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Environmental Chemistry

Effects of arsenite and dimethylarsenic on the growth and health of hydroponically grown commercial Doongara rice

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Environmental context. Arsenic's effect on rice plant health is a critical environmental issue. This study reveals that rice plants absorb inorganic arsenic and dimethylarsenic differently, with dimethylarsenic posing a greater threat to rice plant health. These findings contribute to our understanding of arsenic toxicity in plants, highlighting the need for further research into detoxification strategies for dimethylarsenic.

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ABSTRACT

Rationale. Arsenic toxicity in plants, particularly the effects of different arsenic species, is not well understood. This study investigated the response of juvenile rice plants, grown hydroponically, to prolonged exposure to inorganic and dimethyl arsenic species. The hydroponic system removed complexity by eliminating soil processes. **Methodology.** The accumulation of inorganic As (As_i) and dimethylarsenic (DMA) in hydroponically grown rice was monitored for plants exposed to different As concentrations (0–6.7 μmol L^{-1}). Dose–response experiments were conducted to compare the effects of As species on plant health in terms of growth. **Results.** Plants absorb As_i and DMA linearly, with faster As_i uptake than DMA. As_i exposure leads to higher As concentrations in roots and shoots than DMA. Despite more As_i in roots, its translocation to shoots is lower. As_i and DMA accumulation in shoots remains relatively constant at lower As concentrations. At the highest As concentration, more As_i and DMA accumulate in shoots. Exceeding 1.6 μmol L^{-1}, As_i and DMA reduce plant height and biomass. As_i-exposed plants show little health differences except at the highest concentrations. DMA-exposed plants show more unhealthy instances above 1.6 μmol L^{-1}. **Discussion.** DMA's lower uptake rate aligns with other rice species results, as do lower shoot and root translocation factors. Near constant As concentrations in shoots at low As_i concentrations suggest an As_i exposure threshold before plants lose their As sequestration ability, resulting in reduced growth. DMA exposure increases the number of unhealthy plants, suggesting a greater potential effect on plant health and fitness, differing from As_i-induced stress.

Keywords: arsenic species, dimethylarsinic acid, health effects, hydroponics, rice, rice grain, straight head disease, uptake.

Introduction

Rice is a stable food source for 3.5 billion people (https://www.cgiar.org/research/center/irri/). Arsenic (As) is accumulated in rice from natural sources, e.g. soils and rocks (Palmer *et al.* 2021) and the historical use of herbicides such as cacodylic acid (Limmer and Seyfferth 2020). There is a concern about human exposure to As, particularly for infants, through the consumption of rice and processed rice products (Sohn 2014; Maher *et al.* 2018).

Arsenic is phytotoxic to plants at high concentrations (Tang *et al.* 2016*a*, 2016*b*) and can result in lower plant growth, crop yields and seed germination (Murugaiyan *et al.* 2021). High As concentrations are also associated with straight-head disease (Hua *et al.* 2013). This disease is a physiological disorder in rice with symptoms of sterile spikelets, distorted husks and erect panicles (Tang *et al.* 2020) and results in yield losses of up to 90% (Rahman *et al.* 2008). Two As species are commonly found in most rice species, inorganic arsenic (As_i), arsenite (As^{III}) and arsenate (As^{V}), and dimethylarsenic (DMA)

(Maher *et al.* 2018). As_i is transported to roots by phosphate transporters and through two silicon transporters, Lsi1 (the aquaporin NIP2;1) and Lsi2 (an efflux carrier) (Bienert *et al.* 2008; Li *et al.* 2009; Abedi and Mojiri 2020). As^V is mostly reduced to As^{III} in flooded organic-rich paddy fields (Takahashi *et al.* 2004; Xu XY *et al.* 2008) and within rice plants, effluxed or complexed with phytochelatins (PC) and As^{III}–PC complexes sequestered within vacuoles (Duan *et al.* 2011; Lemos Batista *et al.* 2014). DMA is produced by bacterial methylation of As_i in reduced organic-rich soils (Jia *et al.* 2013; Chen C *et al.* 2019; Geng *et al.* 2023); rice plants cannot methylate As (Ye *et al.* 2012). DMA also enters roots by a silicon transporter, Lsi1 (Li *et al.* 2009; Abedi and Mojiri 2020). DMA uptake into roots is lower than As_i; however, unlike As_i, DMA is efficiently transported to reproductive organs (Zheng *et al.* 2013). DMA is in a dissociated state (Sarwar *et al.* 2021), thus it cannot form PC complexes and poorly interacts with SH groups (Abedin *et al.* 2002). This results in higher DMA concentrations in grain compared to that of As_i (Abedin *et al.* 2002; Shinde and Kumar 2021). DMA is also more toxic to rice (Zheng *et al.* 2013; Tang *et al.* 2016*a*). When this study was undertaken, DMA was suspected of causing straighthead disease, but little direct evidence was available.

Soil properties (pH, REDOX potential, organic matter, S, N, P, Fe, and Mn content) affect the uptake of As by rice plants (Zhao *et al.* 2009; Abedi and Mojiri 2020), thus we conducted hydroponic experiments to eliminate the complexity associated with soils. We used a commercial rice species, Doongara, that is susceptible to straighthead disease (Martin *et al.* 2023) to understand the connection between As and straighthead disease. The aims of the study were to: (a) measure how the uptake and accumulation of As in Doongara varies when exposed to different As^{III} and DMA concentrations and (b) measure how exposure to As^{III} and DMA affects developing Doongara plants in terms of their growth and health.

