Exposure and Health (2020) 12:711–719
https://doi.org/10.1007/s12403-019-00330-y

ORIGINAL PAPER

Global Sourcing of Low-Inorganic Arsenic Rice Grain

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Received: 22 August 2019 / Revised: 21 October 2019 / Accepted: 29 October 2019 / Published online: 12 November 2019

Abstract

Arsenic in rice grain is dominated by two species: the carcinogen inorganic arsenic (the sum of arsenate and arsenite) and dimethylarsinic acid (DMA). Rice is the dominant source of inorganic arsenic into the human diet. As such, there is a need to identify sources of low-inorganic arsenic rice globally. Here we surveyed polished (white) rice across representative regions of rice production globally for arsenic speciation. In total 1180 samples were analysed from 29 distinct sampling zones, across 6 continents. For inorganic arsenic the global $\tilde{x}$ was 66 µg/kg, and for DMA this figure was 21 µg/kg. DMA was more variable, ranging from < 2 to 690 µg/kg, while inorganic arsenic ranged from < 2 to 399 µg/kg. It was found that inorganic arsenic dominated when grain sum of species was < 100 µg/kg, with DMA dominating at higher concentrations. There was considerable regional variance in grain arsenic speciation, particularly in DMA where temperate production regions had higher concentrations. Inorganic arsenic concentrations were relatively consistent across temperate, subtropical and northern hemisphere tropical regions. It was only in southern hemisphere tropical regions, in the eastern hemisphere that low-grain inorganic arsenic is found, namely East Africa ($\tilde{x}$ < 10 µg/kg) and the Southern Indonesian islands ($\tilde{x}$ < 20 µg/kg). Southern hemisphere South American rice was universally high in inorganic arsenic, the reason for which needs further exploration.

Keywords Arsenic · Global · Rice

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Introduction

Paddy rice is the dominant source of the carcinogen inorganic arsenic to the human diet (EFSA 2009; EU 2015; WHO 2014). The concentrations of inorganic arsenic in rice are of such concern that both the WHO (2014) and EU (2015) have set Maximum Levels (MLs). Identifying where and why rice is low in arsenic is important for developing strategies to lower inorganic arsenic in the diet. Regional studies have shown that with respect to regulation of arsenic species in rice grain that there is a complex interaction between plant physiology and genetics, soil biogeochemical cycling, paddy management practice, natural bedrock geology and soil diagenesis and environmental contamination (Meharg and Zhao 2012). In particular, point source paddy contamination through mine spoil contamination (Zhu et al. 2008), other industrial activity (Li et al. 2015) and coal burning (Li et al. 2015) have all been identified as major sources of soil pollution. Irrigation can be important source of arsenic to paddy fields, such as for the Bengal Delta where elevated groundwaters are used for dry season rice cultivation (Williams et al. 2006).

Arsenic, generally, is either naturally or anthropogenically inputted into soils in the inorganic state. Under paddy conditions soil microflora can convert inorganic arsenic to methylated species (Reid et al. 2017), with the dimethylarsinic acid (DMA) so produced being readily translocated to grain (Carey et al. 2010). Geographically, the relative contribution of inorganic arsenic and DMA to grain arsenic is known to vary considerably, with higher concentrations of DMA observed in temperate growing regions (Meharg et al. 2009; Zavala et al. 2008), though the reasons underpinning this variation are not known.

To gain a better understanding of the variation in rice grain arsenic there is a need to determine if there are any global patterns and trends across the growing regions of this crop. In particular, this needs integration of S. American, African and the S.E. Asian archipelago that are either under reported in the literature, or only feature in inter-regional comparisons to a limited extent (Adeyemi et al. 2017; Adomako et al. 2011; Farias et al. 2015; Joy et al. 2017; Otero et al. 2016; Segura et al. 2016). This knowledge is required to source low arsenic rice for sub-populations where the consumption of inorganic arsenic from rice is of particular concern, such as for infants and young children (Carey et al. 2018). Here we set out to determine the variation in rice grain arsenic concentrations and speciation across representative growing regions globally. Twenty-nine cultivation regions, across 6 continents, that spanned the world's major paddy rice growing areas, were selected and market polished (white) rice (to reflect actual dietary consumption) were analysed for arsenic speciation.

