Greatly Enhanced Arsenic Shoot Assimilation in Rice Leads to Elevated Grain Levels Compared to Wheat and Barley

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Paired grain, shoot, and soil of 173 individual sample sets of commercially farmed temperate rice, wheat, and barley were surveyed to investigate variation in the assimilation and translocation of arsenic (As). Rice samples were obtained from the Carmargue (France), Doñana (Spain), Cadiz (Spain), California, and Arkansas. Wheat and barley were collected from Cornwall and Devon (England) and the east coast of Scotland. Transfer of As from soil to grain was an order of magnitude greater in rice than for wheat and barley, despite lower rates of shoot-to-grain transfer. Rice grain As levels over 0.60 μg g^{-1} d. wt were found in rice grown in paddy soil of around only 10 μg g^{-1} As, showing that As in paddy soils is problematic with respect to grain As levels. This is due to the high shoot/soil ratio of ~0.8 for rice compared to 0.2 and 0.1 for barley and wheat, respectively. The differences in these transfer ratios are probably due to differences in As speciation and dynamics in anaerobic rice soils compared to aerobic soils for barley and wheat. In rice, the export of As from the shoot to the grain appears to be under tight physiological control as the grain/shoot ratio decreases by more than an order of magnitude (from ~0.3 to 0.003 mg/kg) and as As levels in the shoots increase from 1 to 20 mg/kg. A down regulation of shoot-to-grain export may occur in wheat and barley, but it was not detected at the shoot As levels found in this survey. Some agricultural soils in southwestern England had levels in excess of 200 μg g^{-1} d. wt, although the grain levels for wheat and barley never breached 0.55 μg g^{-1} d. wt. These grain levels were achieved in rice in soils with an order of magnitude lower As. Thus the risk posed by As in the human food-chain needs to be considered in the context of anaerobic verses aerobic ecosystems.

Introduction

Chronic inorganic arsenic (As) exposure from dietary sources is of mounting concern (*1*). Meacher et al. (*2*) conclude that food followed by drinking water is the greatest source of inorganic As intake for the U.S. population. Rice is the primary source of As exposure in a nonseafood diet, typically possessing higher inorganic As levels than seafood (*3*). The range reported in rice grain As levels varies from less than 0.01 to 2.05 μg g^{-1} (*4*). For typical Asian American diets, As exposure from rice even at inorganic As levels of 0.10 μg inorganic As g^{-1} exceeds ingestion of 1 L of 10 μg L^{-1}, the volume and As concentration on which current U.S. regulations are set (*1*). In a study of inorganic As exposure, surveying predominantly elderly white caucasian males in the mid-western U.S. (typically not a high rice consumer group), food was found to be a significant As exposure route, explaining 37% of total variance (*5*). Arsenic exposure from food is even more important for those reliant on rice subsistence diets, where dietary As exposure from rice alone can be considerable (*4*).

Recent studies have shown that in rice there is large varietal variation in the As sequestered in root surface iron plaque and in As uptake and shoot As transport (*4*, *6–8*), leading to the possibility of breeding cultivars with low grain As levels. In wheat Liu et al. (*9*) showed large differences in seed germination upon exposure to As^{III} and As^{V}, while Geng et al. (*10*) showed cultivar differences in growth resulting from As exposure. There is a clear impetus for furthering the understanding of soil–root, root–shoot, and shoot–grain assimilation and export not just in rice but also in all the major grain crops. In this study As levels for soil, shoot, and grain of temperate rice, wheat, and barley were obtained so that grain As levels could be reviewed in regard to both soil and shoot As levels. This study can be differentiated from other surveys because it is the transfer of As from the rhizosphere soil through the plant to the grain that is explored—not just total grain levels.

In anaerobic paddy soil systems As is more mobile than in aerobic wheat soils; thereby suggesting separate soil As threshold criteria for the different crops. This survey explores the validity of this by comparing rice and wheat collected in the field and also in pot experiments spiked with As.

Methodology

Crop and Soil Survey. Paired grain, shoot, and soil samples from commercially farmed temperate crop fields were surveyed at harvest, with one sample set per field sampled. Rice samples were obtained from the Carmargue (France), Doñana (Spain), Cadiz (Spain), California, and Arkansas. Wheat and barley were collected from Cornwall and Devon (England) and the east coast of Scotland. Each field/sample was formed from the bulking of triplicate grain panicle, shoot, and soil (0–10 cm depth/ 10–20 g) subsamples, with triplicate samples taken at 0.5 m spacings. Regions were selected under the premise of suspected variability in agricultural soil As levels (*1*, *11*, *12*). In each case the soil directly under the sampled plant was sampled.