Experimental

Hydroponic system

The system was a self-contained reservoir, with individual plants growing in individual reservoirs (Fig. 1). The system consisted of three main components: a reservoir, plant housing and aerating system. The reservoir was constructed using 90-mm diameter polyvinyl chloride (PVC) pipes cut to 370-mm lengths. Along the edge, five holes (22-mm diameter) were drilled 55 mm apart. The five large holes were used to insert the plant housing units. A sixth hole was also bored, with a diameter of 10 mm. The smaller hole was used to insert an air hose to aerate the nutrient solution and as an access point to take pH measurements. The capacity of the system was 1.6 L per reservoir.

The plant housing units were constructed using 20-mm diameter piping cut to 80 mm in length to fit into the reservoirs. Each housing unit held a single plant, and the spacing between the plants allowed the plant to grow without the roots becoming entangled with neighbouring plants. In previous hydroponic systems, it has been found that if the plants were not separated, direct competition occurred, leading to roots becoming entangled, resulting in significant experimental variation within treatments. Plants were held in place using styrofoam discs. Styrofoam was used due to its inert properties, thus avoiding the leaching of unwanted contaminants into the nutrient solution. Each nutrient solution and treatment was aerated by pumping compressed air through Teflon tubing and bubbling it into the nutrient solution by air stones through the sixth hole.

The plants were grown in a temperature-controlled laboratory between 20 and 24°C with two ATI T5 power module light systems using T5 full-spectrum fluorescent bulbs suspended from the roof above the plants. The light field was measured using an irradiance sensor (Biospherical Instruments

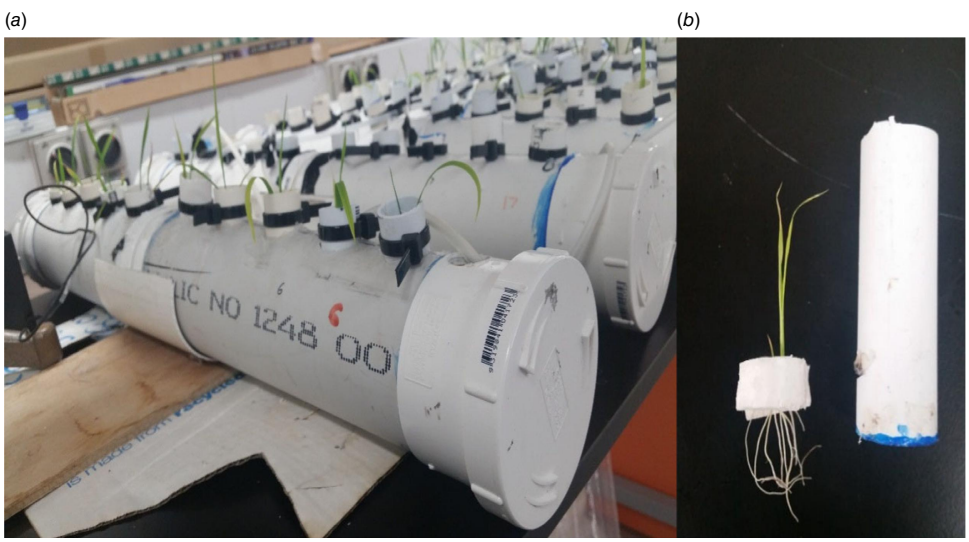

Fig. 1. Hydroponics set up. (*a*) The complete system with growing rice plants. (*b*) An example of the plant housing tube with a rice plant placed in the styrofoam disc.

QSL 2102) across the surface of the hydroponic growing tubes to ensure an even light distribution across the surface.

Seed germination and growth conditions

Rice seeds were surface sterilised utilising a technique adapted from Kim *et al.* (2005). The seeds were sterilised by rinsing with 10% v/v H_2O_2 for 10 min, followed by 70% w/w ethanol for 5 min and a final rinse with deionised water. After surface sterilisation, the seeds were placed in deionised water and then placed in an incubator at 30°C for 48 h to break dormancy and promote germination of the seedlings.

Seedlings were transplanted into the hydroponic system after germination. For the first 14 days following germination, the plants were exposed to a half-strength nutrient treatment to allow the plants to acclimate, followed by full strength. The nutrient media composition was as follows: 396 μmol L^{-1} of KNO_3, 360 μmol L^{-1} of $Ca(Cl)_2$, 290 μmol L^{-1} of $MgSO_4{\cdot}H_2O$, 38 μmol L^{-1} of $NaH_2PO_4{\cdot}2H_2O$, 230 μmol L^{-1} of K_2SO_4, 2.5 μmol L^{-1} of $MnCl_2{\cdot}4H_2O$, 3.2 μmol L^{-1} of H_3BO_3, 0.04 μmol L^{-1} of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.035 μmol L^{-1} of $ZnSO_4{\cdot}7H_2O$, 0.04 μmol L^{-1} of $CuSO_4{\cdot}5H_2O$, 0.037 μmol L^{-1} of $FeCl_3{\cdot}6H_2O$ and 0.04 μmol L^{-1} of $Na_2SiO_2{\cdot}5H_2O$. The pH of the nutrient solution was 5.5. The nutrient solution was similar to that used by Yoshida *et al.* (1976), with the addition of silicon at 0.04 μmol L^{-1} and EDTA at 3.4 μmol L^{-1}. The ETDA was added to prevent the precipitation of iron oxyhydroxide and plaque formation on roots (Jacobson 1951). The nutrient solution and As species were renewed every 7 days. This involved discarding the old nutrient solution and cleaning the tubes to ensure no build-up of mould or algae. The tubes were then topped up with 1.6 L of nutrient solution and appropriate arsenic species. Plants were grown on a 14:10-h day:night cycle at a light intensity of 180 μE m^{-2} s^{-1}.