Materials and Methods

Rice Sample Collection

Polished, locally produced, market rice, $n = 1180$, was purchased from retailers from 29 distinct sampling regions from 6 continents. Surveyed regions and sampling frequency are given in Table 1, and mapped in Fig. 1. Given the variability in rice post-harvest processing and how rice is distributed and retailed from country to country, and the geographic scale of the task (i.e. covering all rice global regions systematically is not possible without enormous resource), approaches to rice collection are dependent on the location and geographic reach of collaborating scientists. Accessible market stalls and supermarkets were targeted to source rice. Also, sample density per region varied as well (Table 1). All, this must be born in mind with respect to the interpretation of the data. This sampling approach is comparable with previous literature publications on market rice arsenic (Meharg et al. 2009; Zavala et al. 2008). Where possible (that sampling density supported the sub-division of samples), data were collated within regional blocks for geographically large rice growing regions such as Brazil, Spain and Vietnam. Sampling structure and density is outlined in Table 1.

Sample Preparation

Rice samples were freeze dried and then milled to a fine powder using a Retsch Planetary ball-mill (Germany), lined with a zirconium oxide coated grinding chamber and milling balls. For As speciation freeze-dried milled rice was weighed accurately to a weight of 0.1 g into 50 ml polypropylene centrifuge tubes to which 10 ml of 1% conc. Aristar nitric acid was added and allowed to sit overnight. Batches of up to 48 samples were prepared which also included 2 blanks and 2 rice flour CRM NIST 1568b in all analytical batches. Samples were then microwave digested in an CEM MARS 6 instrument for 30 min. at 95 °C using a 3-stage slow heating programme: to 55 °C in 5 min. held for 10 min., to 75 °C in 5 min., held for 10 min. to 95 °C in 5 min., held for 30 min.. The digestate, on cooling, was accurately diluted to 10 ml with deionized distilled water and centrifuged at 4500 rpm for 15 min. A 1 ml aliquot was transferred to a 2 ml polypropylene vial and 10 µl of analytical grade hydrogen peroxide was added to convert any arsenite to arsenate to facilitate subsequent chromatographic detection. The NIST rice flour CRM NIST 1568b was included in each batch of rice tissues to ascertain percentage recoveries, and reagent blanks were also included, as per arsenic speciation.

Table 1 Sampling locations, modelled atmospheric air concentrations and grain median, 25th and 75th percentiles, minimum and maximum and number of samples collected for each location

