



Variation in arsenic bioavailability in rice genotypes using swine model: An animal study



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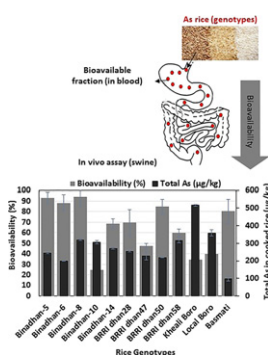
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HIGHLIGHTS

- Arsenic (As) concentrations in rice varies significantly based on genotypes.
- Bioavailability of As from rice is crucial to understand the human health risk.
- Inorganic As has five times higher bioavailability than organic As.
- Rice As bioavailability varied between 25% and 94% based on varieties.
- Salt tolerance and brown rice have higher As content but lower bioavailability.

GRAPHICAL ABSTRACT



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ABSTRACT

An *in vivo* assay using swine was used to measure the absolute bioavailability (AB) of As from cooked rice of twelve genotypes commonly grown in Bangladesh. An assessment of both total As in rice and its bioavailability is crucial for estimating human exposure following dietary intake by the local community. Average As concentrations in each rice genotype ranged from $108 \pm 4 \mu\text{g}/\text{kg}$ to $580 \pm 6 \mu\text{g}/\text{kg}$. Arsenic speciation shows that most of the rice genotype contains 73 to 100% inorganic As. Swine were administered with As orally and *via* intravenous method, *i.e.* injection and fed certain common Bangladeshi rice genotypes (cooked). Swine blood As levels were measured to calculate As bioavailability from rice. Pilot studies shows that for As(III) and As(V), $90.8 \pm 12.4\%$ and $85.0 \pm 19.2\%$ of the administered oral dose was absorbed from the gastrointestinal tract whereas organic As was poorly absorbed resulting in low bioavailability values $20.2 \pm 2.6\%$ (MMA) to $31.2 \pm 3.4\%$ (DMA), respectively. These studies demonstrates that rice genotypic characters influenced As bioavailability in rice grown in As-contaminated areas and the bioavailability varied between 25% and 94%. Arsenic in salt tolerant rice genotypes Binadhan-10 and BRR1 dhan47 as well as brown rice genotypes Kheali Boro and Local Boro has lower bioavailability (<50%) compared to other rice genotypes. The most commonly cultivated and consumed variety (BRR1 dhan28) has As bioavailability of 70%, which poses a significant risk to consumers.

Abbreviations: As, Arsenic; AUC, Area Under Curve; BMDL, Benchmark dose lower confidence limit; CONTAM, Contaminants in Food Chain Panel; CRC CARE, Cooperative Research Centre for Contamination Assessment and Remediation of the environment; DL, Detection Limit; DMA, Dimethylarsinic acid; EFSA, European Food Safety Authority; GCER, Global Centre for Environmental Remediation; GI, Gastrointestinal; ICP-MS, Inductively coupled plasma mass spectrometer; ISIS, Integrated samples introduction system; LOQ, Limit of quantification; MMA, Monomethylarsinic acid; MTDI, Maximum Tolerable Daily Intake; ND, Non Detectable; NIST, National Institute of Standard and Technology; SAHMRI, South Australian Health and Medical Research Institute; SRM, Standard Reference Material; TFA, Trifluoroacetic acid; UniSA, University of South Australia; WHO, World Health Organization.

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Calculation of maximum tolerable daily intake (MTDI) for humans due to consumption of rice based on bio-availability data was higher than those calculated based on inorganic and organic As concentration in rice genotypes.

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1. Introduction

Arsenic exists in nature in a number of different forms and is present in groundwater, soil and food. Substantial As contamination of paddy soils, water and foods are important exposure pathways for As toxicity in humans. People are mostly exposed to As *via* drinking contaminated groundwater and food especially rice where people heavily depend on it for their subsistence. Rice paddies grown in soil using irrigation water contaminated with As resulted in grain As accumulation and hence the consumption of this rice may contribute around 60% daily dietary intake, based on concentrations of As in Bangladeshi rice (Meharg, 2004). In addition, As-contaminated water used for rice cooking also enhanced As concentrations in cooked rice (Ackerman et al., 2005). It indicates that the contribution of As from cooked rice in As-impacted areas needs to be considered while assessing human health risk (Domingo, 2010).

