si concentrations, Figure S6 with grain Fe and Zn concentrations, Figure S7 with total plant biomass and rice yield, Table S1 with Fe plaque mineralogy results, and Table S2 with As speciation in Fe plaque. This material is available free of charge at http://pubs.acs.org.

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FIGURES

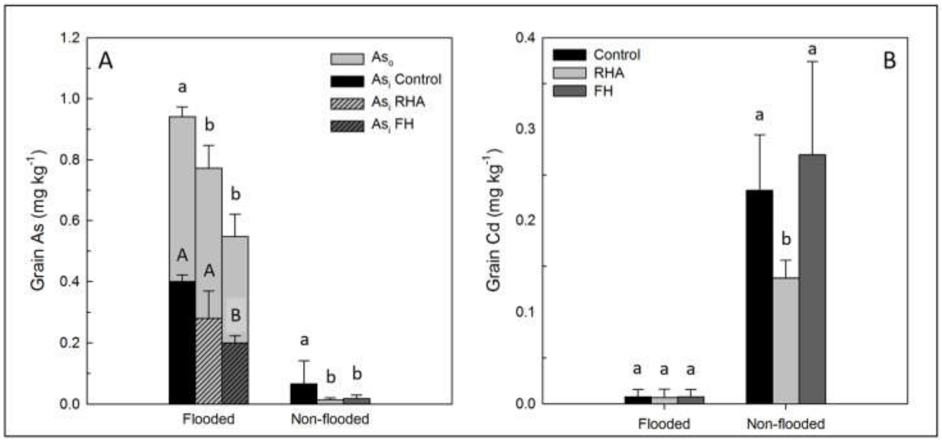

Figure 1. Total and speciated grain As (A), and grain Cd (B) concentrations in unpolished rice grain of rice (*Oryza sativa* L., cv. IR66) grown in amended soil with 1% (w/w) of fresh husk (FH) or rice husk ash (RHA) compared to non-amended control soil under flooded and non-flooded conditions. As_i = inorganic As; As_o = organic As (only DMA was detected). Different letters denote significant differences ($P < 0.05$) for total As (lower case), speciated As (upper case), or total Cd between amended and non-amended treatments. Statistical testing was performed separately for each water management.

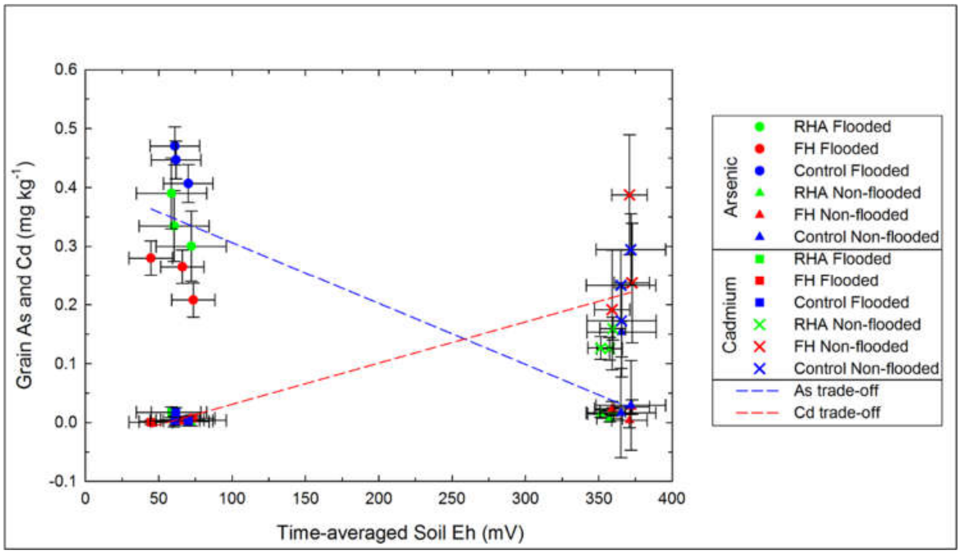

Figure 2. Relations of the trade-off value for time-averaged soil Eh versus total inorganic grain As or total grain Cd concentration of rice grown in non-amended soil or soil amended with 1% (w/w) of fresh husk (FH) or rice husk ash (RHA) under either flooded and non-flooded conditions.