Region	Country	Long. (°)	Lat. (°)	Arsenic species	No. of values	Min. (µg/kg)	25% Per. (µg/kg)	Median (µg/kg)	75% Per. (µg/kg)	Max. (µg/kg)	As air conc. (ng/m^3)
S. America	Argentina	− 58.7	− 27.0	DMA	11	18	29	45	175	222	0.41
				inorg. As		38	47	61	88	94	
				Σ As sp.		64	84	127	260	316	
	Bolivia	− 62.5	− 14.1	DMA	9	2	6	38	74	430	0.15
				inorg. As		5	40	66	114	154	
				Σ As sp.		6	47	147	181	529	
	N. Brazil	− 60.7	2.7	DMA	9	2	7	18	58	79	0.04
				inorg. As		35	43	62	72	81	
				Σ As sp.		36	63	92	112	125	
	C. Brazil	− 61.9	− 11.5	DMA	10	2	15	66	96	690	0.09
				inorg. As		2	38	73	91	105	
				Σ As sp.		3	78	124	177	781	
	S. Brazil	− 53.9	− 29.8	DMA	97	2	57	88	129	614	0.50
				inorg. As		21	62	77	96	245	
				Σ As sp.		39	147	169	211	700	
	Chile	− 70.2	− 33.5	DMA	11	59	105	138	147	189	30.00
				inorg. As		48	59	60	66	68	
				Σ As sp.		112	164	198	207	256	
	Paraguay	− 56.4	− 27.2	DMA	27	14	47	86	117	207	0.41
				inorg. As		48	96	107	141	155	
				Σ As sp.		103	144	183	243	358	
	Uruguay	− 56.3	− 34.7	DMA	8	33	55	113	152	194	0.62
				inorg. As		43	50	67	87	94	
				Σ As sp.		86	107	193	237	244	
W. Africa	Ghana	− 0.3	6.2	DMA	23	2	12	23	32	122	0.14
				inorg. As		6	48	65	104	156	
				Σ As sp.		6	62	93	136	277	
	Cote d'Ivoire	− 5.3	6.8	DMA	87	2	4	13	20	65	0.11
				inorg. As		2	14	49	75	116	
				Σ As sp.		0	20	64	91	180	
	Mali	− 8.3	11.8	DMA	44	2	13	32	40	91	0.07
				inorg. As		2	19	70	90	156	
				Σ As sp.		3	31	111	128	238	

Table 1 (continued)

Region	Country	Long. (°)	Lat. (°)	Arsenic species	No. of values	Min. (µg/kg)	25% Per. (µg/kg)	Median (µg/kg)	75% Per. (µg/kg)	Max. (µg/kg)	As air conc. (ng/m^3)
E. Africa	Malawi	34.3	− 12.9	DMA	19	2	2	3	6	19	0.04
				inorg. As		2	2	5	8	27	
				Σ As sp.		3	3	6	12	40	
	Tanzania	36.0	− 6.8	DMA	88	2	2	3	8	140	− 0.02
				inorg. As		2	5	9	29	86	
				Σ As sp.		3	7	12	38	219	
Mediter	Egypt	31.0	31.0	DMA	22	5	6	19	43	86	0.66
				inorg. As		22	36	48	85	116	
				Σ As sp.		28	42	68	134	176	
	France	7.0	43.7	DMA	6	23	40	52	66	82	0.36
				inorg. As		85	88	114	133	139	
				Σ As sp.		113	126	182	189	192	
	Italy	8.7	44.5	DMA	106	2	33	53	91	324	0.38
				inorg. As		2	68	92	112	244	
				Σ As sp.		3	111	156	194	425	
	C. Spain	− 0.6	38.4	DMA	28	1	18	27	75	259	0.14
				inorg. As		28	48	64	81	175	
				Σ As sp.		37	84	106	164	362	
	S. Spain	− 6.3	37.2	DMA	12	20	38	95	235	286	0.20
				inorg. As		29	51	75	99	130	
				Σ As sp.		68	89	170	330	388	
	Turkey	31.5	40.0	DMA	21	13	27	34	70	368	0.29
				inorg. As		43	68	78	94	280	
				Σ As sp.		57	96	119	159	647	
Asia	Bali	115.2	− 8.6	DMA	15	2	2	2	4	7	0.06
				inorg. As		2	2	13	31	55	
				Σ As sp.		3	3	14	34	63	
	China	117.3	26.4	DMA	39	2	19	27	34	121	15.00
				inorg. As		2	71	82	96	120	
				Σ As sp.		3	96	112	128	198	
	Java	107.6	− 6.9	DMA	47	2	2	4	8	23	0.63
				inorg. As		2	17	35	58	99	
				Σ As sp.		3	18	39	65	120	
	Malaysia	100.0	5.3	DMA	18	37	47	55	72	81	1.30
				inorg. As		55	74	80	89	103	

Table 1 (continued)