For comparison, high As exposure greenhouse pot experiments were conducted for rice and wheat plants to observe shoot–grain As relationships under controlled growing conditions on uniform soil spiked with As for known cultivars. The results were then applied to predict/model shoot and grain As accumulation for serious contamination scenarios.

Pot Experiment. Experimental information for rice are detailed in Abedin et al. (*7*). Seeds for two wheat varieties were germinated and then transferred to 1 L free-draining pots containing clay rich subsoil, as used and characterized in the experiments of Abedin et al. (*7*). The soils were either

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untreated or had monosodium arsenate added to a concentration of 10, 25, and 50 μg As g^{-1} soil dry weight. The plants were then grown in a temperate green house (average temperature 25 °C) with sodium lighting providing a supplement of 150 μ mol m^{-2} s^{-1} photosynthetically active radiation (PAR) until plants had set seed (3 months). Three wheat plants, one per pot, of each variety replicated each treatment. After seed set, seeds and shoots were harvested.

Chemicals. Aristar grade reagents were used exclusively throughout the analysis. Nitric acid was obtained from VWR International. The 1000 mg/L standards As and In were obtained from Merck.

Statistics. General linear modeling (GLM) and the Mann Whitney test were conducted using Minitab v.14 (State College, PA). Data used in the GLM were ranked prior to analyses to normalize distribution.

Sample Preparation. *Plant: Rice, Wheat, and Barley Shoot and Grain.* All material was washed with deionized water to remove soil, air-dried at room temperature, then powdered, and oven dried at 90 °C for 48 h. In the case of rice and barley, any husks (glumellae) were removed. None of the grain was polished; therefore, all bran layers were maintained prior to milling. Approximately 0.200 g d. wt of milled grain and 0.100 g d. wt of milled shoot was weighed out into 50 mL polypropylene digest tubes, and 2 mL of concentrated nitric acid was added. The mixture was left to imbibe overnight. Samples were digested in a microwave oven (CEM Mars 5, CEM Corp., Matthews, NC). The temperature was gently raised, first to 55 °C (and held for 10 min) and then to 75 °C (and held for 10 min). Finally the digest was taken up to 95 °C and maintained for 30 min. Samples were cooled to room temperature, 0.500 g of 100 μg In L^{-1} was added, and then the sample was diluted to a mass of 50.000 g with ultrapure deionized water (18.2 MΩ) obtained from a Milli-Q system (Millipore).

Soil. Soils were oven dried at 90 °C for 48 h, then sieved (mesh size = 2 mm). Subsamples of 0.200 g d. wt were then dispensed into quartz glass tubes and blended with 2 mL of concentrated nitric acid. The mixture was allowed to predigest at room temperature for 12 h, before the addition of hydrogen peroxide (2 mL). Digests were then raised to 140 °C, on a heating block, for 6 h. Once at room temperature the solutions were diluted with ultrapure deionized water, first to a volume of 10 mL, from which a 1 mL aliquot was mixed with 0.1 mL of 1000 μg In L^{-1} and taken to a volume of 10 mL.

NIST certified reference material (CRM) 1568a Arkansas long grain rice flour and NCS ZC73007 and GBW 07405 soil CRMs were used to validate the analyses. Quality controls of CRMs, spikes, and blanks were run with each plant or soil digest batch of ~40 samples—which were determined randomly using Microsoft Excel 2000.

Total Element Detection. An Agilent 7500c (Agilent Technologies, Tokyo, Japan) ICP-MS with a Meinhard nebulizer was used to measure the elements Ti (m/z 49), As (m/z 75), Se (m/z 77, 78, 82), and In (m/z 115). The following m/z 77, 78, 82 were measured in order to identify polyatomic $Ar^{40}Cl^{35}$ interferences on m/z 75. Corrections for interference from $Ar^{40}Cl^{35}$ were not found to be necessary. Samples were randomized prior to analysis. Standards were run after every set of 40 samples. Every tenth sample was digested and analyzed in duplicate (Tables S1−3; all Tables and Figures with an "S" prefix can be found in the Supporting Information.). All sample vials were cleaned then soaked in 10% v/v nitric acid for a minimum of 12 h, then washed with deionized water, and air-dried prior to use. Concentrations were determined using five-point calibrations (Table S4) calculated from a multielement standard.