Experimental design

Two hydroponic growth experiments were conducted with Doongara, a long-grain rice variety that was selected due to its susceptibility to As and straighthead disease (Martin *et al.* 2023). The rice plants were exposed to As^{III} and DMA at four different concentrations: 0.0, 0.8, 1.6, 3.5 and 6.7 μmol L^{-1}. The arsenic concentrations selected were based on the study by Shaibur *et al.* (2006), where As_i toxicity in hydroponically cultivated rice plants was found to be severe above 6.7 μmol L^{-1} of As^{III}. No experiments have reported direct toxicity for DMA in rice, so for this study, the DMA concentrations were matched to that of As^{III} to allow comparison between the two As species. For quality control, the highest arsenic treatment (6.7 μmol L^{-1}) of the alternative arsenic species was included in the As^{III} and DMA experiments.

Treatments were carried out in triplicate for a total of 38 days to allow for substantial growth to occur and to observe As accumulation throughout the vegetative growth stage. The experiment was terminated before the plants reached flowering, thus allowing us to observe the early accumulation of As because studies have shown that the uptake of nutrients and As changes at different growth stages for rice (Zheng *et al.* 2011).

Harvested plants were lightly rinsed with deionised water, and height was recorded from the base of the stem to the tip of the highest leaf. Plant biomass was determined from plant dry mass. Plant roots were also inspected for iron plaque formation; however, no plaque was observed on any of the root systems (Fig. 2).

Arsenic measurement

Total As concentrations

Plant samples were digested in a microwave oven (CEM, MARS) with 2 mL of concentrated HNO_3 and 1 mL of H_2O_2 (30% v/v). Samples were digested in batches of 40,

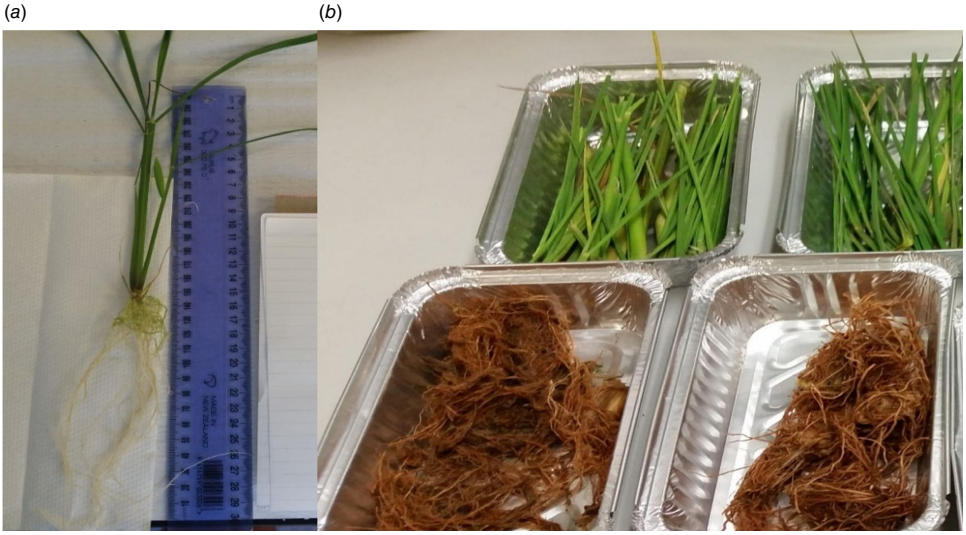

Fig. 2. Photos of rice plants being processed at the end of the experiments. (*a*) A rice plant from the hydroponic experiments. Note the white root structure. (*b*) Plants grown in the field (Martin *et al.* 2023) – the top trays hold the above-ground biomass, and the bottom trays are the roots of the plant that show iron plaque (Chen Z *et al.* 2005). Panel (*a*) illustrates that the rice grown hydroponically exhibited no iron plaque formation (typically an orange colour) on the roots.

containing 37 samples, 2 certified reference materials (CRMs) and 1 blank. Digests were diluted to 10 mL with deionised water. Before analyses, digests were further diluted to 1 in 100 (v/v) with deionised water and internal standards added before analysis by inductively coupled plasma–mass spectrometry (ICP-MS) (Perkin Elmer DRC II) (Maher *et al.* 2013).

Arsenic speciation

Plant samples were extracted with 2% v/v HNO_3 and diluted to 10 mL with deionised water. The extracts were centrifuged for 10 min at 4000 rpm (Eppendorf, 5804R) at room temperature and filtered through 0.45-µm polyethersulfone (PES) syringe filters before analysis. Samples were analysed using high-performance liquid chromatography (HPLC)-ICP-MS (Perkin Elmer) employing a PRPX-100 anion exchange column (Hamilton) with a mobile phase containing 20 mM $(NH_4)_3PO_4$ buffer at a flow rate of 1.5 mL min^{-1} and a column temperature of 40°C (Maher *et al.* (2013). Results below are reported as As_i (the sum of As^{III} and As^{V}). Nearly all the As_i is As^{III}.

