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Arsenic accumulation and speciation in rice grown in arsanilic acid-elevated paddy soil

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ABSTRACT

P-arsanilic acid (AsA) is a emerging but less concerned contaminant used in animal feeding operations, for it can be degraded to more toxic metabolites after being excreted by animals. Rice is the staple food in many parts of the world, and also more efficient in accumulating arsenic (As) compared to other cereals. However, the uptake and transformation of AsA by rice is unclear. This study aimed to evaluate the potential risk of using AsA as a feed additive and using the AsA contaminated animal manure as a fertilizer. Five rice cultivars were grown in soil containing 100 mg AsA/kg soil, after harvest, As species and their concentrations in different tissues were determined. Total As concentration of the hybrid rice cultivar was more than conventional rice cultivars for whole rice plant. For rice organs, the highest As concentration was found in roots. AsA could be absorbed by rice, partly degraded and converted to arsenite, monomethylarsonic acid, dimethylarsinic acid, arsenate. The number of As species and their concentrations in each cultivar were related to their genotypes. The soil containing 100 mg AsA/kg or more is unsuitable for growing rice. The use of AsA and the disposal of animal manure requires detailed attention.

1. Introduction

Para-arsanilic acid (AsA) (4-aminophenylarsonic acid) has been extensively used as an animal feed additive in the poultry and swine industries to promote growth and control diseases. 100 mg As/kg feed has been used since the mid 1940s, and United States use 800–1000 t of AsA every year. Yao et al. (2013) reported that 25.4% of the 146 animal feeds contained organoarsenics, and average concentration of AsA was 21.2 mg of **As**/kg. Morrison (1969) found measurable amounts of As (15–30 mg/kg) in litter and Romina et al. (2011) found that the dog food contained AsA. However, only a small proportion of this compound is actually absorbed by animals and a large part is excreted unchanged through the animal litter (Aschbacher and Feil, 1991). The high correlation coefficient between As concentrations in feed and manure was observed (Kazi et al., 2013).

The toxicity of the As species has been drawn great attention. The inorganic As including arsenite (As(III)) and arsenate (As(V)) are generally considered to be more toxic than organic arsenic, and the toxicity order of As species is as follows: As(III) > As(V) > monomethylarsonic acid (MMA) > dimethylarsinic acid (DMA) (Abedin et al., 2002). Though the toxicity of AsA is low, it can be degraded to more toxic metabolites after animal manure enters the environment (Wang et al., 2014), or when untreated litter is stockpiled or used as organic fertilizer in agricultural sites. Thus, such scenarios could give rise to a great risk of As contamination, lead to the uptake and accumulation of this element by plants growing on contaminated sites, and endanger human health through food chains. Yao et al. (2009, 2010) have reported that vegetables could accumulate organic arsenic compounds, and their degradation products As(III) and As(V). The accumulation of As by rice (*Oryza sativa L.*) is of great attention considering the fact that the dietary intake of rice is potentially a major As exposure pathway in countries where rice is the main staple food, and inorganic arsenic (iAs) concentration increases significantly after cooking (Laparra et al., 2005). Huang et al. (2015) reported that 1.1% of 1653 milled rice samples from 11 provinces exceeded the maximum contaminant level established by Chinese legislation. Mireia et al. (2011) reported that rice presented the highest iAs values in 215 food products and drinks (i.e., seafood, fruits and vegetables, meat products, oils and fats, rice

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and rice products, seasonings, and alcoholic drinks), corresponding to 67% of dietary iAs intake. Total As concentrations in 31 samples (60% were grown in the United States) ranged from 0.090 ± 0.004 to 0.85 ± 0.03 mg/kg with a mean value of 0.275 ± 0.161 mg/kg (n = 31) (He et al., 2012). As concentration in rice grain was 0.5 ± 0.02 mg/kg with the highest concentrations being observed in grains of plants grown on soil treated with 40 mg As/kg soil (Rahman et al., 2008). Moreover, Juskelis et al. (2013) found that the average total As and iAs concentrations in infant rice cereal were 174.4 and 101.4 μg/kg, respectively. The mixed-grain rice cereal contained the least total (105 μg/kg) and iAs (63 μg/kg). The major detected organoarsenical species was DMA. Concentrations of As(III), As(V), and DMA were closely positively correlated with the total As concentrations (Narukawa et al., 2012). It has been demonstrated that As concentrations in different parts of rice plants increase with elevated concentrations of roxarsone and AsA added to soils, the As concentrations vary with the growth stage of the plants (Wang et al., 2006). Syu et al. (2015) found that the translocation of As in rice plants affected the accumulation of As in rice shoots and grains among different rice genotypes in As-contaminated soils. In addition, As phytotoxicity also has an impact on the accumulation and speciation of As in rice grains (Khan et al., 2010). However, the effect of AsA contaminated poultry or pig litter have received little attention although such material is widely used as farmyard manure. The contamination of the soil and water near piggeries and hen yards has been reported (Ghosh et al., 2014). Therefore, there is an urgent and important need to study the transportation, transformation, metabolism and potential toxic properties of AsA in rice plants. However, up to now, there has been little research in this area.

In the current study, a pot experiment was carried out to investigate the uptake, accumulation, transformation and speciation of As in rice plant organs (roots, stems, leaves, ears and grains) grown in AsA - elevated paddy soils. Since As speciation has important toxicological implications once ingested, the ultimate aim of the study was to evaluate the potential risk of using AsA as a feed additive and using AsA contaminated animal manure as a fertilizer.