Mean As recovery from the rice CRM was 104 ± 1% (n = 26). The mean recoveries for the soil CRMs were 84 ± 1% (n = 9) and 82 ± 1% (n = 3), respectively, for NCS ZC73007 and GBW 07405. Plant spike recoveries for 5 μg L^{-1} were 89 ± 2% (n = 15) and for 10 μg L^{-1} 93 ± 3% (n = 11). Soil spike recoveries for 5 μg L^{-1} were 91 ± 1% (n = 5) and for 50 μg L^{-1} 87 ± 2% (n = 7). The presented data has not been corrected for these recoveries. Further quality control data can be found in Table S5.

Results

Summary of As in Grain, Shoot, and Soil. *Grain.* Carmargue (France) rice grain was found to be the most elevated in As. Mean and median grain As levels (n = 22) were 0.32 μg g^{-1} d. wt and 0.34 μg g^{-1} d. wt, respectively, and the maximum recorded level, 0.66 μg g^{-1}d. wt, was the highest found in this survey (Table 1). These levels are comparable to tube-well-irrigated rice grown in Bangladesh's groundwater-As-elevated districts of Chuadanga (mean = 0.32 μg g^{-1} d. wt, n = 27) and Satkhira (0.38 μg g^{-1} d. wt, n = 14) (*13*). Inorganic As grain levels of 0.28 μg g^{-1} d. wt have subsequently been observed in market bought Carmargue rice grain (unpublished data).

Mean grain levels for Arkansas rice were 0.20 μg g^{-1} d. wt, the maximum value observed being 0.43 μg g^{-1} d. wt. The levels in Californian rice averaged 0.13 μg g^{-1} d. wt and did not exceed 0.18 μg g^{-1} d. wt (Table 1). In a related study (*1*) of 134 U.S. supermarket rice samples, the south central (AR, MI, MO, LA, TX) processed grain exhibited significantly higher levels than those from California.

In 1998 over 45 km^2 of land (~30 km^2 farmland), including rice paddies, were affected by As-enriched mine waste, resulting from a breach from a tailings lagoon in Aznalcóllar (southern Spain) that released 5 000 000 m^3 of acidified water and soil (*11*, *14*). Rice was collected from impacted Doñana fields and from Cadiz (an unindustrialized area) to provide a comparison. Our data shows that the rice grain from Doñana is not particularly elevated in As, with mean and median grain levels of 0.16 μg g^{-1} d. wt and 0.15 μg g^{-1} d. wt, respectively (Table 1.). Despite average soil As levels ~4 times lower in As, the grain levels from Cadiz were only fractionally less, with mean and median levels of 0.13 μg g^{-1} d. wt and 0.14 μg g^{-1} d. wt, respectively.

Mean wheat and barley grain As levels from Scotland were 0.03 μg g^{-1} and 0.04 μg g^{-1} d. wt, respectively (Table 1). Wheat and barley from southwestern England were approximately twice as high with mean levels of 0.07 μg g^{-1} and 0.08 μg g^{-1}, respectively. Our data is concurrent with a field survey from The Netherlands that found a mean wheat grain level of 0.05 μg g^{-1} d. wt (assuming 15% water content (*15*)) for 84 samples and a mean barley grain level for 0.08 μg g^{-1} d. wt (assuming 15% water content (*15*)) for 45 samples (*15*). In the U.S. the average grain levels are reported as 0.02 μg g^{-1} d. wt (*16*, *17*); however, levels lower than this are not unusual. In U.K. field experimental plots of wheat grain grown under soil compaction and irrigation treatments, mean grain levels of <0.01 μg g^{-1} in two successive years were recorded (*18*).

The highest Scottish grain was a wheat sample with a level of 0.21 μg g^{-1} d. wt (Table 1). In southwestern England both wheat and barley grains were found with levels of ~0.50 μg g^{-1} d. wt (Table 1). This is higher than the maximum levels found in the Dutch study for wheat and barley at 0.33 μg g^{-1} d. wt and 0.43 μg g^{-1} d. wt (assuming 15% water content (*15*)). However this is still considerably lower than the level of 0.74 μg g^{-1} d. wt observed by Norra et al. (*19*), in a southeastern Asia wheat, which is comparable to grain levels of 0.75, 0.71, and 0.69 μg g^{-1} d. wt found in wheat grown in a greenhouse in 50 μg As g^{-1} spiked soil (current study).

