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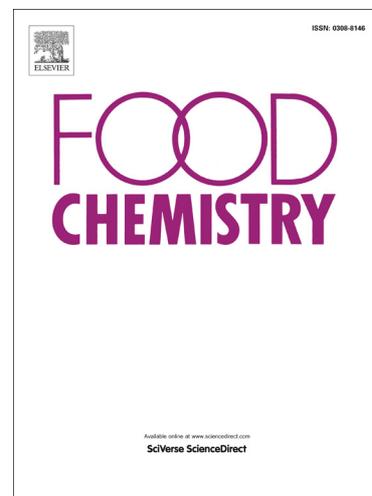
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Food Chemistry

Arsenic and cadmium contents in Brazilian rice from different origins can vary more than two orders of magnitude

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Abstract

Brazil is a major producer of rice, but there is not enough information about As and Cd in rice grown under different conditions in this country. Here, As and Cd were determined by ICP-MS and species of As by HPLC-ICP-MS in Brazilian husked rice, covering diverse cultivars and regions, as well as upland and flooded production systems. Significant differences were observed for both elements contents according to the origin of rice. All samples were below the maximum limit for Cd (400 $\mu\text{g}/\text{kg}$) set by national legislation, while nine samples presented total As above the legislation limit (300 $\mu\text{g}/\text{kg}$). From 24 samples analyzed for As species, 42% showed iAs above the European limit for production of food to infants (100 $\mu\text{g}/\text{kg}$). The total As content in samples from Mato Grosso state presented a maximum value of 6 $\mu\text{g}/\text{kg}$, which combined with low Cd content make interesting further studies.

Highlights

- The total As varied by two orders of magnitude (<2.6 $\mu\text{g}/\text{kg}$ to 630 $\mu\text{g}/\text{kg}$)
- Cd content was always below the maximum limit established by regulatory authorities
- Upland rice from Mato Grosso contain the lowest ever measured As contents
- Brazilian rice from flooded system contains iAs from 68 to 174 $\mu\text{g}/\text{kg}$
- Twenty samples were above the limit of iAs in rice destined to food for infants

Keywords: ICP-MS; HPLC; inorganic arsenic; arsenic speciation; rice cultivars; flooded production; upland production

Chemical compounds:

Arsenic, inorganic arsenic (PubChem CID: 5359596), Cadmium (PubChem CID: 23973), Dimethylarsinic acid (PubChem CID: 2513), monomethylarsonic acid (PubChem CID: 8948)

1. Introduction

Despite being a complementary food, rice can contain toxic elements at undesirable levels, mainly arsenic (As) and cadmium (Cd), which can originate from human activities, contaminated soil and irrigation water, as well as agrochemical products used for disease and pest control (Chi et al., 2018).

Arsenic naturally exists in different chemical forms. The inorganic species arsenite (AsIII) and arsenate (AsV) are about 100 times more toxic than the organic species (dimethylarsinic acid - DMA and monomethylarsonic acid - MMA), being soluble in water and readily metabolized (Kuramata et al., 2013). Studies showed that rice and its products are one of the main sources of inorganic arsenic (iAs) in the human diet, since the rice plant is efficient at accumulating As, with large amounts in the grains (Williams et al., 2005; Torres-Escribano, Leal, Vélez & Montoro, 2008; Zhao, Zhu & Meharg, 2013). When considering rice cultivated in flooded systems, water produces a higher mobilization of the As contained in the soil, increasing the accumulation of this element (Takahashi et al., 2004).

Exposure to As can cause skin lesions, cardiovascular disorders, immune system diseases and diabetes (Kuramata et al., 2013). The International Agency for Research on Cancer (IARC) classified iAs compounds as 'carcinogenic to humans' (Group 1), associated with skin, lung, bladder and kidney cancers (IARC, 2012). There is evidence of negative impacts on fetal and infant development. According to European Food Safety Authority (EFSA), children under three years are the most exposed to iAs (EFSA, 2014). Pre-cooked, milled rice is a main carbohydrate source to weaning babies up to one year of age (Meharg et al., 2008). The Commission Regulation (EU) 2015/1006 established a maximum limit of iAs for rice destined to the production of food for infants [100 µg/kg]. For husked rice, the European limit of iAs is 250 µg/kg. A Joint FAO/WHO Expert Committee on Food Additives had established a provisional tolerable weekly intake (PTWI) of 15 µg iAs per kilogram of body weight, but in 2011 the PTWI for iAs was withdrawn (WHO, 2011). In order to reduce the exposure of the population in Brazil, ANVISA (Brazilian Health Regulatory Agency) stipulated the maximum limit of 300 µg/kg for total As in rice (ANVISA, 2013).

Cadmium is a toxic and carcinogen metal, known as one of the major environmental pollutants, with serious effects on food safety and public health (Uraguchi & Fujiwara, 2012). Because Cd is easily transferred from soil to plants, the major route of its uptake in humans is through ingestion of food (Satarug et al. 2003; Uraguchi & Fujiwara, 2012). In the case of rice, studies show that aerobic (upland) cultivation condition increased Cd compared to flooded crop (Li et al., 2009).

The ingestion of Cd can cause diabetic renal complications, hypertension, cancer of lung, kidney, bladder, pancreas, breast and prostate, renal dysfunction and osteoporosis (Satarug et al. 2003). The EFSA established a provisional tolerable monthly intake (PTMI) of 25 µg of Cd per kilogram of body weight (WHO, 2013). In Brazil, the maximum limit of Cd in rice is 400 µg/kg (ANVISA, 2013), while the Commission Regulation (EU) 1881/2006 has established a maximum limit of 200 µg/kg.

