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Regional variability of arsenic content in Uruguayan polished rice

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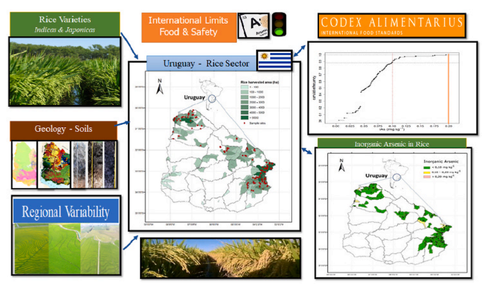
HIGHLIGHTS

- Country spatial variability of arsenic accumulation in rice grain was identified.
- The average proportion of inorganic arsenic from total arsenic was 35%.
- Levels of inorganic arsenic were below the international limits of 0.2 mg kg^{-1}.
- Arsenic levels were influenced by the geological material and rice variety.
- Consumption of rice by male and female adults is safe in Uruguay.

GRAPHICAL ABSTRACT



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ABSTRACT

Characterization of the country internal variability of arsenic (As) accumulation in rice grain across different rice production regions is very important in order to analyze its compliance with international and regional limits. A robust sampling study scheme (n = 150 samples) was performed to determine total arsenic (tAs) and inorganic (iAs) levels from polished rice grain covering all rice producing regions along two growing seasons.

The mean and median concentration of tAs were 0.178 mg kg-1 and 0.147 mg kg-^{1}, with a minimum and maximum value of 0.015 mg kg^{-1} and 0.629 mg kg^{-1}, respectively and a coefficient of variation of 63.6%. The mean and median concentration of iAs were 0.062 mg kg^{-1} and 0.055 mg kg^{-1} respectively ranging from 0.005 mg kg^{-1} up to a maximum of 0.195 mg kg^{-1} and a coefficient of variation of 51.5%. A moderate correlation was revealed within iAs and tAs. Levels of iAs in all of the samples were below the international limits of 0.2 mg kg^{-1} according to the international limits for human health by the Codex Alimentarius (FAO and WHO, 2019).

Rice fields cultivated on soils originated from igneous geological material reported lower arsenic levels accumulated in rice grain in relation to sedimentary soils. *Japonica* cultivars presented significantly lower tAs and iAs concentrations than *Indica* ones ($p = 0.0121$ and $p < 0.0001$; respectively).

Consumption of rice by male and female adults in Uruguay is safe according to its level of annual consumption and based on the mean iAs levels determined in this study.

1. Introduction

Rice is a major staple food consumed at a global scale being the main carbohydrate source for billions of people worldwide, with an average consumption of 53.9 kg of grain per person. It is also the second more extensively cultivated cereal in the world (FAOSTAT, 2018). Arsenic

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(As) content in rice presents a risk to human health as it has been classified as a carcinogen class 1 and its toxicity depends on its chemical form. Different species of As are grouped into organic (oAs) and inorganic (iAs) and both constitutes the "total arsenic" (tAs) content. The iAs forms arsenite As^{III} and arsenate As^{V}, being more toxic for human health than the organic forms, such as monomethylarsonate (MMA) and dimethylarsinate (DMA) (Meharg et al., 2012; Mei et al., 2009; Wu et al., 2011). The relative percentage of iAs or oAs species of tAs in rice grain can vary from region to region (Meharg et al., 2009; Carey et al., 2020; Majumder et al., 2019). The major inorganic species of tAs in rice grain are As^{III} and As^{V}, which are associated with negative health impacts like cancers (IARC, 2004), hypertension, neurological effects, diseases of the respiratory system, diabetes, obstetric problems and premature births (Abhyankar et al., 2012). Arsenic levels in water and food are concerning as they are frequently associated with high risk factors in food nutritional safety (Meharg et al., 2012; Islam et al., 2016; Menon et al., 2020).

Arsenic is a natural component in primary minerals; therefore, it is also found naturally in soils. The levels of As and their forms in rice grain can be affected by irrigation, varieties, fertilization and by natural presence in air, soils and waters (Meharg et al., 2012; Islam et al., 2016; Shrivastava et al., 2015).

Arsenic levels in food are strongly regulated and international standards are being continuously revised for human health issues. Recommended limits of iAs levels for milled and husked rice in the Codex Alimentarius are 0.2 mg kg^{-1} and 0.35 mg kg^{-1}, respectively (FAO and WHO, 2019). Regional Mercosur technical regulation on maximum limits of inorganic Arsenic in foods are 0.30 mg kg^{-1} (Mercosur, 2011; Mondal et al., 2020). The 0.30 mg kg^{-1} is the maximum inorganic As permitted content to the edible part of the food product. This Technical Regulation does not apply to foods for infants and young children. . The iAs concentration for infant rice products limit is below 0.10 mg kg^{-1} in the USA (FDA, 2020) and European Union (EUEuropean Union, 2021).

Compliance with these standards influences access to international markets which is crucial for exporting countries like Uruguay.