Region	Country	Long. (°)	Lat. (°)	Arsenic species	No. of values	Min. (µg/kg)	25% Per. (µg/kg)	Median (µg/kg)	75% Per. (µg/kg)	Max. (µg/kg)	As air conc. (ng/m^3)
				Σ As sp.		102	119	132	155	178	
	S. Korea	128.0	36.5	DMA	56	3	11	16	19	59	3.60
				inorg. As		35	55	64	82	145	
				Σ As sp.		38	67	81	102	171	
	Sri Lanka	80.0	9.7	DMA	142	2	2	2	6	71	0.79
				inorg. As		2	24	36	54	134	
				Σ As sp.		3	26	41	60	171	
	N. Vietnam	105.5	20.5	DMA	71	7	18	26	34	132	5.20
				inorg. As		36	57	69	91	399	
				Σ As sp.		49	76	101	128	440	
	S. Vietnam	107.0	11.5	DMA	46	2	15	26	39	131	0.74
				inorg. As		2	55	70	79	107	
				Σ As sp.		3	71	95	112	210	
N. America	Mexico	− 100.4	20.0	DMA	8	25	39	88	136	236	4.10
				inorg. As		79	83	91	102	125	
				Σ As sp.		104	140	181	226	326	
Australasia	Australia	141.9	− 37.3	DMA	13	14	44	102	171	308	− 0.05
				inorg. As		21	55	58	79	102	
				Σ As sp.		39	130	169	226	385	

Chemical Analysis

To speciate As in rice the digested rice solutions were analysed using a Thermo Scientific IC5000 Ion Chromatography (IC) system, with a Thermo AS7, 2×250 mm column (and a Thermo AG7, 2×50 mm guard column), with a gradient mobile phase (A: 20 mM ammonium carbonate, B: 200 mM ammonium carbonate—starting at 100% A, changing to 100% B, in a linear gradient over 15 min., with a flow rate of 0.3 ml/min), interfaced with a Thermo ICAP Q ICP-MS that monitored m/z^+ 75, using He gas in collision cell mode. The resulting chromatogram was compared with that for authentic standards; DMA, arsenate, monomethylarsonic acid, tretramethylarsonium and arsenobetaine. The arsenic present under each chromatographic peak was calibrated using a DMA concentration series. This calibration was also used to calculate the Limits of Detection (LoD). The rice flour CRM NIST 1568b was used in all rice speciation analytical batches ($n=76$), with DMA have 100±2.7% median and standard error recovery; and inorganic arsenic 99±1.1% recovery. LoD was 3 µg/kg for grain inorganic arsenic and DMA.

Statistical Analysis

All statistical analysis was performed on SPSS v. 25. The Kruskal–Wallis test was used to test medians between sample groups.

Results

Globally, inorganic arsenic in polished rice varied from < 2 to 399 µg/kg, with a global median ($\tilde{x}$) of 66 µg/kg (Table 1; Fig. 1). Median values significantly varied between regions ($P<0.0001$). The lowest inorganic arsenic rice region was East Africa with Malawi and Tanzania having $\tilde{x}$ concentrations of 5 and 9 µg/kg, respectively. West African rice had circa an order of magnitude higher inorganic arsenic than East African, with the main West African regions being in the northern hemisphere, and East African in the southern hemisphere (Fig. 1). The only other region that approached East Africa with respect to low arsenic concentrations were in the equatorial, southern hemisphere locations of Bali ($\tilde{x}=13$ µg/kg) and Java ($\tilde{x}=35$ µg/kg). South American rice was universally high in inorganic arsenic, not showing the trend observed for Africa and Asia where concentrations decreased towards the equator.