Brazil is the ninth consumer and producer of rice (FAOSTAT, 2017). According to official statistics (CONAB, 2018), the Brazilian rice production is concentrated in the south region, where the flooded system predominates, in the states of Rio Grande do Sul (responsible for 70% of total country crop) and Santa Catarina (10%). Some relevante production is also found in the north and central regions, with upland system, comprising the states of Mato Grosso (4%) and Maranhão (3%). This study investigated the occurrence of total and inorganic As, and Cd in Brazilian husked rice, covering different cultivars and geographic regions, as well as both flooded and upland systems, with an emphasis on identifying cultivars that accumulate low As and Cd contents so that they could be a possible source of rice destined to infants. Hojsak et al. (2015) published an extensive consensus statement about As in rice, and some of conclusions were: the iAs content of dietary products used by infants needs to be regulated; in areas of the world where rice consumption is high, authorities should declare which of the rice cultivars have the lowest As content; the As content in rice should be globally monitored and rice with the lowest content shall be used for the preparation of infant foods. Therefore, the disclosure of the data of the present study are of current importance.

2. Materials and methods

2.1 Sampling and sample preparation

Samples were collected directly from producers of certified rice, providing information guaranteed in relation to cultivar, location and production system. Sampling included the main producing states of Brazil (Mato Grosso MT, Rio Grande do Sul RS, and Santa Catarina SC), thirteen cultivars and both upland and flooded systems, as shown in Table 1. Only one sample was obtained for some cultivars, since they are less used and producers are scarce. The husk was removed using the rice mill Yanmar ST 50. The husked rice was used in this study because it usually has more As than polished rice. Afterwards, the rice samples were dried in oven at 50 °C for 12 h, and grinded in a cryogenic mill (Freezer Mill 6870). Prepared samples were placed in polyethylene bottles and stored at 18°C under controlled humidity and luminosity conditions. The evaluation of residual moisture was performed using analytical portions of 1g in triplicate, dried in oven at 80°C for 6 h.

Table 1. Brazilian rice samples collected for this study

State	Production system	Group	Cultivar	Samples
Mato Grosso	Upland	Japonica	Esmeralda	4
			Sertaneja	4
			AN Cambará	1
			ANA 6005	1
Rio Grande do Sul	Flooded	Indica	Guri Inta	8
			Puitá Inta	8
			IRGA 424	6
			IRGA 424 RI	6
Santa Catarina	Flooded	Indica	Tio Taka	3
			Andosan	2
			Epagri 109	3
			SCS 112	1
			118 Marques	1
TOTAL				48

2.2 Total arsenic and cadmium

The digestion and ICP-MS determinations were according to the methodology proposed by Williams et al. (2007). Portions of 0.1 g were digested direct in 50 mL polypropylene vials (Corning) in a CEM Mars 5 Microwave System, using 2 mL of concentrated HNO₃ (70% v/v) and 1 mL of ultrapure water (Milli-Q). The programmed temperature was: 2 minutes ramp to 50°C, 5 minutes holding, 2 minutes ramp to 75°C, another 5 minutes holding, 2 minutes ramp to 95°C, 30 minutes holding, and finally

cooling for 30 minutes. Blanks and certified reference materials were also included. After the digestion, samples were diluted with 10 mL of ultrapure water (Milli-Q).

Total As and Cd were determined in the ICP-MS equipment Agilent Technologies 8800. All the operation conditions were described in Figure S1 at the Supplementary Material. Rhodium (10 µg/L) was run on an external line as the internal standard and H₂/O₂ was used as reaction gas. A rice flour certified reference material (SRM 1568a) was used to validate the analysis. The elements measured were As (m/z 75), Cd (m/z 111) and Rh (m/z 103). As was measured using hydrogen in the reaction cell, removing the potential polyatomic interference from ArCl.

2.3 Arsenic speciation

The arsenic speciation was made by HPLC-ICP-MS in 24 samples that presented the largest total As contents. An aliquot amount of homogenized sample (0.2 g) was added into a 50 mL polypropylene vial (Corning) with 10 mL of Aristar HNO₃ 1% and H₂O₂ 1% (v/v), followed by microwave-assisted extraction in CEM Mars microwave system, using the same heating program for total As (according to Method A described by Calle et al. 2011). The H₂O₂ oxidizes arsenite to arsenate, improving chromatographic resolution as arsenate elutes at some distance from MMA and DMA.

According to methodology used by Williams et al., 2005, after extraction, the supernatant was centrifuged using an Eppendorf Minispin Centrifuge, and 600 µL transferred to HPLC plastic vials. Arsenic species were identified and quantified using HPLC (Agilent 1290 HPLC) ICP/MS (Agilent Technologies 8800). The HPLC equipment was connected to a strong anion exchange column PRP X 100 Hamilton. The mobile phase consisted of ammonium carbonate buffer (30 mM, pH 9.2). Rhodium (0.01 mg/kg) in 1% (v/v) nitric acid was added during the analysis as an internal standard. Two certified reference materials of rice flour (SRM 1568a and ERM-BCR 211) were used throughout for speciation analysis. Solutions containing known amounts of DMA (0.1 µg/kg to 20 µg/kg) were prepared from stocked standard and subjected to HPLC-ICP-MS under the same conditions as the supernatants. Peak areas from these measurements were used to construct a calibration curve (Williams

et al., 2005). DMA was used since this compound is much more stable in comparison to arsenate and it shows a reasonable retention time (3 - 4 minutes). A mixture containing 10 µg/L of DMA, MMA and arsenate was analysed to check the retention time. The species resulted to be well separated and peaks well resolved (retention times of 3 minutes for DMA, 4 minutes for MMA and 6 minutes for arsenate). Peak areas were used for quantification of As species. Three samples were digested in triplicate and measured for estimating precision. Each analytical batch also contained procedural blanks.