Rice is the largest irrigated crop in Uruguay with 140.257 ha cultivated annually and an annual grain yield of 8.6 ton ha^{-1}. Uruguayan rice producing sector is divided in three regions: East, North and Central representing in average across years 70%, 20% and 10% of total annually rice planted area (DIEA MGAP, 2020). National total rice production is 1.2 million tons of paddy rice per year, of which 95% is exported worldwide. As such, Uruguay ranks seventh in terms of global rice exports and is one of the main exporters in South America (FAOSTAT, 2018).

A recently published study determined that the main reasons for As contamination are: the biogeochemical weathering of rocks and the release of bound As into the groundwater and the flooded cultivation conditions of rice that favors the accumulation of As in rice grains (Upadhyay et al., 2020). It was also found that soil As bioavailability is reduced with shorter flooding periods, semiarobic and aerobic cultivation causing less As accumulation in rice grains.

AWD (alternate wetting and drying) is an irrigation technique that allows soil water to reduce until the soil reaches an aerobic state determining saturated and unsaturated soil conditions, increasing redox potential. An increase in oxygen concentration in rhizosphere may increase redox potential, limiting As mobilization (Seyfferth et al., 2018). Several research studies have reported that AWD could lead to a reduction in the accumulation of As in grain (Yang et al., 2017; Carrijo et al., 2018; Li et al., 2019), thereby contributing positively to food safety while lowering the environmental impact of rice crops and reducing greenhouse gas emissions (Linquist et al., 2015).

Different As accumulation occurs among cultivars. A field experiment reported that *Japonica* cultivars type varieties (n = 49; 0.0628 mg kg^{-1} tAs) tend to accumulate less As in grain than *Indica* varieties (n = 167, 0.121 mg kg^{-1} tAs) (Jiang et al., 2012). Long cycle cultivars could be more exposed to As in soils as well as allow to achieve lower redox potential at the soil interface when grown under flooded, increasing arsenite and DMA availability in soil (Meharg et al., 2012). Root porosity depends on genotype defining the radial oxygen loss (ROL) that could be related to As accumulation in rice grain. Genotypes with higher ROL accumulate less As in straw and grain (Wu et al., 2011), as a higher redox potential is maintained in the rhizosphere, forming an iron plaque which prevents rice plants to accumulate more As^{III}(Pan et al., 2014). As^{III}, DMA and MMA uptake occurs through silicon transporters (Lsi1/2) (Meharg et al., 2012). Arsenic uptake variability was reported among different genotypes (Chen et al., 2015). Phosphorus transporters are related to arsenic absorption as arsenate (Jiang et al., 2012). Wang et al. (2016), found that mutants in OsPT8 phosphorous transporter absorbed less arsenic than other rice genotypes.

In recent years several studies had approached the analysis of the variability of the rice arsenic content in South American countries. Oteiza et al. (2020), determined for Argentinean milled rice mean concentrations of tAs, oAs and iAs of 0.303 mg kg^{-1}, 0.222 mg kg^{-1} and 0.081 mg kg^{-1}, respectively. Almost 32% of the Argentinean milled rice samples reported tAs $\geq$0.30 mg kg^{-1}. Kato et al. (2019), found that Brazilian arsenic rice content from different rice regions can vary more than two orders of magnitude. Average and median reported tAs husked rice content was 0.174 and 0.11 mg kg^{-1}, respectively. The mean and standard deviation reported iAs husked rice content was 0.123$\pm$0.026 mg kg^{-1}, respectively. Mondal et al. (2020), reported in Peru average total As concentration in rice was 0.168 $\pm$ 0.071 mg kg^{-1} (n = 29; range 0.06839–0.3453 mg kg^{-1}).

Carracelas et al. (2019), concluded that inorganic As accumulated in polished rice grain cultivated in two different locations in Uruguay were found to be below the regional (Mercosur, 2011), and international limits (FAO and WHO, 2019). The study was conducted in two specific sites in the North and East rice growing regions in Uruguay. A recommendation was stated for a more extensive, broader and regional study in order to further understand the spatial variability of grain As levels.

A relative intensive and spatially comprehensive rice grain sampling scheme was implemented during to growing season in Uruguay. The general objective of this study was to quantify the levels and range of tAs and iAs concentrations in polished rice from different Uruguayan rice growing regions. Additionally, investigate and identify factors that are associated and potentially explain the difference in accumulation of tAs and iAs in grain, such as rice variety, cultivar type and the soil geological materials where the rice was cultivated.