2. Materials and methods

2.1. Rice experimental methodology

The soil was collected from the surface soil (0–30 cm) of a paddy field with organic matter of 2.34%, pH equal to 7.62, available potassium, nitrogen and concentration 353, 67.9, 95.7 mg/kg, respectively, with the following background concentrations of heavy metals (Cd: 0.4, Pb: 39.4, Cr: 16.7, Hg: 0.1 and As: 6.2 (AsA: 0) mg/kg, respectively). The soil was air dried, shattered using a spade and hammers, and then passed through a 0.85 mm sieve and mixed well. Plastic pots (24.5 × 21.0 × 29.0 cm) were filled with 8 kg soil and spiked with 100 mg AsA/kg soil. The control was made without AsA added. There were three replicates for each different rice cultivar.

Rice seeds were sterilized in a solution containing 15% sodium hypochlorite solution for 30 min, washed with deionized water and then germinated in Petri dishes containing moist tissue paper for three days. After germination, rice seedlings were transferred to a 0.6 - L beaker and grown in half-strength nutrient solution modified from Zhang et al. (2011) (pH was adjusted to 5.5–6.0 and the solution was renewed every 7 days) for 2 weeks. Five seedlings were then transplanted into each pot of soil. The soil was saturated with water and the water level was maintained at about 3 – 5 cm above the soil surface during the whole period of plant growth. The soil was supplemented with 0.28 g N/kg as NH_4NO_3, 0.073 g P/kg as KH_2PO_4 and 0.078 g K/kg as K_2SO_4 as basal fertilizers. Mature rice was harvested about 90 days after transplantation. The rice was removed with some soil to prevent damaging the roots. They were rinsed with tap water and then with deionized water to remove soil. Later, they were carefully separated into grains, stems, leaves and roots, and then homogenised with a stainless steel waring blender, and stored at −20 °C till analysis.

2.2. Chemical reagents and solutions

All reagents were of analytical reagent grade unless stated otherwise. Ultrapure water (18.25 MΩ·cm) was obtained using an EASY pure treatment system (DUBUQUE, IOWA, U.S.A). Methanol of HPLC grade (J.T.Baker, USA) was used. Both argon (Ar) and nitrogen (N_2) with high-purity (≥99.99%) were purchased from gtobal.com (Guangzhou, China). AsA with a purity of 98% was purchased from Aladdin-e. company. The standard stock solutions of As(III) (75.7 ± 1.2 mg/L), As(V) (17.5 ± 0.4 mg/L), MMA (25.1 ± 0.8 mg/L) and DMA (52.9 ± 1.8 mg/L) presented as the concentration of As were provided by the Chinese National Standard Materials Center (Beijing, China), and stored in the dark at 4 °C. The mixed working standard solutions with different concentrations were prepared step-by-step daily by diluting the solutions with ultrapure water as required.

2.3. Plant digestion and total As analysis

The method of total As extraction and detection was according to GB/T 5009.11-2003 issued by China. Approximately 5.0 g of plant material was digested by 10 mL mixed solution ($V_{saturated\ HNO3}$:V_{water} = 4: 1) in a 50 mL glass beaker. Samples were first allowed to stand overnight at room temperature covered with a watch glass for cool digestion and then placed on a heating block and the temperature increased in steps from 100 to 180 °C, and kept until the solution in the beaker was clear and about 1–2 mL left. After digestion, solutions were cooled, and poured into 25 mL colorimetric tubes. The beaker and the watch glass were washed with deionised water for three times and the washing water was also poured into the colorimetric tube. Each colorimetric tube was also treated with 2.5 mL 50% HCl solution ($V_{saturated\ HCl}$:V_{water} = 1:1) and 2.5 mL of a mixted solution containing 5% ($m_{thiourea}$:V_{water}) thiourea and 5% ($m_{ascorbic\ acid}$:V_{water}) ascorbic acid. The concentration of tubes was then diluted with deionised water to 25 mL, shaken well and left to stand for at least 30 min before analysis by AFS. For quality assurance and quality control purposes, three blanks and two standard reference material samples GSB-5 (cabbage) and GSB-6 (spinach) purchased from the Chinese National Reference Materials Center were included in the whole digestion procedure and sample analysis. During the HG-AFS analysis, the external standards calibration curve using six aqueous As standard solution from 0 to 80 μg/L were run before and after each sample series, and the correlation coefficient exceeded 0.9995, the method's detection limit was 0.01 mg/kg and the recovery (%) was 96.6–104.8%.

An on-line hydride generation step (5% HCl solution ($V_{saturated\ HCl}$:V_{water} = 95:5), 130 mL/min and 1.0% KBH_4 in 0.2% NaOH, 130 mL/min) was used to convert the nonvolatile As compounds into AsH_3 which were detected by an atomic fluorescence spectrometer (AFS8130, Beijing Titan Instruments Co., Ltd., Beijing, China). The running speed of the carrier gas (argon) was 400 mL/min and shield gas 800 mL/min. Photomultiplier tube voltage was 270 V and the hollow cathode lamp current 60 mA (primary) /30 mA (boosted).

All glass vessels used for total As determination were soaked in 20% HNO_3 solution ($V_{saturated\ HNO3}$:V_{water} = 4:1) for 24 h and washed with deionized water and dried.

2.4. Determination of As species in rice by HPLC-ICP-MS

The extraction of As species was based on previous studies (Pizarro et al., 2003). For this, 0.5g samples were weighed into 50 mL centrifuge tubes and 10 mL of 50% (v:v) methanol aqueous solution were added to perform an ultrasound assisted extraction at room temperature for 30 min, followed by centrifugation at 3170 × *g* for 30 min. The supernatant was filtered through a 0.22 μm PET filter using a 13 mm syringe