2.4 Statistical methods

The statistical tests were performed using the Statistic Analysis System SAS. Normality and homoscedasticity of data were tested. Analysis of variance (ANOVA) was used to verify significant differences between the mean content of As and Cd in rice grains from diverse cultivars and geographic regions at the 95% confidential level ($p < 0.05$). When significant differences were identified by ANOVA, the Duncan's new multiple range test was applied.

3. Results and discussion

3.1 Quality control

Total As and Cd

The mean value and standard deviation ($n=8$) for total As in the SRM 1568a Rice Flour was 264 ± 14 µg/kg ($n = 8$). Comparing to the certified value of 290 ± 30 µg, the recovery was 91% and fit within the 95% confidence interval. For Cd, the mean value and standard deviation ($n=8$) in the SRM 1568a was 22 ± 1 µg/kg compared to its certified value of 22 ± 2 µg/kg with a 95% confidence interval, therefore, the recovery was virtually 100%.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated from 3 and 10 times the standard deviation of the blank signals, using the slope of the calibration curve and the average dilution factor. The LOD for As and Cd were respectively 2.6 µg/kg and 1.1 µg/kg, while the LOQ was 8.6 µg/kg for As and 3.7 µg/kg for Cd.

Arsenic speciation

The calibration curve of the standards using DMA presented R^2 of 0.999, slope 0.12 and intercept 0.0017. Limits (LOD and LOQ) were calculated via blank samples, as previously described for total As and Cd. For DMA, LOD and LOQ were 0.9 $\mu\text{g}/\text{kg}$ and 3.1 $\mu\text{g}/\text{kg}$, respectively. For iAs, LOD was 1.1 $\mu\text{g}/\text{kg}$ and LOQ was 3.5 $\mu\text{g}/\text{kg}$, and for MMA, LOD was 0.1 $\mu\text{g}/\text{kg}$ and LOQ was 0.3 $\mu\text{g}/\text{kg}$. To evaluate precision, the relative standard deviation (RSD) of three samples analyzed in triplicate was calculated. The average variability was considerably low for DMA (13%), MMA (16%) and iAs (10%).

For the certified reference material ERM BCR-211, results were 136 $\mu\text{g}/\text{kg}$ of DMA and 154 $\mu\text{g}/\text{kg}$ of iAs, whereas the certified values are $119 \pm 13 \mu\text{g}/\text{kg}$, and $124 \pm 11 \mu\text{g}/\text{kg}$. Recovery percentage results were 114% for DMA and 124% for iAs, with a recovery of 118% for total As. Although not certified for species, the SRM 1568a was also analyzed and results were 141 $\mu\text{g}/\text{kg}$, 17 $\mu\text{g}/\text{kg}$ and 84 $\mu\text{g}/\text{kg}$ for DMA, MMA and iAs respectively, with a total content of 242 $\mu\text{g}/\text{kg}$, recovery of 83% comparing to the certified value of As ($290 \pm 30 \mu\text{g}/\text{kg}$). For this material, the average values of DMA and iAs reported by other authors (Williams et al., 2005), i.e. $164 \pm 5 \mu\text{g}/\text{kg}$ for DMA and $96.5 \pm 14 \mu\text{g}/\text{kg}$ for iAs, are about 15% higher than those found here. Such results for DMA and iAs agreed with the 83% recovery value obtained for total As.

Considering all the 24 samples processed for As speciation, the linear correlation between total As determined previously by ICP-MS and total As extracted by HPLC-ICP-MS (sum of DMA, MMA and iAs) was strong, with R^2 of 0.94, showing a good efficiency of extraction and a mean recovery of 82%.

3.2 Total As and Cd in Brazilian rice

The total As varied by two orders of magnitude ($<2.6 \mu\text{g}/\text{kg}$ to $628 \mu\text{g}/\text{kg}$, $n = 48$) and the average, standard deviation and median were $174 \pm 168 \mu\text{g}/\text{kg}$ and $111 \mu\text{g}/\text{kg}$ respectively, with a RSD of 96%. Samples from Rio Grande do Sul had the highest mean values of As, followed by those from Santa Catarina. Both regions presented As statistically higher ($p < 0.05$) than mean values of Mato Grosso (Table 2). Arsenic content in rice from Rio Grande do Sul varied between $20 \mu\text{g}/\text{kg}$ and $630 \mu\text{g}/\text{kg}$,

while in Santa Catarina, As content in rice varied from 70 $\mu\text{g}/\text{kg}$ to 427 $\mu\text{g}/\text{kg}$. The flooded crop system used in these two regions may contribute to the increase of As, nevertheless the large variation of the results within the same state indicates the effect of other factors on As presence in rice. In fact, the characteristics of the cultivation site seems to have a relevant role in As accumulation. Literature has discussed the influence of contaminated soils or soils with natural high levels of As (Yamamura & Amachi, 2014), contaminated irrigation water (Ng, Wang & Shrain, 2003), use of fertilizers and pesticides containing As (Jayasumana et al. 2015), microbiology and chemical properties of the soil (Zheng, Sun & Zhu, 2013), among other factors that should be better studied.