The measured total As concentrations in reference materials (NIES 10a Rice Flour and NIES SRM 1568a) and As speciation concentrations (NIST 1568a Rice Flour) were in agreement with published concentrations (Table 1).

Results and discussion

Arsenic accumulation

Plants exposed to As_i accumulated more As as exposure concentrations increased, and the amount of As accumulated was much higher than in plants exposed to a comparable concentration of DMA (Fig. 3). Because plants were grown for the same period of time, 38 days, accumulated As amounts are directly comparable. When the plant was exposed to either As species, the majority of the As was in

Table 1. Total arsenic and arsenic speciation measured in certified reference materials.

Total arsenic and arsenic speciation	Measured (µg g^{-1})	Certified (µg g^{-1})
Total As concentrations in certified reference material		
NIES 10a Rice Flour	0.18 ± 0.03 (n = 11)	0.17
NIST 1568a Rice Flour	0.279 ± 0.001 (n = 10)	0.29 ± 0.03
Arsenic species in NIST 1568a Rice Flour		
As^{III}	0.088 ± 0.015 (n = 15)	0.062 ± 0.009
DMA	0.157 ± 0.022 (n = 15)	0.163 ± 0.009
As^{V}	0.034 ± 0.013 (n = 15)	0.039 ± 0.005
MA	0.010 ± 0.002 (n = 15)	0.010 ± 0.003

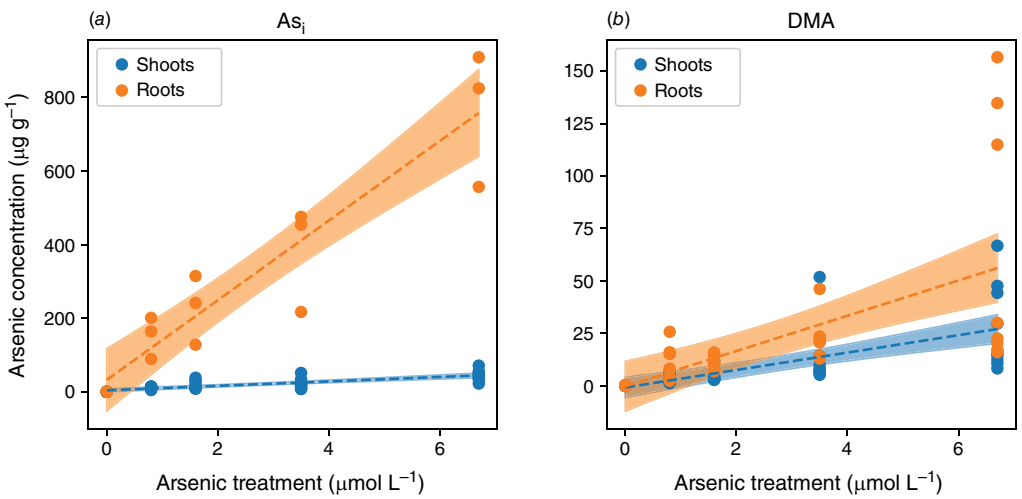

Fig. 3. Arsenic accumulation within different plant segments of rice grown hydroponically with increasing exposure to As_i (*a*) and DMA (*b*). Linear regressions were fitted, as shown by dotted lines, with 95% confidence intervals represented by the shaded areas.

the roots. Specifically, 92 ± 5% of As_i and 52 ± 15% of DMA were found in the roots. Plants exposed to As_i or DMA both showed a linear response to increasing As concentration. The response to increasing As exposure, however, showed two distinct accumulation patterns. The roots of the rice plants accumulated As_i to much higher concentrations than the shoots, whereas plants exposed to DMA had a similar distribution of As between shoots and roots with increasing As concentration (Fig. 3). The differences in uptake observed between As species are in agreement with previously published data (Abedin *et al.* 2002).

Plants exposed to As_i contained up to 13–19 fold higher As concentrations in the roots and 1.6–4 fold higher As concentrations in the shoots than when exposed to DMA. The lower uptake rate of DMA is in accordance with results published for other rice species (Abedin *et al.* 2002). Visual observations at the end of the experiment show that iron plaque did not form on the roots (Fig. 2). In the absence of iron plaque, the high As concentrations measured in the roots for the As_i treatments suggest active sequestration by root cells or As absorption followed by incorporation into root cells (Vázquez Reina *et al.* 2005; Moore *et al.* 2011).

Although root cells took up more As_i, the translocation of As from roots to shoots was significantly lower for As_i than DMA. The ratios of shoot to root As concentrations were 0.06–0.08 and 0.3–0.6 for As_i and DMA respectively. The accumulation of As in the shoots of the plants exposed to As_i remained fairly constant at low As_i concentrations (0.8–3.5 μmol L^{-1}) (Fig. 4). It was only through exposure to the highest As_i treatment (6.7 μmol L^{-1}) that the As concentration significantly increased within shoots ($P < 0.05$). The As concentrations in the shoots increased from 21 ± 14 to 45 ± 14 μg g^{-1} for the 3.5 to 6.7 μmol L^{-1} treatments, whereas for the lower As_i treatments (0.8 and 1.6 μmol L^{-1}), mean As concentrations were 11 ± 3 and 21 ± 14 μg g^{-1} respectively. This poses the question, is there a threshold for As_i exposure before plants lose the ability to sequester As in roots (Hartley-Whitaker *et al.* 2001)?