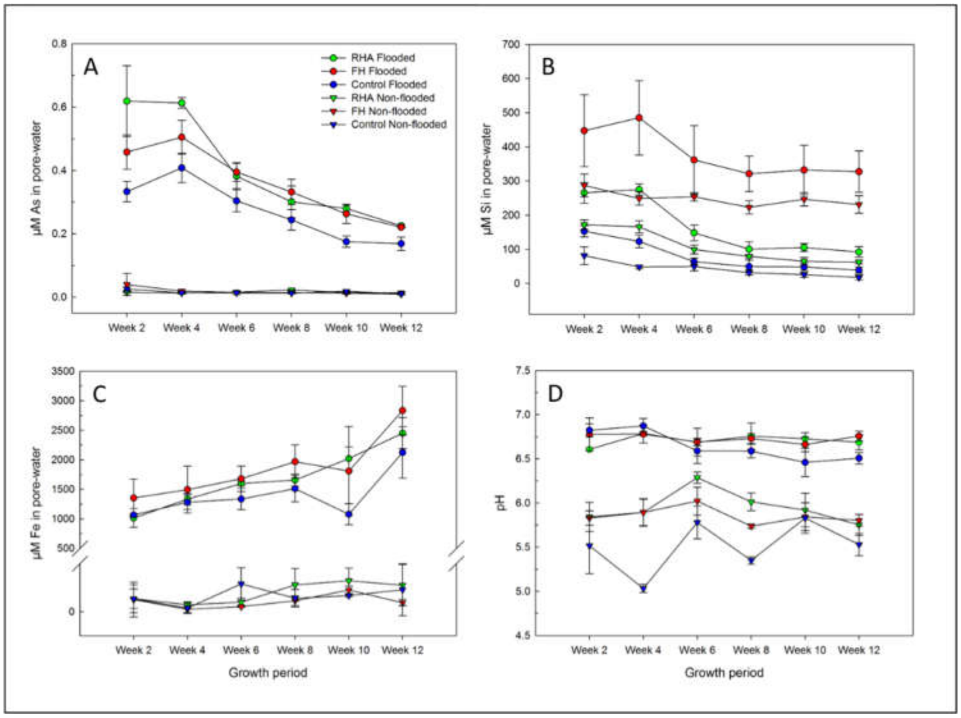

Figure 3. Porewater As (A), Si (B), Fe (C), and soil pH (D) of rice (*Oryza sativa* L., cv. IR66) grown in amended soil with 1% (w/w) of fresh husk (FH) or rice husk ash (RHA) compared to non-amended control under flooded and non-flooded conditions. Values are averages ± 95% confidence intervals, n = 3.

SUPPORTING INFORMATION

Impacts of Si-rich rice residues on grain As and Cd under flooded and non-flooded conditions

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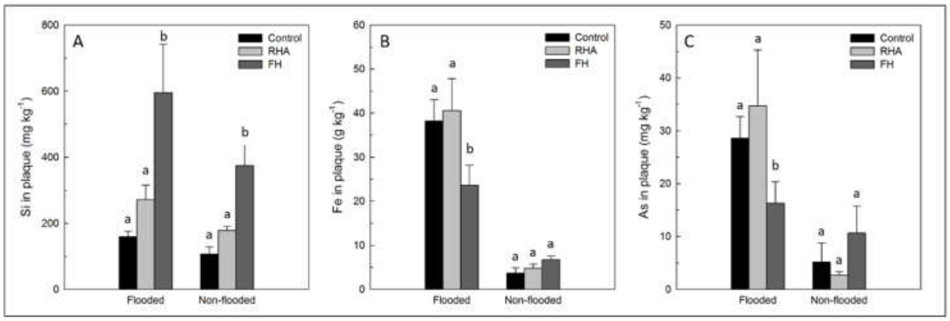

Figure S1. Average DCB-extractable Si (A), Fe (B), and As (C) sampled from root Fe plaque of rice grown in non-amended soil or amended with fresh husk (FH) or rice husk ash (RHA) under flooded and non-flooded treatments. Different letters denote significant differences ($P < 0.05$) between amended and non-amended treatments. Statistical testing was performed separately for each water management.