Table 2. Average and range of total As and Cd in Brazilian husked rice ($\mu\text{g}/\text{kg}$, dry basis). Averages followed by letters in common within the same column do not differ statistically ($p < 0.05$)

State	Total As			Total Cd		
	Average	Min-Max	RSD%	Average	Min-Max	RSD%
Rio Grande do Sul (n=28)	235 \pm 157 ^a	20 - 630	76	18.6 \pm 10.3 ^a	7.4 - 44.4	55
Santa Catarina (n=10)	157 \pm 108 ^a	70 - 427	72	11.8 \pm 7.2 ^b	5.3 - 28.9	61
Mato Grosso (n=10)	4 \pm 2 ^b	<LOD - 6	50	5.9 \pm 3.4 ^c	2.9 - 11.9	57
All regions (n=48)	174 \pm 168	<LOD - 630	98	14.6 \pm 10.0	2.9 - 44.4	68

*LOD As: 2.6 $\mu\text{g}/\text{kg}$

Figure 1 shows that 19% of the rice samples were above the maximum limit of total As in rice established by ANVISA (300 $\mu\text{g}/\text{kg}$), one from Santa Catarina (9% of the cohort) and eight from Rio Grande do Sul (28% of the cohort). Considering the limit established by the European Commission for iAs in husked rice (250 $\mu\text{g}/\text{kg}$), 14 samples should be investigated for iAs because they exceeded this limit for total As, i.e. 12 from Rio Grande do Sul and 2 from Santa Catarina. Meanwhile, considering the limit set by the European Commission for infant foods (100 $\mu\text{g}/\text{kg}$), 19 samples from Rio Grande do Sul and 8 from Santa Catarina should be investigated in relation to iAs. In China, the maximum limit of iAs in husked rice is 200 $\mu\text{g}/\text{kg}$ (Ministry of Health of the People's Republic of China, 2012), while Codex Alimentarius (2018) recommended a maximum limit of 350 $\mu\text{g}/\text{kg}$ of iAs in husked rice.

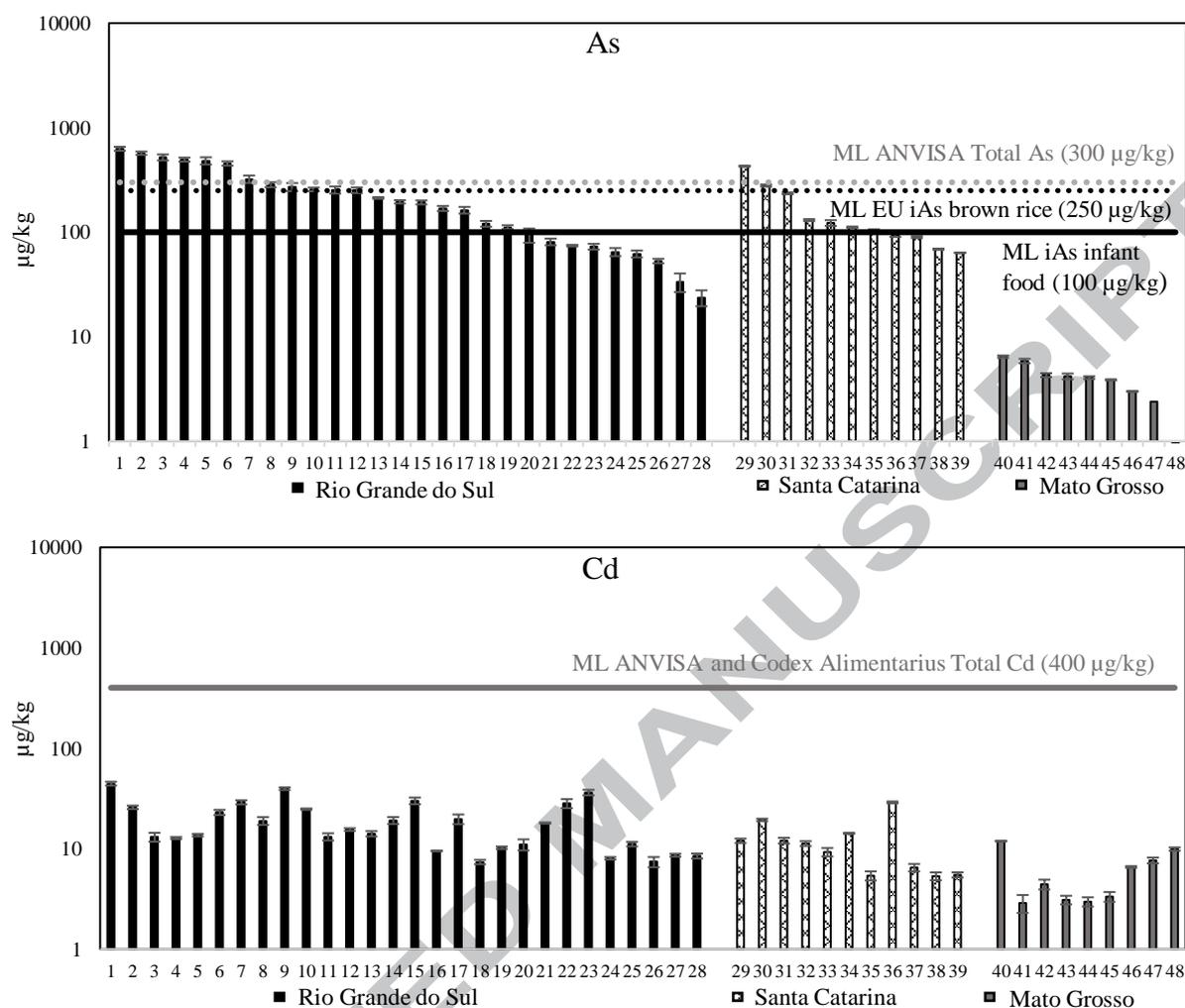


Figure 1. Total As and Cd in Brazilian husked rice from Rio Grande do Sul, Santa Catarina and Mato Grosso states, compared to maximum levels (ML) established by legislation

The Cd content in rice varied much less than the As content, fluctuating only over one order of magnitude, from $2.9 \pm 0.1 \mu\text{g/kg}$ to $44 \pm 1 \mu\text{g/kg}$, with average and standard deviation of $15 \pm 10 \mu\text{g/kg}$ (RSD = 68%) for a median value of $12 \mu\text{g/kg}$. As observed in Figure 1, all rice samples showed Cd level well below the maximum limit of $400 \mu\text{g/kg}$ established by ANVISA (2013). In fact, the results were all lower than other maximum limits defined elsewhere, i.e. $200 \mu\text{g/kg}$ by European Commission (2006), $200 \mu\text{g/kg}$ by China (Ministry of Health of the People's Republic of China, 2012) and $400 \mu\text{g/kg}$ for polished rice by Codex Alimentarius (2018).