2. Methods

2.1. Sample collection

A total number of 150 samples were collected from all Uruguayan rice producing regions which included most planted rice varieties Uruguay is divided into several rice-producing areas known as numeric areas (NA) within the different rice regions (Supplementary Figure 1). Sampling was done during two rice growing seasons: 2017–2018 and 2018–2019, with same number of samples taken in each season (n = 75). Sampling criteria was meticulously planned to have a representative sample of each rice variety planted across regions. In order to carried out the representative sampling the first step consisted in determine the proportion of sowing area that each region represented of the total rice producing area based on the analysis of information provided by Ministry of Livestock, Agriculture and Fisheries of Uruguay (DIEA MGAP, 2020). Rice varieties with less than 4% area for each region were not sampled in this study. All samples used for this study where taken from the traceability system implemented by the Rice Industry Millers Association (GMA) and Rice Growers Association (ACA) in Uruguay. In this procedure each rice sample was taken from every truck accessing to the drying or milling facilities. Each sample had a code with the identification of the farmer, rice variety and geographic coordinates with the location of the field. Geological information was obtained from a digitalized version of Geological Map of Uruguay (MIEM, 1985). All samples were georeferenced using geo-coordinates and geological maps were overlapped using QGis 3.8 software (QGIS, 2020). Average level range of tAs and iAs arsenic were determined considering the rice grain samples taken within each NA and are presented in Fig. 2a and b, respectively. A complete geological description from each sampling site was obtained by joining layers attributes using data management tools. List of all rice varieties, cultivar type (*Indica* or *Japonica*) and number of samples in both growing seasons are presented in Table 1.

2.2. Arsenic speciation analysis (tAs and iAs)

Samples in this study were 200 g each, of 13% moisture paddy rice. Samples were received in plastic bottles. Determination of total arsenic (tAs) and inorganic arsenic (iAs) was done in Technological Laboratory of Uruguay (LATU). Rice samples were processed in a Satake Pilot Plant, taking 100 milling grade as the end point of the production. Milled rice grain samples were frozen until grinding and were grinded with a blade mill to pass a 1 mm sieve. 1g of milled rice was digested with 10 mL of 0.28 M Nitric Acid (Merck, 65% for analysis) in 50 mL plastic tubes, 15 min at 95 °C in a preheated water bath (GLF 1083, Deutschland). The extracts were diluted with 1,5 mL of 30% Hydrogen Peroxide (Carlo Erba, for analysis) and 5,2 mL of deionized water, centrifuged at 3000 rpm for 10 min and filtered with a 0.45 μm nylon syringe. High performance liquid chromatography (Flexar, PerkinElmer, USA) coupled to inductively coupled plasma mass spectrometry (Nex Ion 350 D, PerkinElmer, USA) was used to determine inorganic arsenic as the sum of two inorganic forms of arsenic, arsenite and arsenate and total arsenic as the sum of inorganic arsenic and organic arsenic (monomethylarsonate (MMA) and dimethylarsinate (DMA)) (Narukawa et al., 2017). Gemini reverse phase column (5μ, 4,6, 250 mm) was used, and 1 mM ammonium phosphate dibasic (99.5% pure, Crystals, Mallinckrodt) and 0.05% Methanol (Carlo Erba for analysis) at pH < 2.0 was used as mobile phase. Arsenic was monitored at *m/z* of 75 with standard cell mode. Calibration curves of inorganic arsenic was prepared with arsenite (1001 mg L^{-1}) and arsenate (1000 mg L^{-1}) stock standards from Inorganic Ventures (USA). Calibration curve of organic arsenic was prepared with Monosodim acid methane arsonate sesquihydrate MMA (99.5%) from ChemService (USA) and Cacodylic Acid- DMA (>99.0%) from Sigma Aldrich (USA). Every 20 samples, one blank, two fortified samples, and one certified reference material (1568b Rice Flour, National Institute of Standards and Technology, USA) were included as quality control samples. Certified reference materials (1568b) were used to assess the accuracy of total As concentration and As speciation for rice flour. Certified results correspond to: Total As: Certified value 0.285 mg kg^{-1}. Obtained results: Mean 0.271 mg kg^{-1} (Min 0.243 - Max 0.350) n = 45. Inorganic As: Certified value 0.092 mg kg^{-1}. Obtained results: Mean 0.095 mg kg^{-1} (Min 0.079 - Max 0.11) n = 45.

Table 1
Rice variety, cultivar type and total number of samples collected in each growing season: first season S1 2017–18 and second season S2 2018–19.

Variety name	Cultivar type	N^0 of samples	S1 (2017–18)	S2 (2018–19)
INIA Olimar	*Indica*	37	18	19
EP 144	*Indica*	24	17	7
Guri CL	*Indica*	24	9	15
INIA Tacuarí	*Japonica*	22	12	10
INIA Merin	*Indica*	20	5	15
Inov Cl (hybrid)	*Indica*	16	9	7
CL 212	*Indica*	6	4	2
Quebracho	*Indica*	1	1	0
	Total:	150	75	75

2.3. Statistical analyses

Statistical analyses were performed in R software (R Core Team, 2019). A frequency distribution analysis of iAs and tAs concentration in polished rice grain was performed for total sample dataset. Pearson's correlation analysis was performed between iAs and tAs. A descriptive analysis was performed including mean, coefficient of variation (CV%), minimum and maximum values of iAs and tAs. Analysis of variance (ANOVA) was used to analyze differences between levels of tAs and iAs from the different cultivar type, geological material, rice varieties and producing regions. Rice grain samples extracted from fields from the two different growing seasons follow the representative criteria described above. However, they do not coincide exactly on the same locations. Based on these mixed effects of location and growing seasons the pooled total samples were analyzed. When significant differences were determined by ANOVA ($p < 0.05$), the Duncan's new multiple range test was applied.