As previously described, detoxification of As_i involves either the sequestration of As by the formation of As^{III}–thiol complexes with glutathione (GSH) or phytochelatins (PCs) (Raab *et al.* 2005) in vacuoles (Song *et al.* 2010, 2014) or As being pumped out of cells into the external medium via efflux pathways (Xu J *et al.* 2017). An efficient As_i efflux transporter within rice plants has not yet been identified; however, Lsi1 has been identified as a bi-directional transporter that can efflux a small amount of As^{III} (Zhao *et al.* 2010). Sequestration of As_i in the roots is an effective way to limit the transport of As_i to above-ground tissues, although As_i still has some mobility throughout the plant (Zhao *et al.* 2012). Unlike Lsi1, Lsi2 can control the efflux of As^{III} towards the stele and restrict xylem loading (Ma *et al.* 2008). Further transport is limited by additional vascular sequestration (Chen Y *et al.* 2015). These plant nodes play a critical role in storing As_i and controlling further As_i distribution (Yamaji and Ma 2014; Zhao *et al.* 2014; Chen Y *et al.* 2015).

In plants exposed to DMA, the As concentrations in the roots and shoots are fairly consistent across all As exposures (as shown in Fig. 3 and 4). Specifically, the roots contain 10–23 μg g^{-1}, and the shoots contain 3–13 μg g^{-1} for a DMA treatment concentration range from 0.8 to 3.5 μmol L^{-1}. Approximately half of the arsenic is translocated from the roots to the shoots. Similar to the highest As_i concentration tested, significantly greater amounts of DMA (28 μg g^{-1}) are accumulated in shoots. Our current understanding is that rice plants lack the ability to either efflux or sequester DMA into vacuoles to reduce its mobility within the plant. Mishra *et al.* (2017) exposed rice plants to As^V, monomethyl arsenic (MA) and DMA for 7 days, and DMA

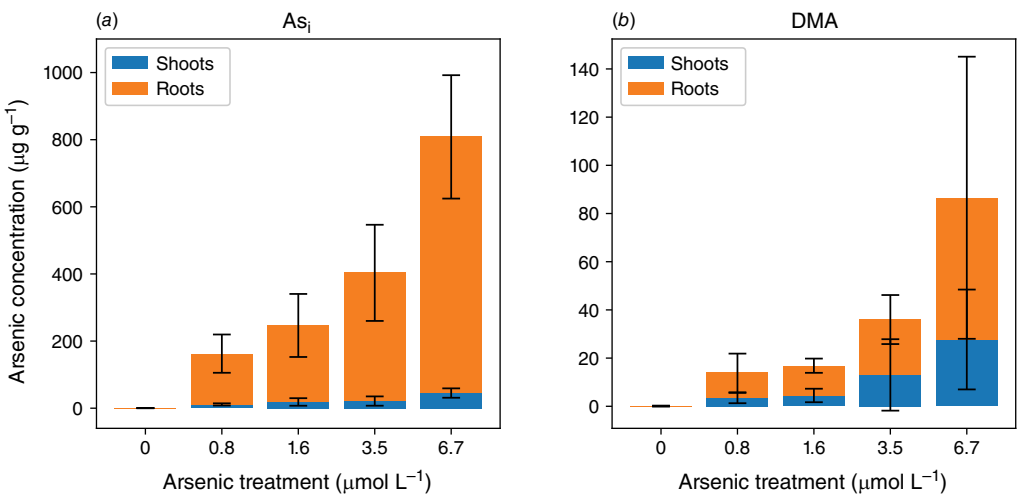

Fig. 4. Mean arsenic concentrations throughout the rice plant for plants exposed to As_i (*a*) and DMA (*b*). Error bars represent 1 s.d.

had the lowest shoot and root translocation factors, and no DMA–PC complexes were detected (Raab *et al.* 2007*a*). DMA, however, was efficiently translocated between the roots and shoots (Raab *et al.* 2007*b*) by both xylem and phloem (Carey *et al.* 2010), with phloem transport believed to be the main pathway for As transport to the grain (Carey *et al.* 2010; Zhao *et al.* 2012; Kumarathilaka *et al.* 2018).

A peptide transporter (OsPRT7) has been identified as being potentially involved in the translocation of DMA (Tang *et al.* 2017). Peptide transporters play an essential role in the transport and remobilisation of nitrogen throughout the plant (Tsay *et al.* 2007) and can affect germination, plant growth and grain yield (Fang *et al.* 2013, 2017). If DMA is transported by peptide transporters, this can offer a plausible mechanism for the translocation of DMA within rice plants (Tang *et al.* 2017). During growth and nitrogen utilisation, DMA could continually accumulate to high concentrations throughout the plant, leading to DMA stress and, in turn, straighthead in rice plants.