No linear correlation was verified for total As and Cd in Brazilian rice samples (Figure 2), although previous data (Li et al., 2009; Hu et al., 2015) indicate that an inverse correlation could be expected.

Table 3 summarizes data from literature for As and Cd in husked rice cultivated in Brazil compared to the results of this study, showing a reasonable agreement. In general, Brazilian rice is low in Cd, even in the upland system, although aerobically grown rice has been reported to have much higher Cd content than those grown anaerobically (Arao et al., 2009; Hu et al., 2015; Liao et al., 2016).

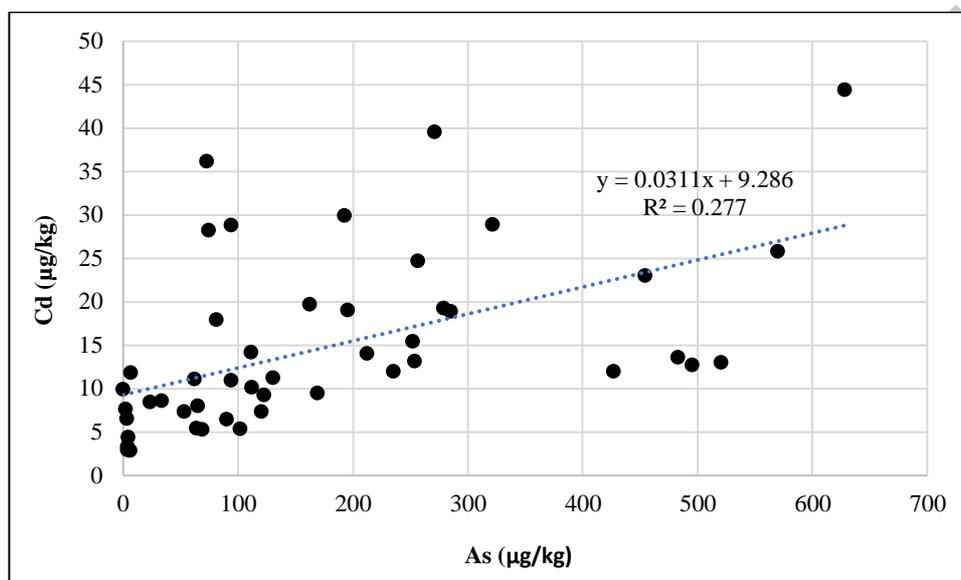


Figure 2. Linear correlation between As and Cd in Brazilian husked rice samples (n = 48)

Table 3. Total As and Cd (µg/kg) reported in the literature for rice samples from Brazil

Samples	As	Cd	Reference
husked rice (n=2)	271 - 428	---	Batista et al. (2011)
husked rice (n = not informed)	189 - 393	12.6 - 27.6	Poletti et al. (2014)
Rice, not specified (n=20)	< 15	< 40	Corguinha et al. (2015)
husked rice (n=3)	189 - 463	---	Costa et al. (2015)
rice, not specified (n=16)	59 - 782	---	Ciminelli et al. (2016)
husked rice (n=8)	101 - 660	<40	Mataveli et al. (2016)
husked rice (n=16)	138 - 342	---	Segura et al. (2016)
husked rice (n=2)	115 - 124	---	Santos et al. (2017)
husked rice (n=48)	<2.6 - 628	2.9 - 44.4	This study

Statistically comparing ($p < 0.05$), rice from Mato Grosso presented the lowest mean content of As ($3.8 \pm 1.8 \mu\text{g/kg}$) and Cd ($5.9 \pm 3.4 \mu\text{g/kg}$), which could be explained by the aerobic condition in the upland system. Arao et al. (2009), investigating the effects of water management in rice paddy on levels

of As and Cd in Japanese rice grains, concluded that flooding increased As concentrations in rice grains, whereas aerobic treatment increased the concentration of Cd. Hu et al. (2015) conducted experiments with rice cultivated in aerobic and anaerobic systems, concluding that aerobic condition caused the decrease of As and increase of Cd. The same conclusion was given by Liao et al. (2016) in a similar study in China. In Brazil, Corguinha et al. (2015) investigated As and Cd contents in soil and rice grains of different cultivars ($n = 20$) from an experimental field located in Minas Gerais state, with aerobic system (upland condition). The soil contained higher levels of As (10 mg/kg) than Cd (0.07 mg/kg), however the rice grains showed As content below the limit of detection, while the mean of Cd content was 29 $\mu\text{g}/\text{kg}$. The results observed in the present study partially disagree with such data reported in literature, because samples of rice grown at aerobic sites showed low levels of both As and Cd. These are very important results to consider, since the levels of As and Cd in rice are a problem currently investigated worldwide. Due to such results, more studies should be carried out, collecting soils, plants and water to better understand the reasons for the low content of the two toxic elements.

Comparing As content in rice of different cultivars grown in Rio Grande do Sul, Guri Inta had the highest mean value ($319 \pm 163 \mu\text{g}/\text{kg}$), differing statistically ($p < 0.05$) from the cultivar IRGA 424 ($187 \pm 142 \mu\text{g}/\text{kg}$). Despite the statistical difference, the standard deviation within samples of the same cultivar is high, indicating a large spatial variability and the influence of growing conditions, as soil and irrigation water, on the As content. Rice of different cultivars grown in Santa Catarina did not differ ($p > 0.05$) for As. All samples from Mato Grosso showed exceptionally low As contents, so far the lowest range recorded for rice worldwide. Although presenting low total As, rice samples of diverse cultivars grown in Mato Grosso were statistically different ($p < 0.05$), with the cultivar Esmeralda presenting the lowest As mean ($3.5 \pm 1.8 \mu\text{g}/\text{kg}$).