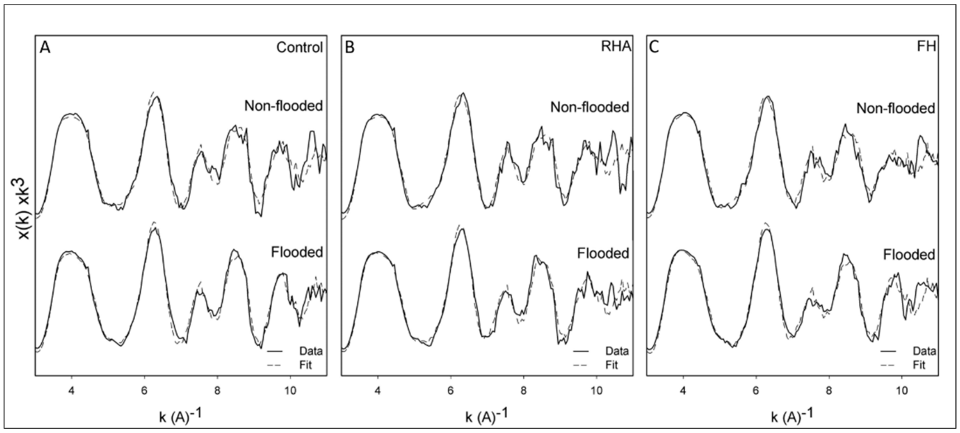

Figure S2. Linear combination fits (LCF) of k^3 weighted Fe EXAFS spectra of Fe plaque sampled from rice roots of rice grown in non-amended soil (A) or amended with rice husk ash (RHA, B) or fresh husk (FH, C) under flooded and non-flooded treatments.

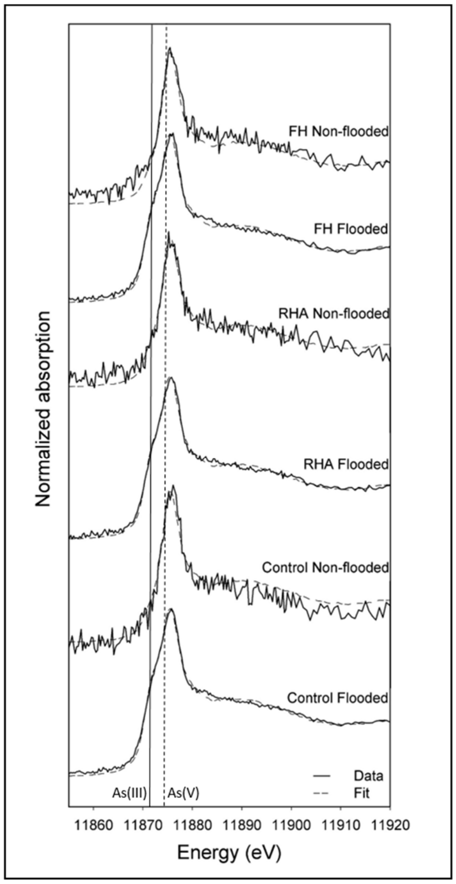

Figure S3. Linear combination fits (LCF) of normalized As K-edge XANES spectra of Fe plaques sampled from rice roots of rice grown under flooded and non-flooded treatments and amended with fresh rice husk (FH) or rice husk ash (RHA) (1% w/w) compared to non-amended control soil.