The global $\tilde{x}$ for DMA in rice, 21 µg/kg, was a third that of inorganic arsenic (Fig. 1). However, DMA was more variable in rice as compared to inorganic arsenic, with DMA in grain ranging from < 2 to 690 µg/kg. Medians between regions were significantly different ($P<0.0001$). Temperate regions tended to have higher DMA than subtropical, and

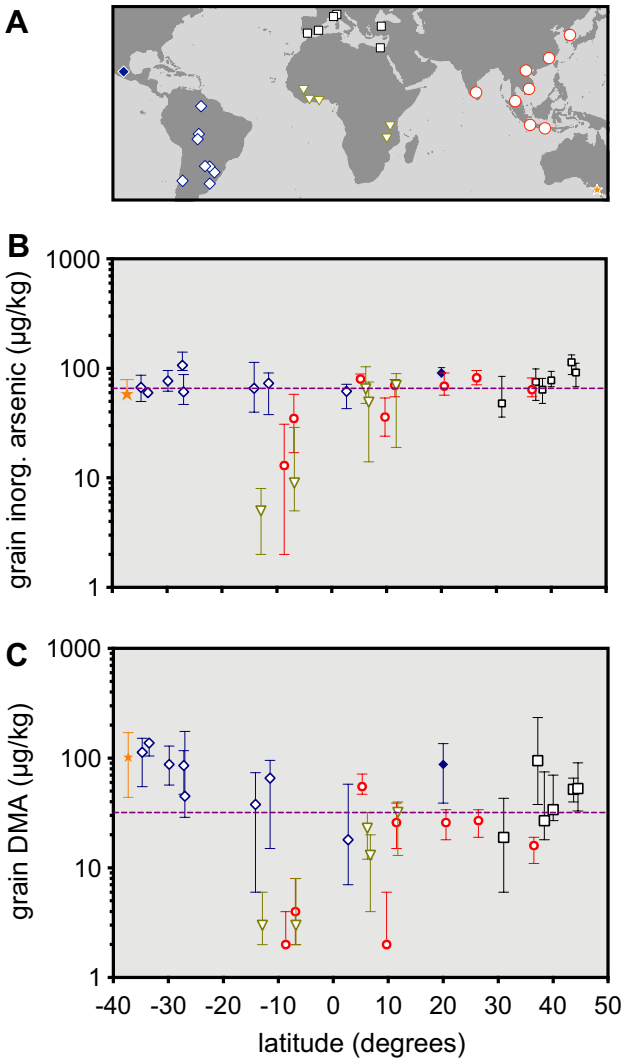

Fig. 1 Centroid locations of the 29 sampling areas (**a**). Concentrations of arsenic species in white (polished) rice across the globe, median concentrations of inorganic arsenic (**b**) and DMA (**c**) in grain at each location, plotted by latitude, with a gold star for Australia, blue diamond for the America's, green inverted triangle for Africa, red circle for Asia, black square for Mediterranean. Bars represent the 25th and 75th percentiles. The purple dashed line is the median for the 29 regions

then decreasing considerably in southern hemisphere tropical regions. Northern equatorial regions tended to have intermediate DMA concentrations, around the global median. The major outlier with respect to grain DMA was Sri Lankan rice with $\tilde{x}=2.0$ µg/kg, but had a typical inorganic arsenic profile for the latitude at 24 µg/kg. Tropical southern hemisphere rice, East African and Indonesian, were very low in DMA with $\tilde{x}$'s < 5 µg/kg. West African and northern Indonesian rice had DMA > 10 µg/kg. South America showed the shallowest cline in DMA with temperate/subtropical South American rice DMA being, $\tilde{x}>40$ µg/kg, decreasing to $\tilde{x}=18$ µg/kg in northern Brazil (Fig. 1). North American,

