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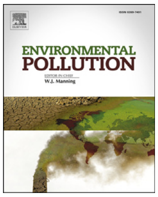

Impact of agronomic practices on arsenic accumulation and speciation in rice grain

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ABSTRACT

Rice is a major source of dietary arsenic (As). The effects of paddy water management, straw incorporation, the applications of nitrogen fertilizer or organic manure, and the additions of biochar on arsenic accumulation and speciation in rice grain were investigated under field conditions over four cropping seasons in Hunan, China. Treatments that promoted anaerobic conditions in the soil, including continuous flooding and straw incorporation, significantly increased the concentration of As, especially methylated As species, in rice grain, whereas N application rate and biochar additions had little or inconsistent effect. Continuous flooding and straw incorporation also increased the abundance of the arsenite methyltransferase gene *arsM* in the soil, potentially enhancing As methylation in the soil and the uptake of methylated As by rice plants. Intermittent flooding was an effective method to decrease As accumulation in rice grain.

1. Introduction

Consumption of rice constitutes a major exposure route of inorganic arsenic (As), a class-one carcinogen (Banerjee et al., 2013; European Food Safety Authority, 2009; Meharg et al., 2009). In the case of Chinese adult population, for example, it has been estimated that rice is the largest contributor, accounting for about 60%, to the total dietary intake of inorganic As (Li et al., 2011). Rice is inherently efficient at accumulating As due to the combination of the biogeochemical characteristics of paddy soil and the relatively high ability of rice plants to absorb and transport As (Zhao et al., 2010). Anaerobic conditions developed in flooded paddy soil is conducive to the mobilization of arsenite (As(III)) (Li et al., 2009; Marin et al., 1993; Takahashi et al., 2004; Xu et al., 2008), which is then taken up inadvertently by rice roots through the highly expressed silicon uptake pathway (Ma et al., 2008). Rice grain contains approximately ten fold higher As concentration than other cereals (Williams et al., 2007b). It is therefore important to understand factors that affect As accumulation and speciation in rice grain in order to develop strategies to minimize its transfer to the food chain.

Rice grain contains both inorganic and organic As species. Typically, As(III) and dimethylarsinic acid (DMA) are the predominant species, with arsenate (As(V)), monomethylarsonic acid (MMA) and, occasionally, tetramethylarsonium being the minor species (Meharg and Zhao, 2012). Health risk associated with As in rice depends on its chemical speciation because inorganic As (As(III) and As(V)) are much more toxic than methylated (organic) As species containing pentavalent As (reviewed by Zhao et al., 2013b). It is clear that measuring total As concentration alone is not enough to assess the risk of As in rice. To protect the population with a high amount of rice consumption, China has set a maximum contaminant level (MCL) of 0.15 mg kg^{-1} of inorganic As (Chinese Food Standards Agency, 2005). In the paddy areas impacted by mining activities, a substantial proportion of rice samples was found to exceed the MCL (Zhu et al., 2008).

There is substantial variation in As speciation among rice produced in different geographical regions and among different rice cultivars (reviewed by Zhao et al., 2013b). Rice produced in Asia generally contains a high proportion of inorganic As, whereas rice produced in the central and southern states of the US tends to have a high proportion of organic As (Meharg et al., 2009; Williams et al.,

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2005; Zavala et al., 2008; Zhu et al., 2008). The reasons for this geographical variation are not clear, but may be related to the differences in the microbial community and in the environmental conditions of the paddy soil (Zhao et al., 2013b). It was previously thought that plants were able to methylate As to produce various forms of organic As (Nissen and Benson, 1982; Wu et al., 2002). However, recent studies have cast doubt on this assumption. Plants such as rice appear to lack the ability to methylate As, but instead take up methylated As from the soil (Jia et al., 2012; Lomax et al., 2012). Variation among rice cultivars in As speciation may therefore reflect differences in the rhizosphere microbial community and/or in the uptake and translocation of methylated As species (Zhao et al., 2013b).