3. Results

3.1. Regional variability of total arsenic (tAs) in rice grain

The cumulative frequency concentrations of tAs species in polished rice samples (n = 150) obtained from the two-year study are shown in Fig. 1a.

The mean and median concentration of tAs in polished rice for the two monitored seasons were 0.178 mg kg^{-1} and 0.147 mg kg^{-1} respectively. The minimum tAs registered value was 0.015 mg kg^{-1} and the maximum tAs value was 0.629 mg kg^{-1}. The coefficient of variation for tAs was 63.6%. Sampling locations and levels tAs spatial variability along the different rice producing regions are shown in Fig. 2a.

3.2. Regional variability of inorganic arsenic (iAs) in rice grain

Cumulative frequency concentrations of inorganic As species in polished rice samples (n = 150) obtained from this two-year study are presented in Fig. 1b.

The mean and median concentration of iAs in milled rice were 0.062 mg kg^{-1} and 0.055 mg kg^{-1} respectively ranging from 0.005 mg kg^{-1} up to a maximum of 0.195 mg kg^{-1}.

Sampling locations and levels of iAs spatial variability along the different rice producing regions is shown in Fig. 2b.

3.3. Relationship within total and inorganic arsenic species

A significant linear relationship ($p < 0,0001$) was found between iAs and tAs for the 150 samples evaluated (tAs = 0.193 iAs + 0.028) with a moderate correlation R^2 of 0,47 between iAs and tAs. (Fig. 3).

3.4. Rice variety and cultivar type effects on the As accumulation in rice grain

Arsenic concentrations (tAs and iAs) of the different rice varieties and cultivar type (*Indicas and Japonicas*) are resumed on Table 2.

Mean concentration of tAs and iAs for *Indica* cultivars where significantly higher than *Japonica* cultivar, 0.188 and 0.066 mg kg^{-1} Vs 0.123 and 0.0035 mg kg^{-1} respectively. Coefficient of variation was high for both tAs and iAs, 63.6% and 51.5%, respectively. Average proportion of iAs to tAs (0.062/0.178 mg kg^{-1}) was 0.348.

Within *Indica* cultivar type no differences in iAs were registered for the different varieties. Regarding tAs Inia Merin that have a longer crop cycle reported significant higher levels than the other varieties. An association between crop cycle duration and level of tAs was observed.

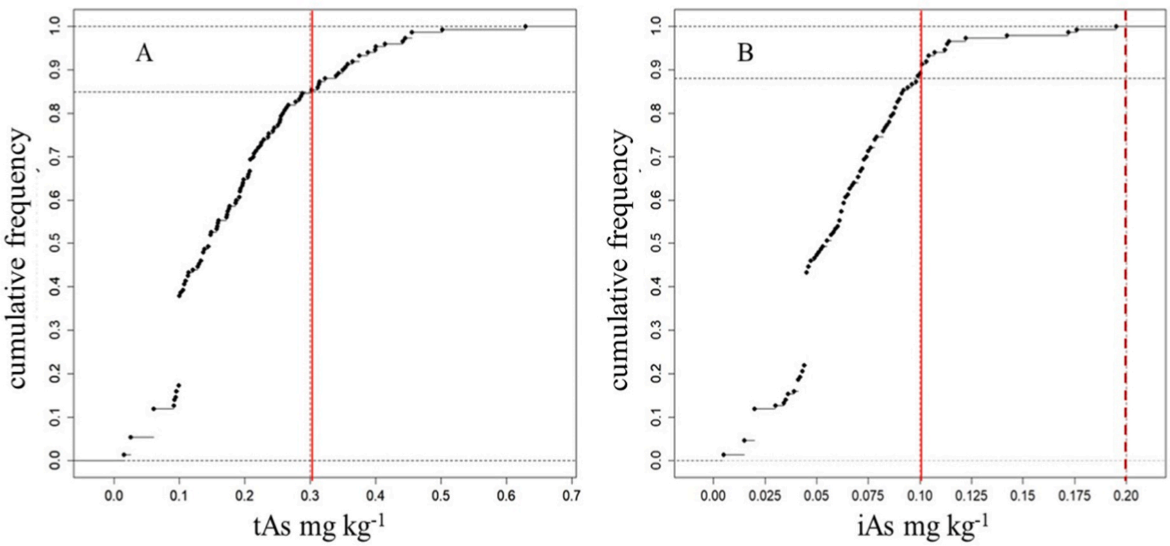

Fig. 1. A. Cumulative frequency of total arsenic (tAs mg kg^{-1}) accumulated in polished rice grain in Uruguay. Vertical red line is indicating the regional tAs limit of 0.3 mg kg^{-1} (Mercosur, 2011). B. Cumulative frequency of inorganic arsenic (iAs mg kg^{-1}) accumulated in polished rice grain in Uruguay. Vertical lines are indicating the arsenic reference levels for infant food products (red) of 0.10 mg kg^{-1} (EUEuropean Union, 2021; FDA, 2020) and 0.20 mg kg^{-1} (red dashed) for Codex Alimentarious (FAO and WHO, 2019). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