Shoot. The As level in shoots was highly variable, albeit in general the levels for rice, wheat, and barley were less than 5 μg g^{-1}. A notable exception to this was the Carmargue rice, whose average (mean and median) levels were over twice that of any other area and the maximum recorded

TABLE 1. Arsenic Distribution in Rice, Wheat, and Barley Grain, Shoot, and Soil by Production Region

crop	country	region		As level, $\mu g\ g^{-1}$ min−max	mean	median	n
rice (*Oryza*)	France	Carmargue	grain	0.12−0.61	0.32	0.34	22
			shoot	1.5−20.6	10.2	6.8	23
			soil	5−10	8	8	23
	Spain	Doñana	grain	0.06−0.29	0.16	0.15	25
			shoot	0.8−9.8	3.3	2.6	25
			soil	4−11	8	7	25
		Cadiz	grain	0.07−0.21	0.13	0.14	10
			shoot	0.4−3.3	1.4	1.2	10
			soil	1−2	2	2	10
	U.S.A.	California	grain	0.08−0.18	0.13	0.11	9
			shoot	0.4−1.3	0.7	0.7	9
			soil	2−4	3	3	9
		Arkansas	grain	0.08−0.43	0.20	0.18	6
			shoot	0.7−3.4	1.5	1.3	6
			soil	4−7	6	6	6
wheat (*Triticum*)	Scotland	East Coast	grain	0.01−0.21	0.03	0.02	29
			shoot	0.0−2.1	0.2	0.1	29
			soil	3−18	7	6	29
	England	Cornwall, Devon	grain	0.01−0.50	0.07	0.04	37
			shoot	0.1−1.6	0.3	0.2	35
			soil	6−201	33	21	37
barley (*Hordeum*)	Scotland	East Coast	grain	0.03−0.05	0.04	0.04	6
			shoot	0.1−0.2	0.1	0.1	6
			soil	6−10	7	7	6
	England	Cornwall, Devon	grain	0.01−0.54	0.08	0.03	29
			shoot	0.1−1.8	0.4	0.2	29
			soil	6−546	57	25	29

sample was 20.6 $\mu g\ g^{-1}$ d. wt. It has been demonstrated that rice has a considerable capacity for assimilating As within the shoot. In a pot experiment by Abedin et al. (*7*), continual irrigation of rice with As-contaminated water resulted in shoot levels in excess of 100 $\mu g\ g^{-1}$ d. wt. Tsutsumi (*20*) observed shoot levels of 149 $\mu g\ g^{-1}$ in rice shoot grown in 312 $\mu g\ g^{-1}$ soils.

In this survey wheat and barley shoot mean and median values were below 1 μg As g^{-1} d. wt in agreement with previous levels found in cereals and straw of U.K. livestock feeds (*21*). However for 5% of the samples levels over 1 $\mu g\ g^{-1}$ were recorded, with the highest being 2.1 μg As g^{-1} d. wt.

The ability of As to accumulate in wheat and barley has already been shown. Greenhouse pot experiments of wheat grown in 50 μg As g^{-1} spiked soil resulted in shoot levels of ~3 $\mu g\ g^{-1}$ d. wt (Table S6), while levels of over 30 $\mu g\ g^{-1}$ d. wt. were found in barley shoot grown in soils amended to 250 μg As g^{-1} d. wt (*22*). Similarly, for wheat and barley grown in As-elevated soil (range 53.8−709 $\mu g\ g^{-1}$), contaminated by the Aznalcóllar mine spill, levels of ~20 $\mu g\ g^{-1}$ d. wt were detected in the shoots (*14*).

To establish if grain and shoot As were a consequence of surface contamination by soil (i.e., incorporation of soil particles in the analyses of grain and shoot) or direct plant uptake, Ti levels were monitored. Ti, being a nonessential trace element in crops, is found at very low relative levels in plants compared to soil. Regressions for grain and shoot Ti against grain and shoot As were found to be nonsignificant (shoot: $R^2 = 0.0009$; slope = −0.0400; $p = 0.6295$) (grain: $R^2 = 0.0095$; slope = −0.0850; $p = 0.1277$), indicating the unlikelihood of surface contamination (Figure S1.).