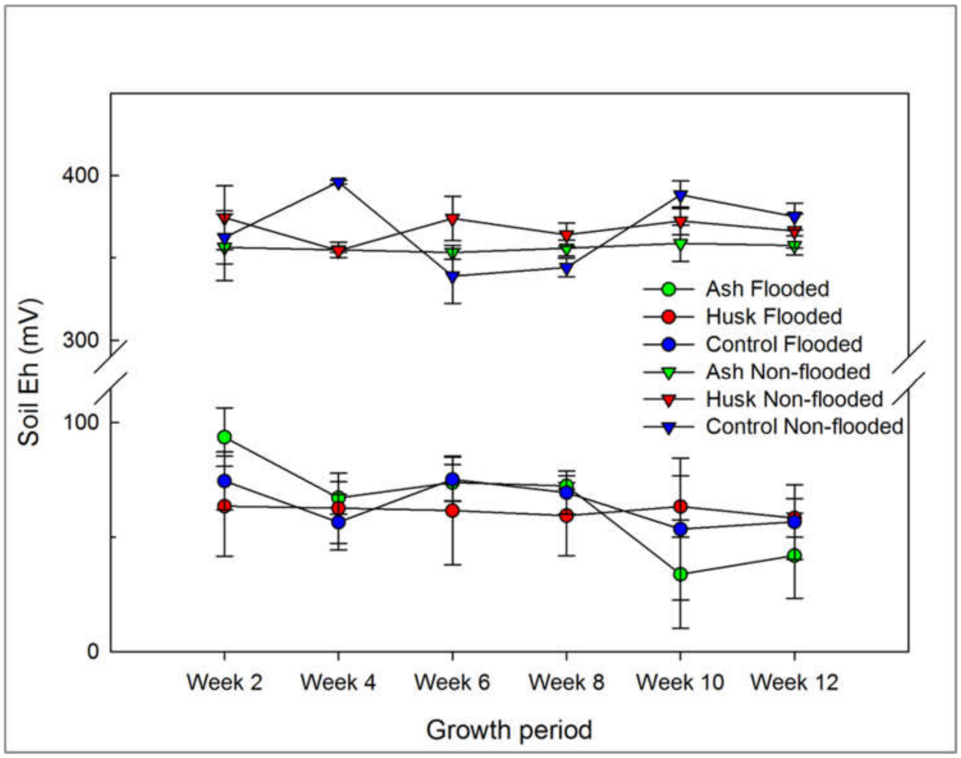

Figure S4. Average (+/- 95% confidence intervals, n = 3) soil Eh (mV) of rice grown in a well-weathered soil amended with fresh rice husk (FH) or rice husk ash (RHA) compared to a non-amended soil.