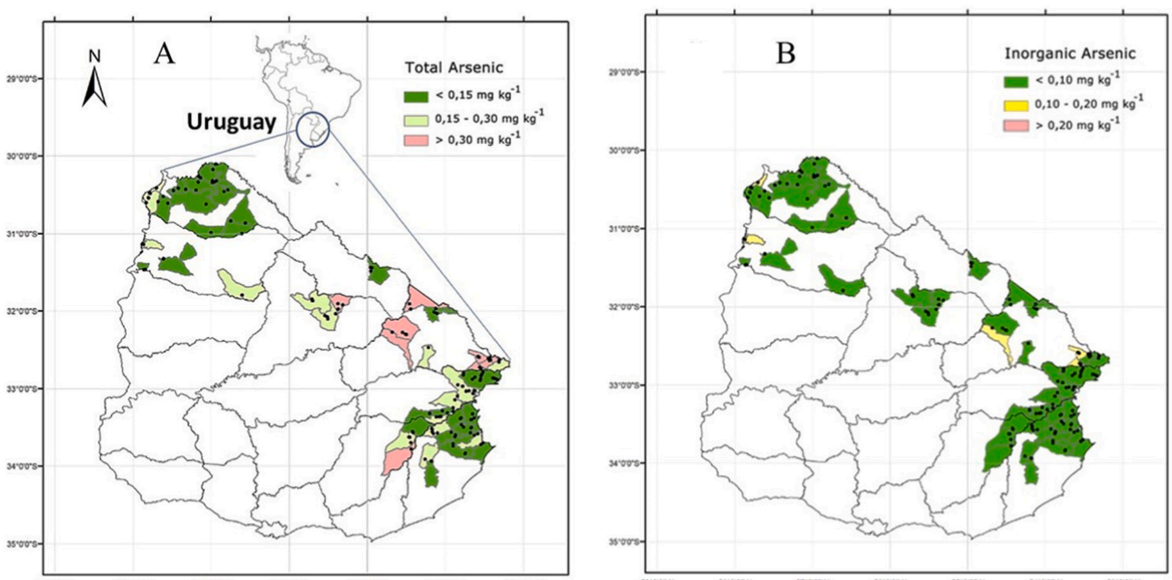

Fig. 2. A. Total Arsenic (mg kg^{-1}) levels recorded by sampling site (black dots). Different colors are indicating the range of all samples included in each rice-producing areas of Uruguay (NA). (green: <0.15, yellow: 0.15–0.30 and red: >0.30 mg kg^{-1}). **B.** Inorganic Arsenic (iAs mg kg^{-1}) levels recorded by sampling site (black dots). Different colors are indicating the range of all samples included in each rice-producing areas of Uruguay (NA). (green: <0.10, yellow: 0.10–0.20 and red: >0.20 mg kg^{-1}). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.5. Total and inorganic arsenic levels in different geological material and rice growing regions

In the three rice growing regions: north, central and east rice is grown over soils originated from two major geological materials: sedimentary (larger proportion) and igneous.

Rice fields over sedimentary geological material reported significantly higher concentration of tAs and iAs in rice grain than rice fields over igneous type (0.198 mg kg $^{-1}$ tAs and 0.067 mg kg^{-1} iAs vs 0.084 mg kg $^{-1}$ tAs and 0.039 mg kg^{-1}, respectively) (Table 3).

Central Rice Region reported significantly higher concentrations of tAs and iAs in the rice grain (Table 3). Rice form the Northern Region reported significantly lower levels of tAs and iAs in the rice grain.