Plant growth

Plants exposed to As_i showed a significant decrease in plant height ($F_{(5,47)} = 13.04$, $P = 5.65 \times 10^{-8}$) over the As concentration range tested. At As_i concentrations below 1.6 μmol L^{-1}, plant height was not affected (Fig. 5); however, when As_i concentrations exceeded this value, a significant decrease in plant height occurred (1.6 μmol L^{-1} = 155 ± 84 mm, 3.5 μmol L^{-1} = 61 ± 23 mm, 6.7 μmol L^{-1} = 95 ± 31) when compared to the control, (230 ± 73 mm). For the plants exposed to DMA, there was no significant change in plant height across treatments ($F_{(5,46)} = 2.37$, $P = 0.053$); however, when DMA concentrations exceeded 1.6 μmol L^{-1}, the mean plant heights decreased (Fig. 5).

The effect of exposure to As_i and DMA on rice plant biomass showed a similar trend to that observed for plant heights (Fig. 6). Plants exposed to As_i showed a significant decrease ($P < 0.05$) in plant mass when exposed to increasing As_i concentrations ($F_{(5,84)} = 11.04$, $P = 3.43 \times 10^{-8}$). Like plant heights, exposure to the lower As_i concentrations resulted in no significant decrease in mean plant biomass, whereas the higher As_i exposures showed a significant decrease in plant biomass relative to controls.

Plants exposed to DMA also showed a significant decrease in plant mass when exposed to increasing DMA concentrations ($F_{(5,66)} = 3.133$, $P = 0.0134$). Again, when DMA concentrations exceeded 1.6 μmol L^{-1}, the mean plant biomass decreased (Fig. 6).

To determine if exposure to the As species had any effect on plant health, the final plant heights were used as a measure of plant fitness. Both modelled dose–response curves and Z scores were used to investigate the effects on plant health (see Eqn 1):

$$Z = \frac{X - \bar{X}}{s} \quad (1)$$

where $\bar{X}$ and s respectively represent the mean and standard deviation (s.d.) of the control group for each experiment, and X is the individual value. Z scores were used to normalise plant height to the control in each experiment. The percentage of plants that fell below the Z-scores for each treatment were deemed to be unhealthy (Table 2).

Plant height significantly decreased with increasing As_i dosage with $r^2 = 0.89$ when a second-order polynomial was fitted (Fig. 7). The DMA treatments displayed no significant change with increasing DMA concentration, $r^2 = 0.21$, when a second-order polynomial was fitted (Fig. 7). A 10% reduction in plant height (EC_{10}) is predicted when plants are exposed to 0.7 mmol L^{-1} As_i, and a 50% reduction in height (EC_{50}) is predicted for As_i concentrations above 2.5 mmol L^{-1} As_i (Fig. 7). The rice plants treated with DMA demonstrated no overall consistent reduction in height with increasing DMA exposure (Fig. 7); however, due to the high variability within each DMA concentration treatment, it was not possible to calculate meaningful EC_{10} and EC_{50} results.

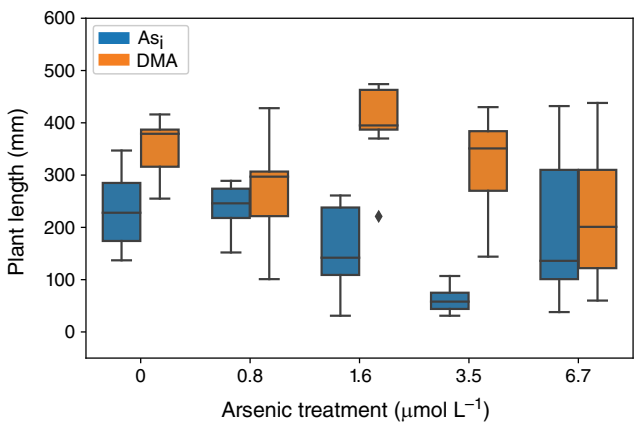

Fig. 5. Plant height at the completion of the growth experiments. Error bars represent 1 standard deviation, $n = 45$ (As_i), $n = 44$ (DMA).

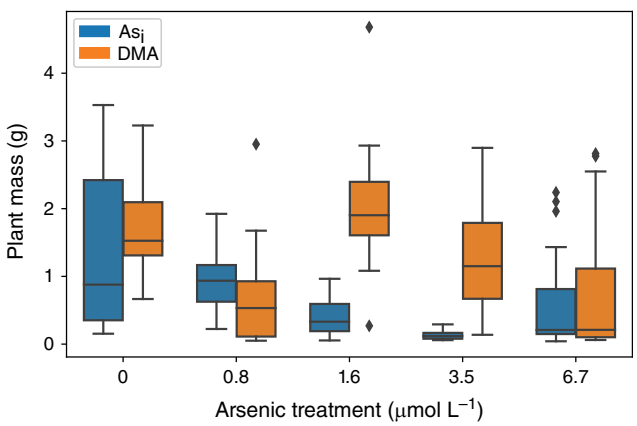

Fig. 6. Plant dry mass at the completion of the growth experiments Error bars represent 1 standard deviation, $n = 45$ (As_i), $n = 44$ (DMA).

Table 2. Percentage (%) of unhealthy plants.