In relation to Cd, it was also observed a high variability within the same cultivar, indicating influence of growing conditions on the concentration. For rice grown in Rio Grande do Sul, the cultivar Puitá Inta had the highest mean ($25 \pm 14 \mu\text{g}/\text{kg}$). Rice of diverse cultivars from Santa Catarina did not differ ($p > 0.05$) for Cd, occurring the same with the rice from Mato Grosso.

3.3 As species in Brazilian rice

The DMA, MMA and iAs (sum of As^{III} and As^V) contents for 24 rice samples can be seen in Figure 3. The range for iAs was 68 µg/kg to 174 µg/kg, with mean and standard deviation of 123 ± 26 µg/kg, RSD of 21%. The relative value of iAs in the total As has varied from 27% to 89%, the average being 58%. All samples were below the maximum limit of 250 µg/kg of iAs in rice established by European Commission. However, 80% of samples were above the maximum limit (100 µg/kg) of iAs in rice destined to the production of food for infants and young children, being inappropriate for this purpose. In fact, after the introduction of the European legislation in January 2016, a market survey in the United Kingdom identified that most baby rice products still exceed the maximum level set (Signes-Pastor et al., 2017). In such context, it should be emphasized that the nine samples from Mato Grosso presented total As content well below the maximum level of iAs set by the European legislation (Figure 1).

DMA ranged from 10 µg/kg to 303 µg/kg, with mean and standard deviation of 110 ± 85 µg/kg, RSD of 77%, indicating high variability of DMA between samples. The relative value of DMA in the total As has varied from 11 to 70%, the average being 41%. According to Figure 2, those samples presenting the highest value of total As actually have more DMA, as in the case of samples 1, 2 and 5, from the same producer in São Gabriel city, Rio Grande do Sul state. Raab et al. (2007), studying the uptake and translocation into shoots of As^V, methylarsonate (MA), and DMA by 46 different plant species including rice, concluded that most of the studied plants have higher transfer factors for methylated-As than for iAs. This means that the soil conditions (soils that promote the methylation of As) or the application of methylated-arsenic herbicides may result in higher As levels in shoots of plants. MMA ranged from 0.5 to 9.4 µg/kg, with 10 samples below the LOD (0.1 µg/kg) and LOQ (0.3 µg/kg).

Similar results of iAs in fifteen Brazilian husked rice samples (31.3 to 161.6 µg/kg) were found by Segura et al. (2016). Evaluating As species in two husked rice samples from Brazil, Batista et al. (2011) found iAs contents higher than the values of the present study, i.e. 185.5 µg/kg and 190.5 µg/kg, with 70.1 µg/kg – 206.2 µg/kg of DMA and 14.2 µg/kg – 18.0 µg/kg of MMA.

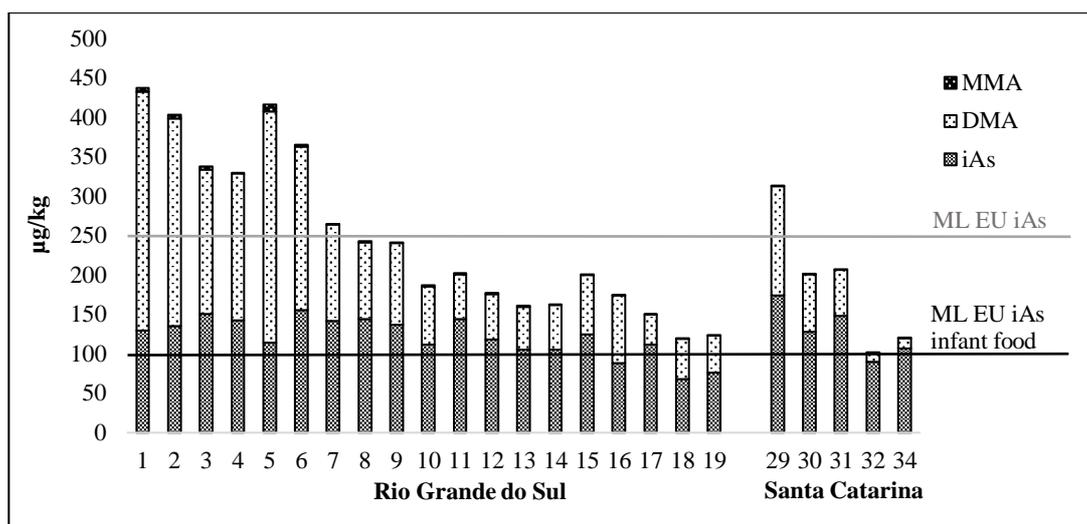


Figure 3. Results of iAs, DMA and MMA in husked rice samples, compared to the maximum levels of iAs established by European legislation

4. Conclusions

There were large variations in the As and Cd contents in husked rice from the main producing regions of Brazil, being the highest average values observed in samples from Rio Grande do Sul (flooded production) and the lowest in samples from Mato Grosso (upland production), for both elements. Such significant differences between regions may originate from the production system (flooded or upland), however several factors can contribute, i.e. soil properties, water, cultivar and other local characteristics. In fact, some significant differences occurred for As and Cd when comparing cultivars grown in a same region and the large variation observed between samples of each cultivar indicated that local field conditions also had important influence on the concentrations of both elements.