Bioavailability of As and its speciation play a vital role in human health risk assessment. The toxicity of As depending on the chemical forms and its oxidation states but also depends on other factors such as physical state, the rate of absorption into cells, the rate of elimination, and the nature of chemical substituents in the toxic compound *etc.* The relative toxicity order of As was $iAsIII > monomethyl\ arsine\ oxide\ (MMAOIII) > DMA\ arsenotriglutathione\ (DMAIIGS) > DMAV > MMAV > iAs(V)$ (Hindmarsh et al., 1986; Vega et al., 2001). When released into the gastrointestinal (GI) tract, a certain percentage is absorbed and the remainder passes through the GI tract. The main objectives of all bioavailability studies are to obtain the best possible estimate of the amount of available As that poses potential risk to human health. However, most methods especially *in vitro* studies have major interpretational limitations. A limited number of *in vivo* studies were conducted using animals but they are all related to As in soil with incidental high levels of As used (Bradham et al., 2011; Brattin and Casteel, 2013; Denys et al., 2012; Laird et al., 2013). While the release of As present in rice grains in the human gut is apparent with the exception of a single study conducted by Naidu and his co-researchers (Juhász et al., 2006) using an animal model and greenhouse grown rice, no other study has been conducted thus far especially using rice genotypes grown in naturally As-contaminated areas. In this study, a range of different rice genotypes commonly grown in Bangladesh were tested to investigate the total As concentration and speciation. Finally, the bioavailability of As was determined for assessing human health risk.

2. Materials and methods

2.1. Rice varieties, cultivation, and preparations

Twelve rice varieties were used for this study, specifically Binadhan-5, Binadhan-6, Binadhan-8, Binadhan-10, Binadhan-14, BRRI dhan47, BRRI dhan50, BRRI dhan58, Kheali Boro and Local Boro. In this study, we have tested local, high yielding and aromatic rice varieties. Rice varietal characteristics are presented in Table 1. All rice varieties were cultivated under field conditions in a severely As-impacted district (Faridpur) in Bangladesh during January–May in 2014. Conventional water management, *i.e.* continuous flooding was employed for rice cultivation and recommended doses of fertilizers (N, P, K and S) were applied to each plot. The concentrations of chemical elements in soil and irrigation water are presented in Table 2.

The soil used in this study was clay loam in texture, having pH 7.6, organic matter 1.98%, total N 0.13%, available P 13700 $\mu\text{g}/\text{kg}$, available S 13700 $\mu\text{g}/\text{kg}$, exchangeable K 0.11 meq 100 per g, cation exchange capacity 10.1 meq 100 per g soil and total As 15.69 mg/kg. The irrigation water used to irrigate the rice contained high levels of As (255.4 $\mu\text{g}/\text{L}$). At maturity, the rice panicles were harvested, air dried and finally dehusked by a stainless-steel thresher. All rice grain samples were ground with a coffee grinder before digestion for analysis. Rice was cooked using absorption methods (1:2 v/v rice to water) with MQ water (ELGA Lab pure) using a rice cooker. A comparative study using supermarket-purchased rice (Basmati - Adelaide, South Australia, BRRI dhan28 - Dhaka, Bangladesh) was also conducted.

2.2. Sample preparations for total and speciated As

For total As analysis, trace analytical grade HNO_3 (70%), obtained from Fisher Chemicals was used for the digestion of rice samples as done by Rahman et al. (2009). The digests were diluted to 10 mL using 0.1% HNO_3 and then passed through a 0.45 μm syringe filter (MCE, Agilent Technologies) and then 10 mL aliquot of the digest from each tube was transferred to a plastic tube for instrumental analysis.

Arsenic speciation analysis was conducted after extracting samples with 2 M trifluoroacetic acid (TFA) as undertaken by Abedin et al. (2002). Briefly, 0.25 g of ground samples were weighed in to glass tubes, and 2 mL of TFA (2 M) was added. The digestion vessel was then positioned on a heating block and heated at 100 °C for 6 h. The

Table 1
Varietal characteristics of selected rice genotypes.

Rice variety	Growth duration (days)	Plant height (cm)	Yield (t/ha)	Special characters
Binadhan-5	150–155	110–115	7.0	Lodging tolerant (grain: long slender and bright in color)
Binadhan-6	160–165	110–115	7.5	High yielding (grain: long, medium bold)
Binadhan-8	130–135	91–95	5.5–8.5	Salt tolerant (grain: medium bold)
Binadhan-10	127–132	104–108	5.0–6.0	Salt tolerant (grain: medium long slender and bright in color)
Binadhan-14	120–130	85–88	6.9	Protein content 9.8% (grain: long slender)
BRRI dhan28	140	90	5.5–6.0	Early maturing (grain: medium slender, white)
BRRI dhan47	152	105	6.0	Salt tolerant (grain: medium bold, white)
BRRI dhan50	155	82	6.0	Aromatic, protein content 8.2% (grain: long slender, white)
BRRI dhan58	150–155	100–105	7.0–7.5	High yielding (grain: medium slender, white)
Kheali Boro	130–135	108–112	3.0–4.0	Grain: Brown, medium slender
Local Boro	128–132	105–108	3.0–4.0	Grain: Brown, medium slender
Basmati	–	–	–	Aromatic (grain: long slender, white)

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