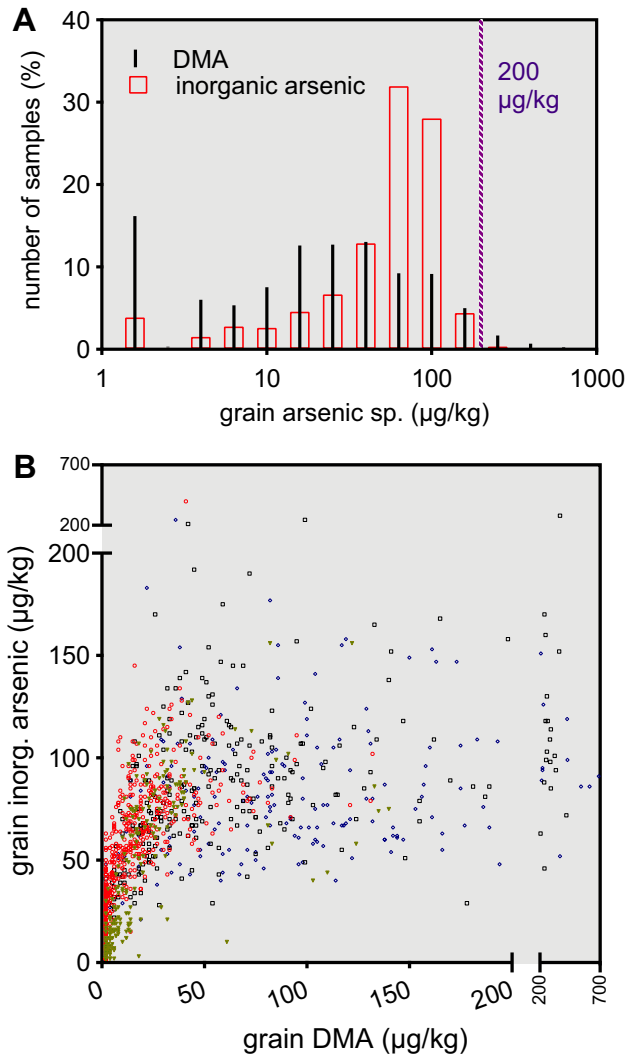

Fig. 2 Relationships between inorganic arsenic and DMA in grain from the global survey: distribution of inorganic arsenic versus DMA for all the samples (**a**), and relationship between inorganic arsenic and DMA in individual samples, with each point labelled as for Fig. 2 (**b**)

Asian and European temperate regions also had high DMA (> 20 µg/kg), similar to S. America.

The distribution of inorganic arsenic and DMA in global rice is shown in Fig. 2. There is a peak at < 2 µg/kg for both inorganic arsenic and DMA, below the LoD. Besides the samples below LoD, both species followed a unimodal distribution. For DMA this distribution was normal, but for inorganic arsenic it was asymmetric, skewed towards higher concentrations. However, the DMA curve was more extended with respect to higher concentrations. A plot of inorganic arsenic versus DMA for the entire global dataset showed that inorganic arsenic dominated when the sum of inorganic arsenic and DMA was low, but this situation reversed at higher grain concentrations (Fig. 2). The higher grain concentrations are dominated by South American and Mediterranean rice production regions, which can reach up to 700 µg/kg DMA. South East Asian and African rice never exceeded 150 µg/kg DMA.

Discussion

This current investigation has identified that only eastern hemisphere, southern latitude rice production regions have low-grain inorganic arsenic. In contrast, rice from the Americas was universally elevated in arsenic, with little geographical variation. The concentrations reported in the literature from surveys of Asia (Chen et al. 2018; Williams et al. 2006; Zavala et al. 2008), West Africa (Adeyemi et al. 2017; Adomako et al. 2011), Europe (Signes-Pastor et al. 2016; Sommella et al. 2013) and the USA (Williams et al. 2007; Zavala et al. 2008) are similar to what we report here.

There has been limited survey of arsenic in African rice (Adeyemi et al. 2017; Adomako et al. 2011; Joy et al. 2017). The finding that East African rice is low in arsenic confirms a study which reported that Malawian paddy rice was particularly low in total arsenic (Joy et al. 2017). The underlying geology of the East African region surveyed, stretching from southern Malawi to northern Tanzania, has a diverse topology and geology (McConnell 1972), yet is universally low in inorganic arsenic. East African rice production tends to be low-input and rain-fed (Meerens et al. 2003). As such East African rice production will have limited input from irrigation waters (Williams et al. 2006), pesticides (Williams et al. 2007) and fertilizers (Hartley et al. 2013; Zhuang and McBride 2013).