4. Discussion

Arsenic levels in food are strongly regulated and international standards are being continuously revised. According to the Codex Alimentarius iAs levels for polished and husked rice should be below 0.2 and 0.35 mg kg^{-1}, respectively (FAO and WHO, 2019). Compliance with these standards influences access to international markets which is crucial for exporting countries like Uruguay. Regional Mercosur technical regulation on maximum limits of As in foods are 0.30 mg kg^{-1} (Mercosur, 2011). The 0.30 mg kg^{-1} is the maximum total As permitted

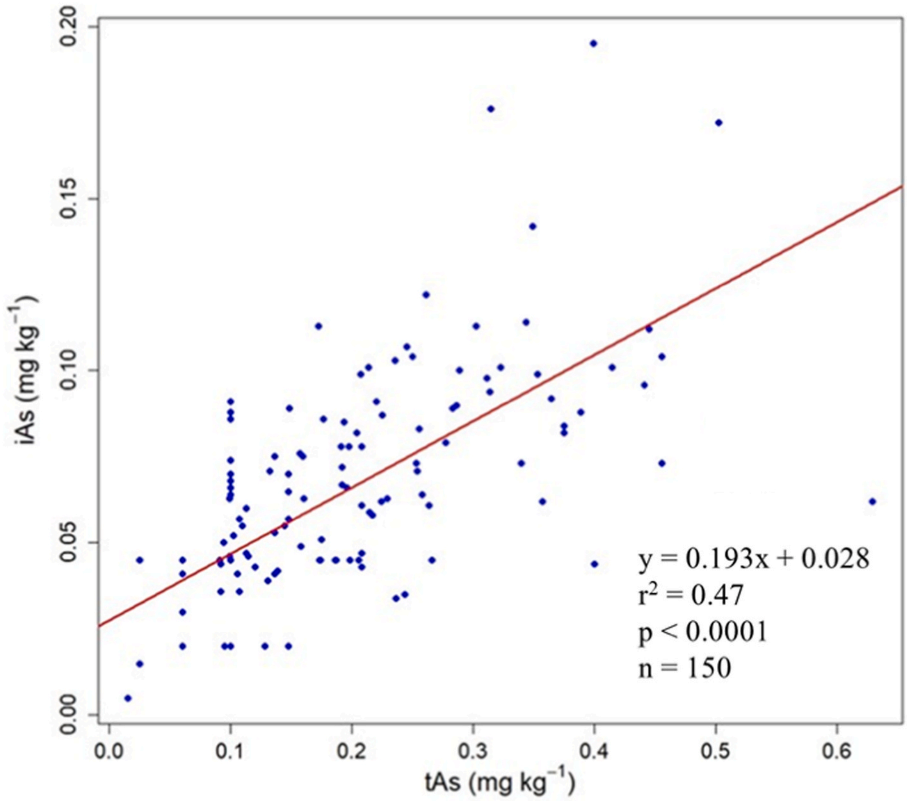

Fig. 3. Correlation between tAs and iAs species (mg kg^{-1}) in150 samples of polished rice grain.

Table 2
Total arsenic (tAs) and inorganic arsenic (iAs) mean concentration (mg kg^{-1}) in polished rice grain for different varieties and cultivar type. Values between parenthesis indicate minimum and maximum.

Cultivar Type	tAs (mg kg^{-1})	iAs (mg kg^{-1})
Indica (n = 128)	0.188 ^{a} (0.015–0.629)	0.066 ^{a} (0.005–0.195)
Japonica (n = 22)	0.123 ^{b} (0.025–0.266)	0.035 ^{b} (0.015–0.046)
CV (%)	63.6	51.5
Average	0.178	0.062
P < 0.05	*	***
Rice Varieties+		
INIA Olimar (n = 37)	0.137 ^{b} (0.015–0.455)	0.058 ^{a} (0.005–0.142)
EP 144 (n = 24)	0.188 ^{ab} (0.025–0.445)	0.066 ^{a} (0.045–0.176)
Guri CL (n = 24)	0.178 ^{b} (0.06–0.455)	0.059 ^{a} (0.020–0.103)
INIA Tacuarí (n = 22)	0.123 ^{b} (0.025–0.266)	0.035 ^{b} (0.015–0.046)
INIA Merin (n = 20)	0.245 ^{a} (0.060–0.629)	0.061 ^{a} (0.020–0.122)
CV (%)	66.1	49.7
Average	0.169	0.056
P < 0.05	***	**
Inov Cl (hybrid)* (n = 16)	0.231 (0.1–0.502)	0.098 (0.045–0.195)
CL 212* (n = 6)	0.215 (0.1–0.414)	0.080 (0.045–0.107)
Quebracho* (n = 1)	0.339	0.073

Means followed by different letters are significantly different with a probability less than 5% (P < 0.05). Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05; NS: non-significant differences. CV: coefficient of variation. +Varieties with number of samples below n = 20 were not considered for the rice varieties statistical analysis.

content to the edible part of the food product. Currently, this regulation is under evaluation with an interest to move to a set of iAs limits. This Technical Regulation does not apply to foods for infants and young children. The iAs concentration for infant rice products limit is below 0.10 mg kg^{-1} in the USA (FDA, 2020) and European Union (EUEuropean Union, 2021).

Table 3
Mean concentration (mg kg^{-1}), range, coefficient of variation (CV%) of total and inorganic arsenic in polished rice according to geological material and rice region.