Arsenic species	Treatment (μmol L^{-1})	Percentage of unhealthy plants (-2.0 *Z*-score)
As^{III}	0	0
	0.8	0
	1.6	22
	3.5	89
	6.7	22
	Reference (DMA, 6.7)	25
DMA	0	11
	0.8	38
	1.6	11
	3.5	22
	6.7	25
	Reference (As^{III}, 6.7)	44

Plants are deemed unhealthy when the height falls below a *Z*-score of 2.

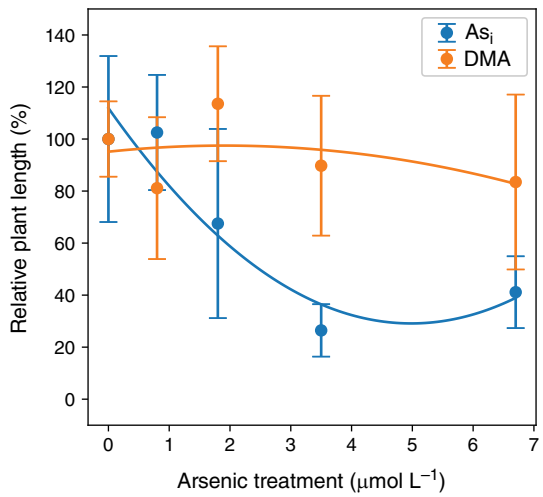

Fig. 7. Dose–response curve for rice plants treated with As_i (blue) and DMA (orange). Plant height (%) relative to the control *v.* arsenic concentration. Error bars represent 1 standard deviation, $n = 45$ (As_i), $n = 44$ (DMA).

Plants exposed to an As_i concentration greater than 1.6 μmol L^{-1} showed an increasing number of unhealthy plants (Fig. 8). Plants exposed to DMA had a consistent number of unhealthy plants across all DMA exposures (Fig. 8) with a greater number of unhealthy plants at the higher exposure concentrations that contain higher As concentrations.

Arsenic exposure has been reported to cause a reduction in both plant growth and root elongation (Han *et al.* 2015; Seneviratne *et al.* 2019). In this study, a delayed response has been observed with plant heights and mass decreasing after exposure to As concentrations greater than 1.6 μmol L^{-1}, indicating either that the rice plant has some tolerance to As_i (Song *et al.* 2014; Xu J *et al.* 2017) or that As_i is interrupting key metabolic pathways where the effects are not immediately observed (Kamiya *et al.* 2013). The rice plants exposed to the lower concentrations of As_i appear to display a degree of tolerance. As_i is most likely sequestered into the vacuoles as part of a detoxification mechanism (Song *et al.* 2014). Although As_i can cause oxidative stress to the rice plant, rice appears to handle low-level exposure, between 0.8 and 1.6 μmol L^{-1}. When exposed to between 1.6 and 3.5 μmol L^{-1}, reduction in growth and plant mass are observed; however, these plants still show the ability to regulate and limit As transport to above-ground tissues. A significant reduction in growth and increased As concentrations in the shoots was measured for the 6.7 μmol L^{-1} As_i treatment, corresponding to a substantial increase of As transport from the roots to shoots (and other plant tissues). Thus, the higher As_i concentrations used in this study could be approaching concentrations that are toxic for hydroponically grown rice. Shaibur *et al.* (2006) also found As_i toxicity to be induced in hydroponically grown rice at 6.7 μmol L^{-1}.

It is well documented that As^{III} uses silicon transporters (Ma *et al.* 2008; Katsuhara *et al.* 2014; Chen Y *et al.* 2017) and As^{V} uses phosphate transporters (Wang P *et al.* 2016) for uptake and translocation (Kumarathilaka *et al.* 2018), thus As can lead to the disruption of signalling or metabolic pathways. As^{III} also has a high affinity to sulfhydryl groups (–SH), and readily reacts with enzymes and proteins (Dixit *et al.* 2015), inhibiting enzyme activity (Chen W *et al.* 2010) affecting plant growth and metabolism (Jha and Dubey 2004). As^{V} can cause a reduction in photosynthetic activity and may delay the effects of arsenic on the health of the plant (Mateos-Naranjo *et al.* 2012; Abbas *et al.* 2018). The reduction in height and biomass (Fig. 5 and 6) observed may also be caused by an increase and imbalance in reactive oxygen species (ROS), resulting in oxidative stress throughout the plant (Stoeva *et al.* 2005; Shri *et al.* 2009). ROS are signalling compounds and intermediates produced by metabolic pathways in cells that play essential roles throughout the plants' lifecycle, including plant growth, germination and grain development (Mhamdi and Van Breusegem 2018). Other studies have reported how plants respond to high As_i concentrations, with both As^{V} and As^{III} inducing the production of ROS (Finnegan and Chen 2012) and inducing oxidative stress in the plant and eventual cell death (Hartley-Whitaker *et al.* 2001; Tripathi *et al.* 2012), with As^{III} typically having a more pronounced effect (Pessarakli and Tan 2010). Oxidative stress can cause a range of effects in plants (Finnegan and Chen 2012). Generally, this is a function of excess ROS production, leading to DNA damage, protein modification or lipid peroxidation, which results in impaired cellular function and potential cell death (Pessarakli and Tan 2010).