Soil. The counties of Devon and Cornwall in southwestern England have soils naturally elevated in As due to As mineralization surrounding the granitic intrusions, with As levels further elevated, considerably in places, by millennia of base and precious mining and smelting, including specifically an As extraction and processing industry (*12*). Total As in over half the soils in this survey from southwestern England were above normal baseline levels. Out of 66 samples, 53% recorded levels over the guideline values set in the U.K. for residential and allotment soils of 20 μg As g^{-1} d. wt (*23*). In Canada, regulatory limits of As contamination in agricultural soils are 25 $\mu g\ g^{-1}$ d. wt (*24*). Samples in excess of guideline values do not necessarily indicate a direct health risk but act as a trigger where further investigation is required; in some cases, remediation or changes in land use must be implemented (*25*). In the case of 5% of the samples, highly elevated levels over 100 $\mu g\ g^{-1}$ were detected, with the maximum level being 546 $\mu g\ g^{-1}$ (Table 1). Similarly, high levels have been observed in other studies such as a field trial on a farm in Cornwall, formally an As smelter, and the total soil level was found to be 748 $\mu g\ g^{-1}$ d. wt (*26*).

Conversely the average As levels from French, Spanish, and U.S. paddy soils and the Scottish wheat and barley soils were below 10 $\mu g\ g^{-1}$ d. wt (Table 1). Despite the high shoot and grain As levels found in Carmargue rice, the total soil concentration was comparably low (5−10 μg As g^{-1} d. wt). Cadiz and California had the lowest median soil levels of 2 $\mu g\ g^{-1}$ d. wt and 3 $\mu g\ g^{-1}$ d. wt, respectively (Table 1).

Discussion

Arsenic Transfer from Soil to Shoot and Grain. Some regional differences existed in average As shoot/soil transfer factors (S^{hoot}/S_{oil} TFs). The proportional transfer of As from soil to wheat and barley in Cornwall and Devon was lower than that of Scotland, indicating reduced bioavailibity. For rice, regional median S^{hoot}/S_{oil} TFs were more variable. The transfer of As from soil to shoot followed the order Carmargue > Cadiz > Doñana > California > Arkansas (Table S7). The range of S^{hoot}/S_{oil} TFs was considerable (Tables S7 and S8). Minimum and maximum S^{hoot}/S_{oil} TFs for rice were 0.05−3.84; for wheat, 0.002−0.142; and for barley, 0.002−0.095. There was good agreement between mean and median S^{hoot}/S_{oil} TFs for wheat (mean = 0.018; median = 0.013) and barley (mean = 0.011; median = 0.010) (Tables S7 and S8, Figure S2.). In rice the median S^{hoot}/S_{oil} TFs were nearly 50 times