Classification criteria	tAs (mg kg^{-1})	iAs (mg kg^{-1})
Geological material		
Sedimentary (n = 124)	0.198 ^{a} (0.025–0.629)	0.067 ^{a} (0.015–0.195)
Igneous (n = 26)	0.084 ^{b} (0.015–0.197)	0.039 ^{b} (0.005–0.091)
P < 0.05	***	***
Rice Region		
North (n = 37)	0.121 ^{c} (0.015–0.441)	0.053 ^{b} (0.015–0.122)
Central (n = 28)	0.257 ^{a} (0.099–0.502)	0.082 ^{a} (0.035–0.172)
East (n = 85)	0.178 ^{b} (0.025–0.629)	0.059 ^{b} (0.015–0.195)
CV (%)	63.6	51.5
Average	0.178	0.062
P < 0.05	**	***

Means followed by different letters are significantly different with a probability less than 5% (P < 0.05). Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05; NS: non-significant differences. CV: coefficient of variation.

4.1. Arsenic concentrations in polished rice

According to the regional Mercosur regulations and limits, 85% of the samples (n = 127) of this study reported tAs concentration below 0.30 mg kg^{-1} (Fig. 1a). A low proportion of the samples located in the east and central rice growing regions (15%, n = 13) exceeded the 0.30 mg kg^{-1} limit (Fig. 2a). In comparison with other countries in South America, the average tAs concentration reported in this study of 0.178 mg kg^{-1} (n = 150) ranging from 0.015 to 0.629 mg kg^{-1} was slightly higher than values reported in husked rice from Peru (Mondal et al., 2020) and from milled rice values from Ecuador (Otero et al., 2016) (Table 4). Values reported in this study were similar to tAs in Brazilian husked rice from Santa Catarina and lower than the tAs concentrations

Table 4
Total arsenic (tAs) and inorganic arsenic (iAs) mean concentration (mg kg^{-1}) in different studies in South America.

Citation	Country	tAs (mg kg^{-1})	iAs (mg kg^{-1})
Oteiza et al. (2020)	Argentina	0.303	0.081
Kato et al. (2019)	Brasil	0.174	0.123
Mondal et al. (2020)	Peru	0.168	–
Otero et al. (2016)	Ecuador	–	0.120
Carracelas et al. (2019)	Uruguay	–	0.070
Current study	Uruguay	0.178	0.060

registered in rice from Rio Grande do Sul (Kato et al., 2019). As mentioned, average values of tAs reported in Argentina were lower than Uruguay. However, the percentage of samples below the 0.3 mg kg^{-1} regional limit was smaller in Argentina (70%) than reported in this study (85%). The relationship found in this study between average tAs and proportion of samples below regional limit reflects that variability especially towards the higher end is larger. Further investigations are needed to quantify either agricultural or genetic practices associated to this variability.

In the case of iAs concentrations, levels of all rice samples where below the 0.2 mg kg^{-1} international Codex Alimentarius limits (Figs. 1b and 2b). This information is similar with previous experimental results reported in Uruguay by Carracelas et al. (2019).

In respect with the infant rice food limits, 89% of the analyzed samples (n = 133) were below the limit of 0.1 mg kg^{-1} (EUEuropean Union, 2021; FDA, 2020).

A moderate correlation was revealed within iAs and tAs (Fig. 3, $r^2 = 0.47$, $y = 0.193x + 0.028$, $p < 0.0001$). According to these results it would be not very accurate to assume a certain fixed percentage of tAs as iAs. Very similar results were presented by Oteiza et al. (2020) in Argentina. Coefficient of correlation of 0.47 reported in this study is even lower than the average across a worldwide dataset reported by Meharg et al. (2012) (r2 = 0.768).

The ratio between average iAs to tAs was 35% similarly with most of the study done in south America. However, this magnitude of proportion of iAs of tAs (30–35%) is quite smaller done what was found in other rice region of the world. Proportionality of iAs to tAs varies regionally (Zhao et al., 2010; Meharg et al., 2012).

Total As concentrations presented a slightly higher coefficient of variation (Table 2: CV% = 63.6%) compared to iAs levels (CV% = 51.5%) following other similar studies (Otero et al., 2016; Kato et al., 2019; Oteiza et al., 2020). The relative high magnitude of the CV's reflects the characteristically natural high variability of As accumulation in rice grain and the potentially large number of variables and conditions that may be involved (Majumder et al., 2019).