The effects DMA has on rice health are less clear and have not been thoroughly studied. Elevated DMA concentrations

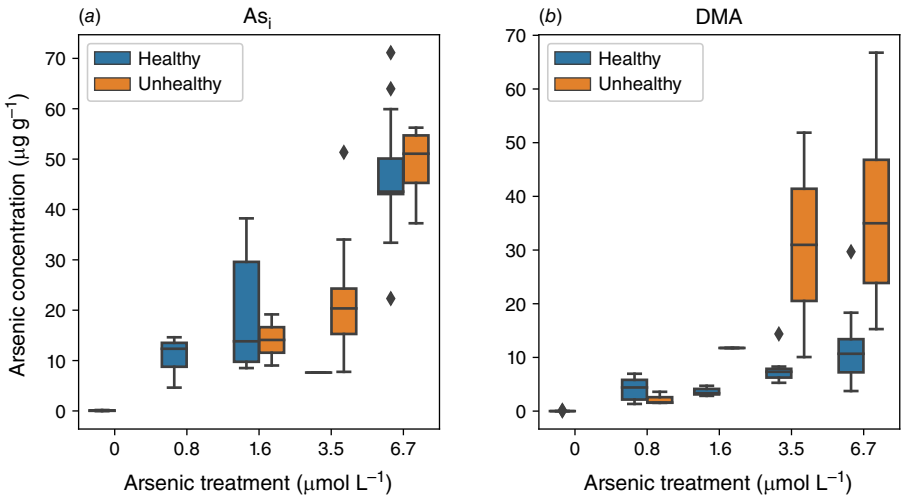

Fig. 8. Arsenic concentration in the shoots of healthy and unhealthy rice plants exposed to (*a*) As_i ($n = 45$) and (*b*) DMA ($n = 44$). Divided by healthy (blue) and unhealthy (orange) plants.

in soil have been linked to straighthead disease in rice (Yan *et al.* 2005); however, the critical role DMA plays has not been established (Meharg and Zhao 2012). Recently, several studies have found that DMA is more phytotoxic to plants than As_i (Tang *et al.* 2016*a*, 2016*b*), primarily due to its mobility within plants (Raab *et al.* 2007*b*; Carey *et al.* 2011) and the plant's inability to detoxify DMA (Tang *et al.* 2016*a*). For each DMA exposure, many plants displayed a significant reduction in growth (Fig. 8). At the higher DMA treatment concentrations, the plants with reduced growth had higher DMA concentrations in their shoots. Tang *et al.* (2016*a*) found that plants exposed to DMA exhibited significantly more oxidative stress (lipid peroxidation), particularly in the shoots, compared to plants exposed to As^V or MA.

The higher translocation of DMA could cause stress to plants by localised accumulation of DMA in different sections of the plant. This could also explain the varying effects of DMA on plant health documented in the literature (Finnegan and Chen 2012). In this study, when plant height was used as a proxy for plant health, we observed that unhealthy plants had higher DMA concentrations compared to healthy plants (Fig. 8). This trend was not observed for As_i-exposed plants. This may indicate that different As species cause different types of stress within plants, or it could demonstrate that some plants can detoxify As_i to a certain extent compared to DMA. Once a specific As concentration is reached, the production of ROS results in oxidative stress and, in some cases, cell death. Unlike As_i, which has limited translocation throughout the plant, the high mobility of DMA could result in many different parts of the plant being susceptible to DMA-induced oxidative stress, which has the potential to disrupt vital metabolic pathways.

The exact mechanism of how DMA induces toxicity in plants is still unclear. Typically, DMA^V is being analysed when DMA is quantified. DMA^{III} is unstable under aerobic conditions and is oxidised to the more stable DMA^V (Jiang *et al.* 2003). The toxicity of DMA may be induced by DMA^{III}; however, changes in DMA^{III} concentrations cannot be detected using standard methods (Garbinski *et al.* 2019; Kerl *et al.* 2019). In animal cells, the trivalent organic arsenic species (DMA^{III} and MA^{III}) are more cytotoxic than As^{III} and As^V (Petrick *et al.* 2000; Styblo *et al.* 2000). Oxidative stress can be induced through the redox cycling of DMA^{III} and DMA^V (Naranmandura *et al.* 2007). In this hydroponic experiment, due to the nutrient solution being continuously aerated DMA^{III} was unlikely to be present.

DMA may be the major cause of straighthead disease. Zheng *et al.* (2013) found that DMA was toxic to reproductive tissues, and Carey *et al.* (2011) showed that DMA is remobilised and transported to reproductive tissues at the beginning of grain formation. Under these conditions, highly localised DMA accumulation is likely to occur and cause stress to the plant.

Conclusions

Doongara rice plants responded differently when exposed to either As_i or DMA. As_i was taken up at a much faster rate into roots than DMA. Rice plants, however, were able to limit the internal transport of As_i, potentially sequestering a large amount of arsenic into vacuoles. This mechanism allowed the plant to tolerate 'low' concentrations of As_i. Rice plants exposed to DMA showed reduced ability to control the distribution of DMA within the plant once accumulated, resulting in greater translocation from roots to shoots, thus illustrating the high mobility and relative lack of detoxification strategies for DMA. Rice plants exposed to As_i and DMA both showed an overall decrease in mean plant heights and masses when exposed to increasing As concentrations, although rice plants exposed to the lower As_i concentrations display a degree of tolerance.

The results presented here highlight that DMA may have a more significant effect on rice plants than previously

thought. DMA has the potential to influence the plant's overall health and fitness, and these effects are different from the stress induced by As_i.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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