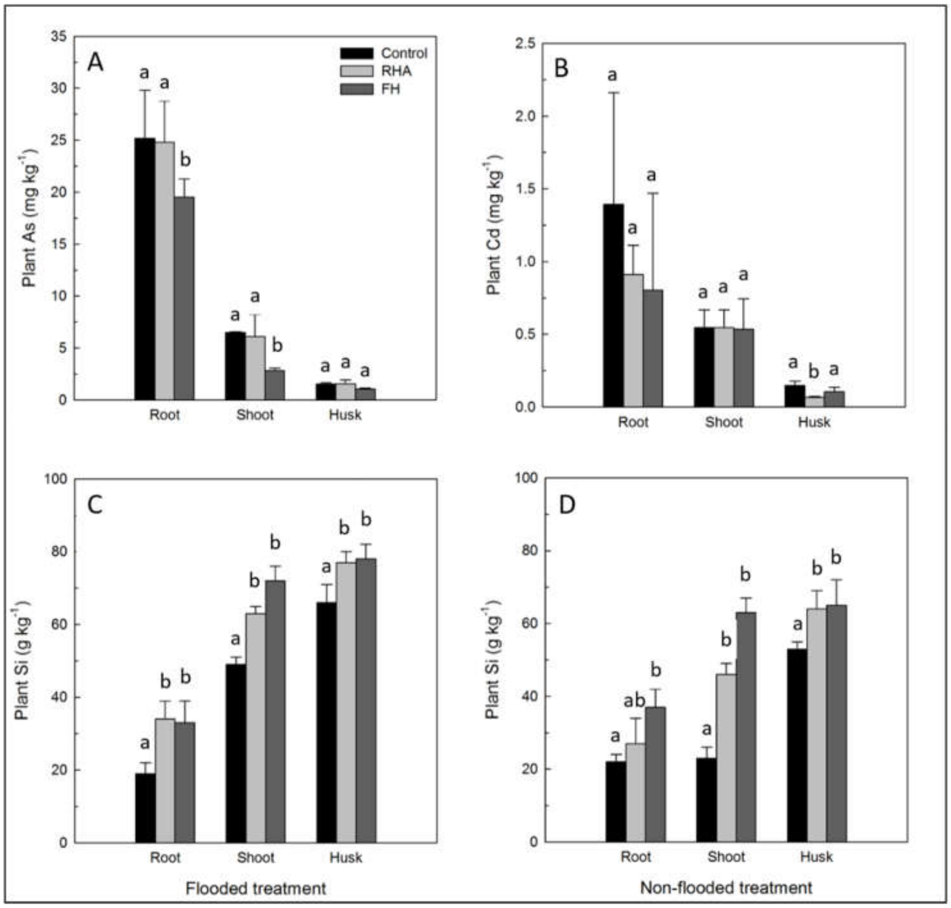

Figure S5. Concentration of As (A), Cd (B), and Si (C-D) in rice tissues for rice grown in non-amended soil or amended with fresh husk (FH) or rice husk ash (RHA) under flooded (A and C) and non-flooded (B andD) treatment. Different letters denote significant differences ($P < 0.05$) between amended and non-amended treatments. Statistical testing was performed separately for each plant organ.

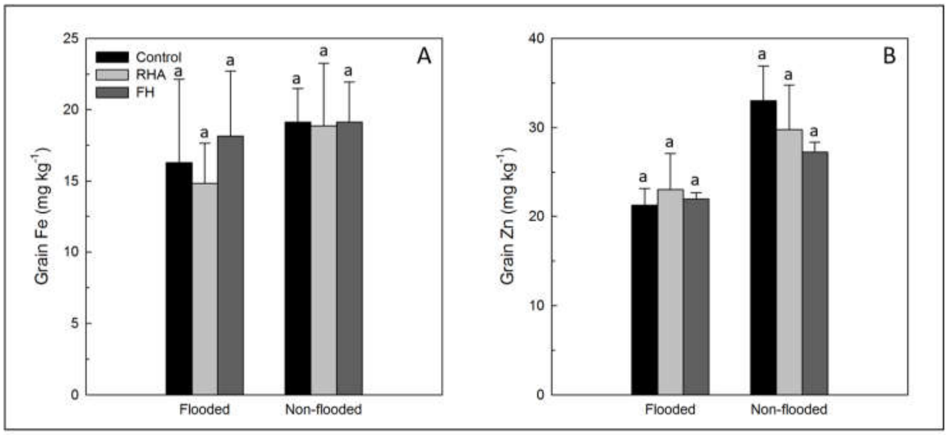

Figure S6. Grain Fe (A) and grain Zn (B) concentrations in unpolished rice grain grown in amended soil with 1% (w/w) of fresh husk (FH) or rice husk ash (RHA) compared to non-amended soil under flooded and non-flooded treatments. Different letters denote significant differences ($P < 0.05$) between amended and non-amended treatments. Statistical testing was performed separately for each water management.