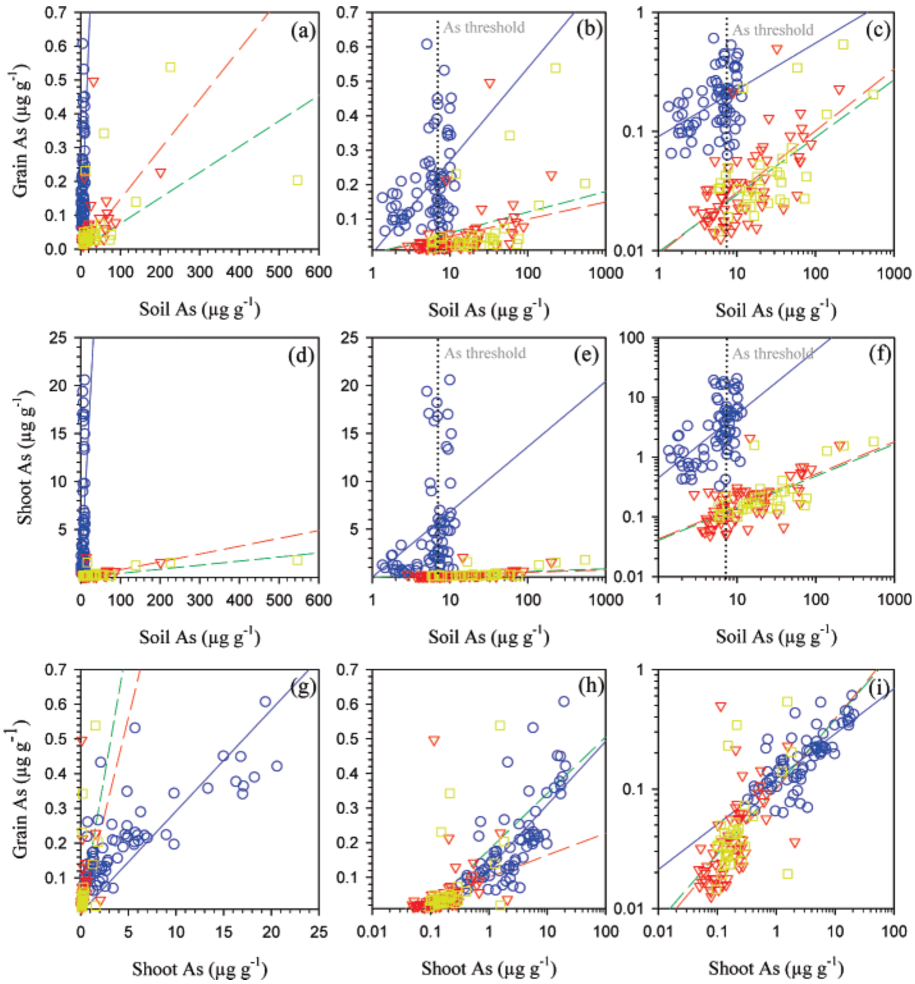

FIGURE 1. Arsenic transfer in grain crops. Regression analysis of (a−c) soil vs grain, (d−f) soil vs shoot, and (g−i) shoot vs grain. Data points: rice (blue ○); wheat (red ▽); barley (yellow □). Regression plots: rice = solid blue line; wheat = dashed red line; barley = dashed green line.

higher than in the wheat or barley. This is shown in Figure 1 (d−f). A similar trend was observed in As grain/soil transfer (G^{rain}/S_{oil} TF) (Figure S2) with rice having higher median values 13 and 20 times greater than median values for wheat and barley, respectively (Tables S7 and S8, Figure S2). Figure 1a−c shows this in a graphical context. There was little variation in regional G^{rain}/S_{oil} TFs for rice, wheat, or barley. This highlights the importance of ascertaining shoot As levels when considering the relationship between soil and grain.

The fate of As in soil is governed by key physiochemical properties (e.g., pH, Eh, organic carbon, texture, Fe/Al/Mn oxides, and S, P, and As concentration) (*27*−*29*), factors that can differ markedly between soil types (*25*), growth seasons, and land use (*18*). Song et al. (*25*) found a 49-fold difference in the EC10 (As dose causing 10% inhibition) in barley root elongation assays of 16 European soils. Zhu et al. (*30*) found considerable differences in the inorganic phosphate transporters of wheat cultivars to discriminate against As^V. Yet despite the widespread variation associated from the collection of soils from distinct geogenic origins and from plants of differing parentage, regressions of log grain As versus log soil As levels were highly significant ($p < 0.001$). R^2 values for rice, wheat, and barley were 0.17, 0.41, and 0.38, respectively (Table S11). Modeling soil As transfer into plants was greatly improved by regressing log shoot As versus log soil As levels, and each was still highly significant ($p < 0.001$), explaining 31%, 43%, and 52% of the variation for rice, wheat, and barley, respectively (Table S11).

Arsenic valency determines bioavailability (*29*), with As^{III} mobility being greater than As^V, because of desorption from Fe (hydro)oxides (*27*). In the anaerobic or anoxic bulk soil environment of a rice paddy, As^{III} predominates (*27*, *29*). In soils favorable for wheat and barley cultivation, oxygen availability is high creating aerobic or (sub)oxic conditions, where As^V prevails. Generic As soil thresholds based on total As levels are therefore superfluous if As sorption−desorption equilibria are not taken into consideration. Flooded soils with a total As level of 10 $\mu g\ g^{-1}$ can result in rice with highly elevated grain being produced (Table 1). In contrast, soil As thresholds set for rice would be unnecessary and uneconomical in the context of aerobic crop systems.

Differences between inter- and intraspecies specific variation in soil solution transfer of As across root plasma membranes is also of importance. Comparisons of root As transporter efficiency (based on the V_{max}(maximum transport velocity)/K_m (Michaelis constant)) of As uptake reveals that rice As uptake is more efficient than in wheat (Figure S13) (*8*, *9*). In addition, the efficiency of As^{III} and As^V are similar in wheat while there is disparity between the species in uptake in rice roots, with the efficiency of As^V transport being 2−3 times higher than As^{III} (Figure S13) (*8*, *9*). In a recent experiment by Raab et al. (*31*), there was ∼2 fold increase in root As^V concentration between As tolerant and As susceptible rice cultivars when exposed to a solution of 1 mg L^{-1} As^V for 24 h.