Many soil microorganisms are able to methylate As, a process catalysed by arsenite methyltransferases (arsM) (Qin et al., 2006; Ye et al., 2012). Soil microbes containing *arsM* genes are phylogenetically diverse (Jia et al., 2013a). It has been shown that As methylation in soil was enhanced by factors that promote the anaerobic conditions, such as soil flooding and the additions of organic matter (Jia et al., 2013b; Mestrot et al., 2009; Norton et al., 2013). Arsenic speciation in rice grain is also strongly influenced by soil flooding and additions of organic manure (Arao et al., 2009; Li et al., 2009; Norton et al., 2013; Xu et al., 2008). In a study that used a single soil, the abundance of the *arsM* gene was higher in the rhizosphere of rice roots than in the bulk soil and was also enhanced by the addition of rice straw, with a significant correlation between the *arsM* copy number and the concentration of methylated As species in the soil solution (Jia et al., 2013a). In contrast, in a study that compared multiple soils, the concentration of methylated As species in the soil solution was found to be correlated with soil conditions such as pH and the concentration of dissolved organic carbon (Zhao et al., 2013a).

Compared with water management and organic matter inputs, the potential effects of other agronomic practices on As accumulation and speciation in rice have been little studied. The form and the addition rate of nitrogen (N) have been found to influence the reductive dissolution of iron oxides/hydroxides in a paddy soil and the formation of iron plaque on rice roots in a pot experiment, thus indirectly affecting As uptake by rice (Chen et al., 2008). Whether N fertilization can be a useful tool to control As accumulation in paddy rice under field conditions remains unknown. The use of biochar to reduce the mobility and bioavailability of heavy metals in contaminated soils has attracted much attention in recent years. However, the effect of biochar addition on the mobility of As was inconsistent among different laboratory studies (Beesley and Marmiroli, 2011; Beesley et al., 2011).

Many of the above-mentioned studies were based on pot experiments under greenhouse or controlled-environment conditions. There is a need to investigate the factors that affect As accumulation and speciation in rice under field conditions. This would allow a more realistic assessment on the mitigation strategies to minimize As contamination in rice. The objective of the present study was to evaluate the effects of agronomic practices, including paddy water management, straw incorporation, the applications of N fertilizers and organic manure and the additions of biochar, on As content and speciation in field-grown rice over four cropping seasons. The abundance of the *arsM* gene in the soil was also determined to provide a better understanding of the factors controlling As speciation in rice.

2. Materials and methods

2.1. Experimental site

Field trials were conducted in a paddy field in Jinjing town, Changsha County, Hunan province, China. The area is located in the subtropical region and has a long history of double rice cropping system. The soil is classified as Stagnic Anthrosols derived from granite red soil (Shen et al., 2014). The soil (0–20 cm) has a total organic C content of 18.9 g kg^{-1}, total N of 2.08 g kg^{-1}, total P of 0.39 g kg^{-1}, available P (by the Olsen method) of 18.7 mg kg^{-1}, total As of 6.5 mg kg^{-1}, ammonium phosphate-extractable As of 0.36 mg kg^{-1}, and amorphous iron oxides of 3.3 g Fe kg^{-1}. The air-dried soil is acidic with a pH of 5.08. More details of the experimental site were described by Shen et al. (2014).

2.2. Experimental design

Field experiments were carried out in both early and late rice seasons during 2012 and 2013. There were two sets of field experiments for each of the four rice crop seasons. The first was a factorial combination of water management methods (continuously flooded vs intermittently flooded) and straw incorporation (none vs 6 t rice straw ha^{-1}). The intermittently flooded (IF) treatment is the locally adopted practice of paddy water management. In this method, the paddy field remained flooded for approximately 30 days after rice transplanting, followed by a 10-day mid-season drainage when plants were at the late tillering stage, followed by intermittent irrigation until one week before rice harvest when water was drained. In the continuously flooded (CF) treatment, the paddy field remained flooded until one week before rice harvest. The amount of straw incorporated into the soil (6 t ha^{-1}) was similar to that of rice straw produced per cropping season. Straw was cut into approximately 10 cm pieces before being incorporated into the soil.