Although the results are mostly in accordance with current Brazilian legislation, it should be noted that rice is an significant source of iAs in the population diet. Therefore, it is important for Brazil to establish a maximum limit for iAs in rice. Twenty out of 24 samples showed the following abundance of As species: iAs>DMA>MMA. Considering rice destined to the production of food for infants and young children, 20 samples were above the European limit of 100 µg/kg iAs. The rice grains from Mato Grosso showed extremely low As and Cd contents in the four cultivars analyzed, which may make them target

product for exporting to the European Union, in order to satisfy the low maximum limit of iAs in rice destined to infants. Given the results, it would be interesting to study more deeply the soil, cultivars and other agronomic aspects of rice cultivated in the Mato Grosso region, as to better understanding the factors contributing for the lower contents of As and Cd.

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Conflict-of-interest statement

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

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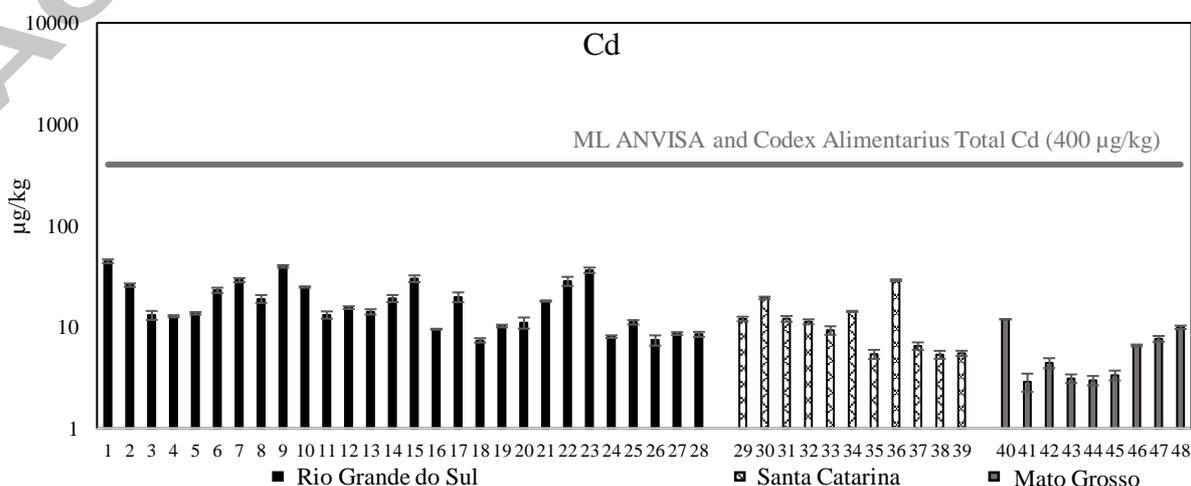
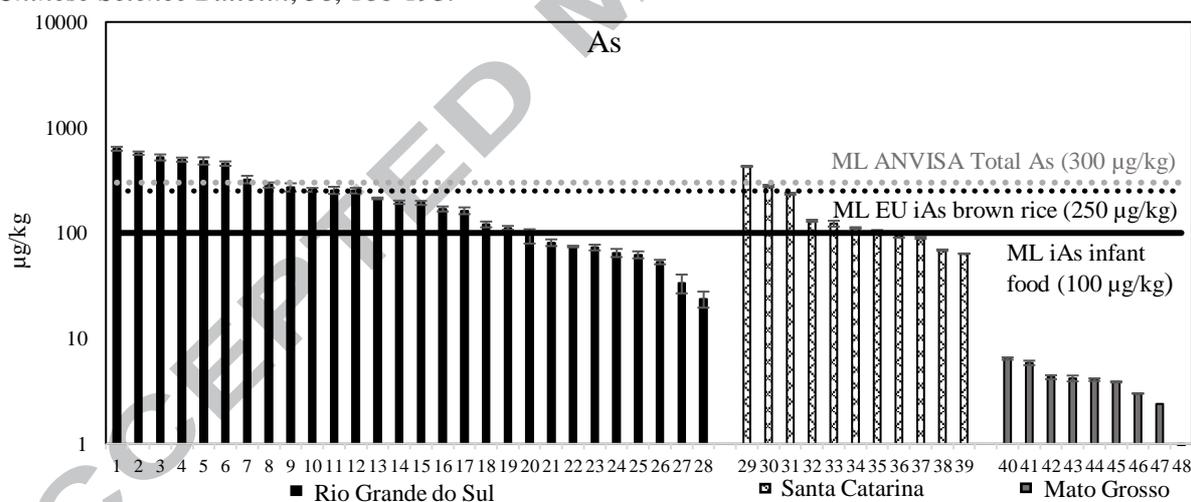


Figure 1. Total As and Cd in Brazilian husked rice from Rio Grande do Sul, Santa Catarina and Mato Grosso states, compared to maximum levels (ML) established by legislation