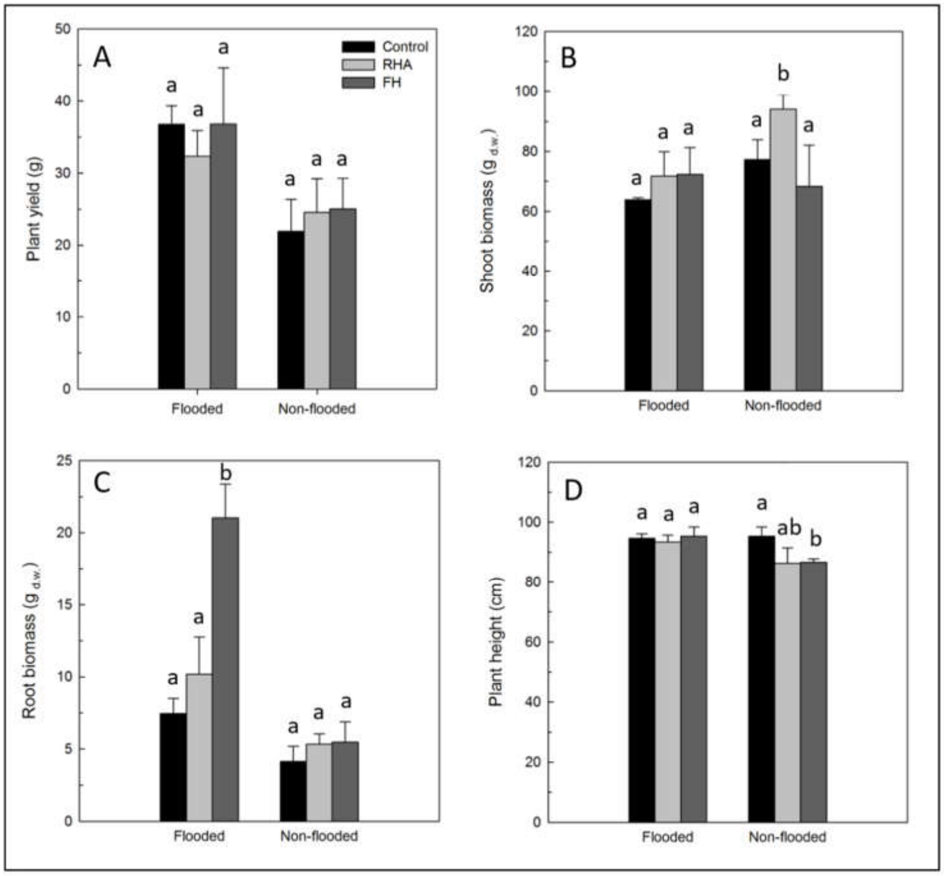

Figure S7. Average dry weight plant yield (A), shoots (B), roots (C), and height (D) of rice grown in non-amended soil or amended with fresh husk (FH) or rice husk ash (RHA) under flooded and non-flooded treatments. Different letters denote significant differences ($P < 0.05$) between amended and non-amended treatments. Statistical testing was performed separately for each water management.

TABLES

Table S1. Linear combination fitting results of first shell Fe EXAFS spectra and goodness of fits (R value) obtained on root-system Fe plaque.

Treatment	**Average percent of fitted species**					**Average R value**
	$Fe(OH)_3$·*n*H2O	**α-FeOOH**	**α-Fe_2O_3**	**γ-FeOOH**	**$FeCO_3$**	
Control (F)	31.9 ± 6.2^{a}	37.6 ± 6.8^{a}	3.5 ± 2.0^{a}	17.9 ± 2.8^{a}	5.9 ± 1.3^{a}	0.023
RHA (F)	43.9 ± 1.5^{b}	35.6 ± 1.1^{ab}	2.8 ± 1.5^{a}	14.6 ± 0.6^{a}	4.5 ± 1.2^{a}	0.026
FH (F)	49.0 ± 5.2^{b}	30.4 ± 3.5^{b}	4.1 ± 3.1^{a}	15.2 ± 2.9^{a}	3.6 ± 1.4^{a}	0.024
Control (NF)	29.7 ± 2.0^{a}	44.3 ± 2.5^{a}	5.6 ± 1.1^{a}	12.4 ± 0.5^{ab}	8.1 ± 1.1^{a}	0.036
RHA (NF)	35.8 ± 2.0^{a}	42.2 ± 0.8^{a}	4.8 ± 1.4^{a}	8.6 ± 1.2^{b}	8.5 ± 3.7^{a}	0.031
FH (NF)	47.2 ± 0.3^{b}	31.8 ± 1.8^{b}	4.2 ± 2.5^{a}	7.4 ± 1.5^{b}	9.8 ± 2.5^{a}	0.030

Values followed by the same letter are not statistically significant at the 0.05 level.

Table S2. Linear combination fitting of normalized As K-edge XANES spectra of Fe plaques from rice roots.

Treatment	**Average percent of fitted species**		**Average R value**
	As(V)	**As(III)**	
Control (F)	57	43	0.005
RHA (F)	59	41	0.006
FH (F)	61	39	0.006
Control (NF)	86	14	0.062
RHA (NF)	81	19	0.060
FH (NF)	80	20	0.073