Arsenic Transfer from Shoot to Grain. Table S9 and Figure 2 shows that there is good agreement in the shoot-to-grain transfer of As from field-collected and high As-spiked pot experiment samples for rice and wheat. Comparison of the regression gradients (Table S9) of field and pot rice are

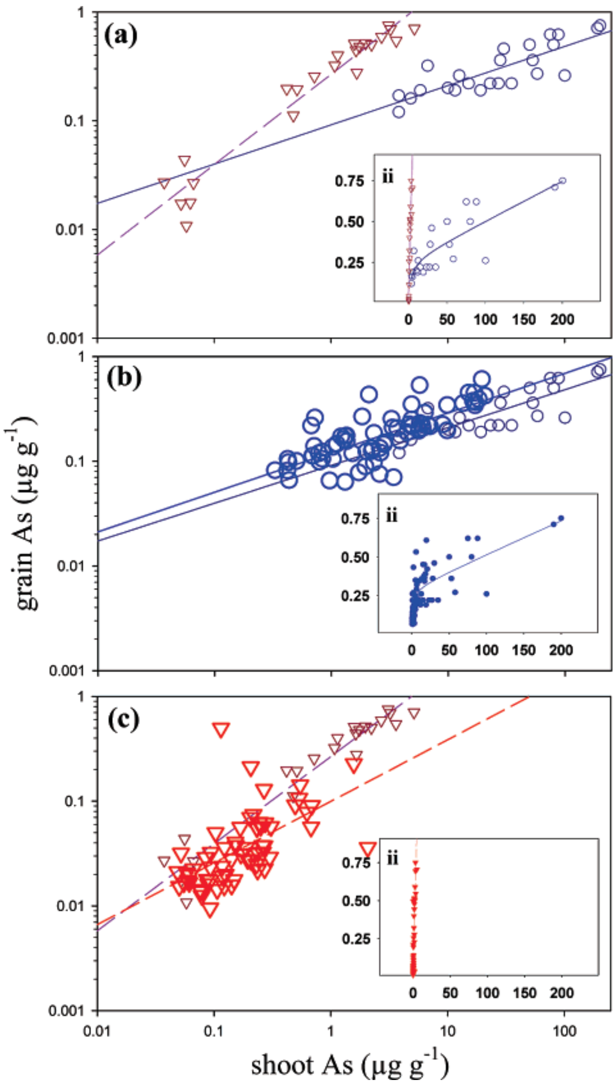

FIGURE 2. Comparison of field and pot experimental data: regressions of shoot and grain As. (a) Pot experiment: rice = blue ○; wheat = purple ▽; ii = unlogged data. (b) Rice: field samples = blue ◯; pot experiment = blue ○; ii = field and pot (unlogged data). (c) Wheat: field samples = red ▽; pot experiment = purple ▽; ii = field and pot (unlogged data).

close, 0.38 and 0.36, respectively. A slight variation existed between field and pot regression gradients for wheat; however, both were consistently higher than those calculated for rice, indicating a greater shoot-to-grain export of As. Median grain/shoot transfer factors (G^{rain}/S_{hoot} TFs) were comparable between pot experiment and field-collected samples (Figure S3.).

Large variation exists in the shoot export of As to the grain. This is illustrated by minimum and maximum grain/shoot transfer factors (G^{rain}/S_{hoot} TF) of 0.02−0.36 in rice, 0.017−4.335 in wheat, and 0.01−1.59 in barley (Tables S7 and S8). However, median G^{rain}/S_{hoot} TF values for field rice were ~0.05, for wheat ~0.2, and for barley ~0.2, indicating that G^{rain}/S_{hoot} TFs approaching or exceeding 1 were uncommon (Figure S3).