In the second set of experiments, there were six treatments consisting of no nitrogen fertilizer (N0), full dose of N fertilizer (N), half dose of N fertilizer (½N), half dose of N fertilizer with organic manure (½ N + M), full dose of N fertilizer with 24 t biochar ha^{-1} (N + LB), and full dose of N fertilizer with 48 t biochar ha^{-1} (N + HB). The full dose of N, applied as urea, was 120 and 150 kg ha^{-1} for early and late rice, respectively. Organic manure was applied as composted pig manure at 5.25 and 6.56 t ha^{-1} for early and late rice, respectively; the amounts of N contained in the manure were equivalent to half of the full N dose. Biochar was produced from wheat straw by pyrolysis and ground to pass a 5 mm sieve before application. The amounts applied (24 and 48 t ha^{-1}) were approximately 1% and 2% of the soil mass in the top 20 cm depth. Water management in the second set of experiments was IF for all treatments.

All treatments in both experiments received the same basal rates of phosphate (40 kg P_2O_5 ha^{-1} as calcium superphosphate) and potassium (100 kg K_2O ha^{-1} as potassium sulphate) fertilizers. All treatments were replicated in triplicate plots in a randomized block design. The size of plot was 35 m^2 (5 × 7 m). The cultivars of rice were Xiangzao 45 and T-you 207 for early and late rice, respectively; both belong to the indica type with the latter being a hybrid rice.

2.3. Chemical analyses

Grain samples were taken from each plot at crop maturity and dried at 60 °C. Grain were dehusked and ground to fine powder. Grain samples were digested with concentrated HNO_3 using a microwave digester. Total As concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Nexion 300 ×) in the He gas collision mode to minimize polyatomic interferences. Indium was added to the samples as the internal standard. Blanks and a certified reference material (NIST 1568b rice flour) were included for quality assurance. Repeated analysis of NIST 1568b gave 0.28 ± 0.02 mg As kg^{-1} (mean ± SD, $n = 10$), which was in good agreement with the certified value of 0.29 ± 0.03 mg As kg^{-1}.

To determine As speciation in rice grain, flour samples were extracted with 1% HNO_3 in a microwave oven according to Zhu et al. (2008). The extracts were filtered through a 0.22 μm filter and analysed using HPLC-ICP-MS. Arsenic species were separated using an anion exchange column (Hamilton PRP X-100, 250 mm). $NH_4H_2PO_4$ (6 mM, pH 6.0) was used as the mobile phase which was run isocratically at a flow rate of 1 mL min^{-1}. Indium was added to the post-column solution and measured by ICP-MS as the internal standard. ICP-MS was set up in the He gas collision mode. Four As species were identified, including arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), with As(III) and DMA being the dominant inorganic and organic species, respectively. In the present study, As(III) and As(V) were summed as total inorganic As, and DMA and MMA as organic As. Repeated analysis of NIST 1568b rice flour gave (mean ± SD, $n = 7$): As(III) 0.084 ± 0.005 mg kg^{-1}, As(V) 0.025 ± 0.002 mg kg^{-1}, DMA 0.189 ± 0.007 mg kg^{-1}, MMA 0.013 ± 0.002 mg kg^{-1}, with a sum of As species of 0.31 ± 0.008 mg kg^{-1}. Although the NIST 1568b reference material has no certified values for As speciation, the values obtained in our study were comparable to previous reports (Williams et al., 2007a; Xu et al., 2008; Zhu et al., 2008). There was also a good agreement between the sum of As species (x) and the total As concentration (y) determined by ICP-MS following digestion with concentrated HNO_3 ($y = 0.015 + 1.06\,x$, $r^2 = 0.81$, $n = 108$, $P < 0.001$; Supplementary Fig. S1).

2.4. Assays of the copy numbers of bacterial 16S rDNA and arsM genes

Soil samples were collected from each plot after the harvest of early rice in 2012. Soils were air dried and ground to <2 mm. pH was determined using a glass electrode in a soil:water (1:2.5) suspension. To determine the bacterial 16S rDNA and *arsM* copy numbers, soil (10 g) was reconditioned with distilled water (6 ml) and