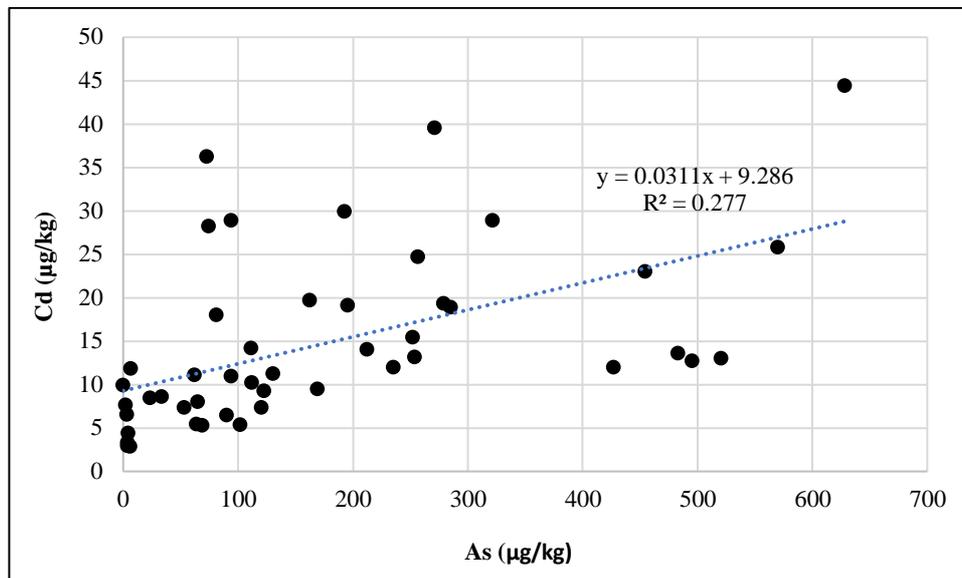


Figure 2. Linear correlation between As and Cd in Brazilian husked rice samples (n = 48)

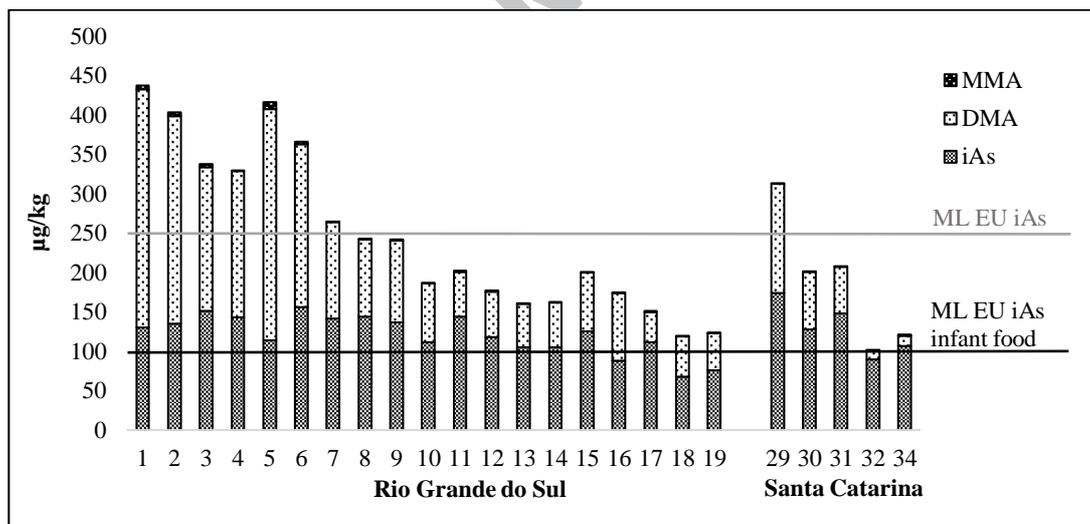


Figure 3. Results of iAs, DMA and MMA in husked rice samples, compared to the maximum levels of iAs established by European legislation

Table 1. Brazilian rice samples collected for this study

State	Production system	Group	Cultivar	Samples
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Mato Grosso	Upland	Japonica	Esmeralda	4
			Sertaneja	4
			AN Cambará	1
			ANA 6005	1
Rio Grande do Sul	Flooded	Indica	Guri Inta	8
			Puitá Inta	8
			IRGA 424	6
			IRGA 424 RI	6
Santa Catarina	Flooded	Indica	Tio Taka	3
			Andosan	2
			Epagri 109	3
			SCS 112	1
			118 Marques	1
TOTAL				48

Table 2. Average and range of total As and Cd in Brazilian husked rice ($\mu\text{g}/\text{kg}$, dry basis). Averages followed by letters in common within the same column do not differ statistically ($p < 0.05$)

State	Total As			Total Cd		
	Average	Min-Max	RSD%	Average	Min-Max	RSD%
Rio Grande do Sul (n=28)	235 ± 157^a	20 - 630	76	18.6 ± 10.3^a	7.4 - 44.4	55
Santa Catarina (n=10)	157 ± 108^a	70 - 427	72	11.8 ± 7.2^b	5.3 - 28.9	61
Mato Grosso (n=10)	4 ± 2^b	<LOD - 6	50	5.9 ± 3.4^c	2.9 - 11.9	57
All regions (n=48)	174 ± 168	<LOD - 630	98	14.6 ± 10.0	2.9 - 44.4	68

*LOD As: $2.6 \mu\text{g}/\text{kg}$

Table 3. Total As and Cd ($\mu\text{g}/\text{kg}$) reported in the literature for rice samples from Brazil

Samples	As	Cd	Reference
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husked rice (n=2)	271 - 428	---	Batista et al. (2011)
husked rice (n = not informed)	189 - 393	12.6 - 27.6	Poletti et al. (2014)
Rice, not specified (n=20)	< 15	< 40	Corguinha et al. (2015)
husked rice (n=3)	189 - 463	---	Costa et al. (2015)
rice, not specified (n=16)	59 - 782	---	Ciminelli et al. (2016)
husked rice (n=8)	101 - 660	<40	Mataveli et al. (2016)
husked rice (n=16)	138 - 342	---	Segura et al. (2016)
husked rice (n=2)	115 - 124	---	Santos et al. (2017)
husked rice (n=48)	<2.6 - 628	2.9 – 44.4	This study

Highlights

- The total As varied by two orders of magnitude (<2.6 $\mu\text{g}/\text{kg}$ to 630 $\mu\text{g}/\text{kg}$)
- Cd content was always below the maximum limit established by regulatory authorities
- Upland rice from Mato Grosso contain the lowest ever measured As contents
- Brazilian rice from flooded system contains iAs from 68 to 174 $\mu\text{g}/\text{kg}$
- Twenty samples were above the limit of iAs in rice destined to food for infants