Median G^{rain}/S_{hoot} TFs for wheat and barley (0.19 and 0.20, respectively) were 4 times higher than rice (0.05) (Tables 1 and 7, Figure S3), although median shoot As levels were lower. To determine the difference of crop type on grain level a GLM was employed, using shoot As level as a covariate. Six outliers were removed to normalize the distribution. The results showed no significance for crop type ($p = 0.098$), but

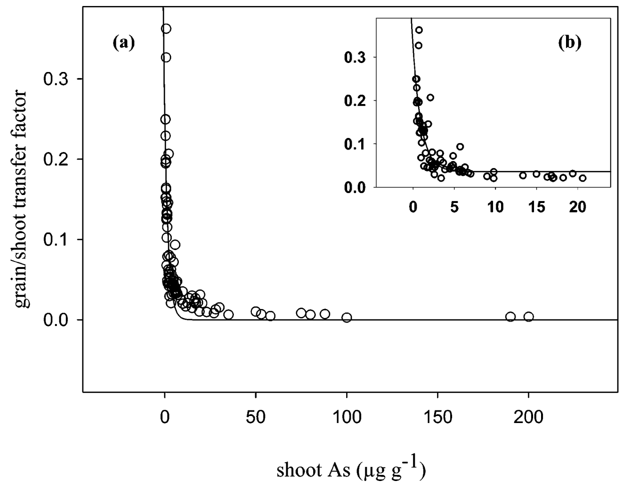

FIGURE 3. Grain/shoot As transfer factor in rice decreases with increasing shoot As. (a) Field and pot experimental data. (b) Field data.

a shoot As level−crop type interaction was highly significant ($p < 0.001$). In direct comparisons between the grain As level of different crops, shoot As should be considered. In a biplot (Figure 3) for rice, shoot As levels against G^{rain}/S_{hoot} TFs samples followed an exponential decay/decrease in G^{rain}/S_{hoot} TFs with increases in shoot As. At shoot As levels lower than 5 μg g^{-1} d. wt, G^{rain}/S_{hoot} TFs ranged from 0.02 to 0.36; above 5 μg g^{-1} d. wt, G^{rain}/S_{hoot} TFs were less than 0.1.

In a field experiment investigating As uptake in rice from soil amended by municipal solid waste compost and cow dung manure, G^{rain}/S_{hoot} TFs of ~0.70 were observed in plants grown under the manure treatment with shoot As levels of 0.50 μg g^{-1} d. wt (*32*). G^{rain}/S_{hoot} TFs were halved when urea was included with the manure. In an As-irrigated pot experiment growing rice, by Islam et al. (*33*), G^{rain}/S_{hoot} TFs were halved as shoot As levels increased from 2.29 μg g^{-1} to 6.53 μg g^{-1} d. wt. Nitrogen levels in the shoot additionally increased, with rises in shoot As levels concurring with Williams et al. (*4*). Differences in G^{rain}/S_{hoot} TFs were not as apparent in wheat and barley, although in general higher shoot As levels were consistent with low G^{rain}/S_{hoot} TFs.

Graphical analysis of combined field and pot experiment data for rice (Figure 3) suggests that the concentration dependency of shoot-to-grain transfer is modeled by an exponential decay curve with an inflection point at shoot levels of between ~2−5 μg g^{-1} d. wt. Fitting of a regression exponential decay model to the 96 samples and using shoot As level to predict subsequent grain As accounted for 63% of total variation (Figure S4). Down regulation of the shoot−grain export of As at higher shoot levels could explain why regressions of grain and soil As are often poor (*34*). A linear model best predicted wheat grain levels ($R^2 = 0.76$) (Figure S5), indicating no suppression of shoot−grain transfer at the shoot As levels detected in this survey.

The nature of any physiological trigger controlling shoot−grain As transfer is speculative. Lipid peroxidation and other tissue damage caused by reactive oxygen stress induced by As levels in the shoot could be important. Geng et al. (*35*) show clear varietal differences in the ability of rice to cope with physiological stress resulting from As exposure. P-deprivation increases the toxicity of As in rice (*35*). Nutritional dynamics within the plant may also determine shoot−grain export of As. Increases in shoot N levels appear concurrent with lower G^{rain}/S_{hoot} TFs (*4*, *32*, *33*). This link should be further explored. Sequestration of As in shoot tissue is unclear, and further understanding of the labile pool of As that is available for grain export is yet to be established. Additionally, speciation must also be considered. Raab et al. (*31*) have shown that in general methylated species are rapidly moved from the roots into shoot tissue; therefore given the differences in relative toxicity between organic and inorganic As, calculating species specific G^{rain}/S_{hoot} TFs would be of value in the screening of cultivars that produce grain with a low inorganic As content.

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Supporting Information Available

All Tables and Figures with an "S" prefix can be found in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org

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