The widespread of elevation of arsenic in rice throughout South America reported here equates to previous more spatially limited investigations, such as those for Argentina (Farias et al. 2015), South Brazil (Segura et al. 2016) and Ecuador (Otero et al. 2016). From the current study, and from literature investigations (Meharg et al. 2009; Zavala et al. 2008), the South American rice grain inorganic arsenic concentrations are similar to those found through the major rice growing belts of S.E. Asia and Europe.

A Chinese study found no trend in inorganic arsenic from south to north of that county (Chen et al. 2018). The DMA versus inorganic arsenic relationships found for Chinese rice confirmed our finding that DMA was prevalent at higher sums of arsenic species in grain (Chen et al. 2018). Both South American (as reported here) and North American (Meharg et al. 2009; Zavala et al. 2008) rice production regions also had elevated grain DMA. The underlying regulation of DMA in soils will be related to the balance between methylation/demethylation (Yoshianga et al. 2011), and to different paddy management regimens (Norton et al. 2013). The paddy soils of the America's are generally managed using directly sowed rice, where those of Africa and Asia

are dominated by transplanted rice. These have very different water management regimes, which regulates both soil redox and rice rooting patterns (Farooq et al. 2011). Redox is a strong regulator of both arsenic availability in soils and of methylation activity (Meharg and Zhao 2012; Norton et al. 2013; Reid et al. 2017; Yoshianga et al. 2011). Having identified major global trends in DMA production provides impetus to identifying the factors involved in soil microbial methylation of arsenic. Sri Lankan rice was the only country that had an anomalous DMA profile, being low despite having moderate inorganic arsenic concentrations. Sri Lanka was surveyed across that country's diverse climate, geology and topology (De Silva et al. 2007), with paddy cultivation systems that traverse from lowland irrigated systems to upland rain-fed Meharg and Raab 2010). Sri Lankan rice is relatively high in inorganic arsenic but low in DMA. This suggests that upland/lowland cultivation of rice, per se, does not seem to be a major determinant of grain inorganic or DMA concentrations. However, more fine-scale investigations are required to investigate more subtle differences between Sri Lankan paddy agroecosystems.

MLs have been set for inorganic arsenic rice at 200 µg/kg by both the EU (EU 2015) and WHO (2014). Only 5 out of 1180 samples analysed, 0.4% of samples, failed these limits. The considerations on the suitability of these standards have been made elsewhere by the authors (Carey et al. 2018; Meharg and Raab 2010). The concentrations of inorganic arsenic present in the global supply-chain are problematic in the EU for the infant standard (100 µg/kg), which is half the adult standard (EU 2015). From our study 17.6% of rice grain samples would fail this infant standard. Note that the standard is set at the concentration of inorganic arsenic in the rice used in formulating infant products, rather than in the final concentration of those products. That is, 17.6% of rice in the global supply chain cannot be used for infant foods in the EU.

This knowledge garnered in this current study adds to our understanding of geographically limited surveys that compare the variation in arsenic speciation between growing regions (Meharg et al. 2009; Zavala et al. 2008), and more limited regional studies that have been conducted (for example: Adeyemi et al. 2017; Adomako et al. 2011; Farias et al. 2015; Joy et al. 2017; Otero et al. 2016; Segura et al. 2016). Specifically, the current findings of this show that given the correct growing environment paddy rice can be low in inorganic arsenic. Southern hemisphere, at eastern latitudes, is the location that low arsenic rice can be sourced for products for sub-populations where arsenic exposure is of concern, such as for infant foods (Carey et al. 2013). Identifying why East African and Southern Indonesian rice cultivation are outliers with respect to rice globally will hold the clues of how to produce low arsenic rice elsewhere. This may include climate, geology, cultivation practice and any potential pollution sources. It also ascertains that S. American rice is high in arsenic species and opens up important considerations about elevation in DMA in certain growing regions.

Acknowledgements We thank Edward Joy for the Malawian samples used in this study.

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