4.1.1. Health risk analysis for three different scenarios

In recent years increasing concerns have being raised on the issue of heavy metals on food and particularly on arsenic levels in groundwater in Uruguay (Machado et al., 2020; Mañay et al., 2019; Falchi et al., 2018). High levels of As in groundwater have been reported in some wells located in aquifers in the south-west region of Uruguay (Mañay et al., 2019). Falchi et al. (2018) reported in the main rice region (east) lower groundwater As levels which were below local and international limits. Arsenic concentration levels in water is unlikely to be an issue as rice is not cultivated in the south -west region and no underground water from aquifers is pumped for irrigation purposes in the rice sector. Main water sources for rice irrigations are rivers, lagoons, and dams (DIEA MGAP, 2018). Having this context and the relatively large number of grain arsenic measurements gathered in this study a health risk analysis for Uruguay consumers was performed following the same procedure and scenarios used by Menon et al. (2020). This risk assessment procedure takes in account the following factors: per capita consumption (daily intake), body weight, lifetime cancer risk which assumes daily exposure over an entire lifetime. Three scenarios were analyzed: Scenario 1 is based on current average per capita rice consumption rate 0.032 kg d^{-1} reported for Uruguay (FAOSTAT, 2018) and the average iAs content of the 150 samples examined in this study (0.062 mg kg^{-1}). Scenario 2 is based on the calculation of the maximum average daily consumption rate to avoid lifetime cancer risk. Scenario 3 is also based on the maximum average daily consumption rate of the Target Hazard Quotient and Margin of Exposure. Details of the procedure, calculations and analyses are explained in the supplementary information document (Supplementary Table 1).

Based on the combination of the three scenarios, average rice consumption and levels of iAs reported in this study the actual level of annual rice consumption is almost three times and two times lower for male and female, respectively than the limit necessary to reach a health risk threshold. Actual level of annual rice consumption is 11.5 kg for male and female while their maximum allowed levels would be 32.5 kg and 25.2 kg for male and female, respectively. Daily safe consumption could reach 0.089, 0.069 and 0.010 kg d^{-1} for male adults, female adults and 1-year old infants, respectively.

Following these considerations and based in the level of consumption of rice and the mean iAs levels determined in this study there is no risk for any of the target populations (male, female and 1-year old infants).

4.2. Cultivar type and varieties effects on the accumulation of arsenic in rice grain

The iAs levels in *Indica* varieties where significantly higher than the *Japonica* one (0.066 mg kg^{-1} vs. 0.035 mg kg^{-1}) (Table 2). This differences in varieties where also found in other previous studies. Carracelas et al. (2019), studying irrigation alternatives on arsenic concentrations also in Uruguay worked with five different cultivars, three *Indica* and two *Japonica* varieties and found that Japonica's INIA Tacuarí and Parao accumulated lower arsenic inorganic concentrations in rice grain. In addition, other field studies worldwide have shown a substantial genetic variation in grain arsenic concentration as well as in arsenic speciation (Meharg et al., 2012). Similarly, results were also reported by Jiang et al., 2012, in China, where iAs and tAs values were lower in *Japonica* rice types than *Indica* ones. tAs levels showed a similar trend differences: tAs in *Indica* varieties was 52.8% higher than *Japonica* varieties (0.188 mg kg^{-1} vs 0.123 mg kg^{-1}) (Table 2).

A limitation of the present study is that rice samples for the only Japonica rice type (INIA Tacuarí) is drastically less represented on the overall number of samples (22 of 150, Table 1). In addition to this INIA Tacuarí is only planted on a spatially concentrated area on the East part of the Country.

Within the evaluated *Indica* varieties, no significant differences in the iAs levels were registered.

An association between crop cycle duration and level of tAs was observed similarly with what reported by Meharg et al. (2012). INIA Merin with the larger crop cycle duration (155 days) presented significantly higher levels of tAs than all other varieties.

Further research is required to better understand the relation of crop cycle duration and grain filling length and the level of accumulation in tAs in grain for different *Indica* and *Japonica* cultivars.

4.3. Geologic and regional variation

Rice samples obtained from fields over soils formed from sedimentary geological material showed significantly higher levels of iAs and tAs (Table 3) like what was reported by Fu et al. (2011). This author found a high correlation between Fe–Mn oxides concentration in soils to As grain levels in rice related to sedimentary rocks on a research developed in Hainan Island in China. The mean value of iAs in rice samples from sedimentary rocks was 71.8% higher than rice samples grown over soils formed from Igneous rocks. Same results were obtained comparing tAs mean values for samples taken from soils formed from sedimentary rocks had a 136% increase in tAs comparing to samples taken from soils formed from igneous rocks (0.198 mg kg^{-1} vs 0.084 mg kg^{-1}).

This result are aligned with information reported by the National Academy of Science and Biologic Effects of Environmental Pollutants report in 1977 (National Research Council, 1977); that listed lower concentration of arsenic in igneous than sedimentary rocks, Bundschuh et al. (2008), on an extensive review of the typical values of arsenic concentrations related to rocks, sediments and soils determined that igneous type of rocks frequently have lower concentrations than sedimentary rocks, when comparing to Fe and Mg oxides. Igneous rocks location in Uruguay are associated to higher slopes and higher positions in the topography than sedimentary rocks. Ferrando et al. (2002), studied the concentration of iron oxides and its reactivity related to phosphorous dynamics in rice fields from Uruguay. They concluded that soils originated from sedimentary rocks presented higher Fe oxides reactivity and low crystallinity species than soils originated from igneous rocks, even when total concentration of Fe oxides where higher in soils generated from igneous rocks. This author relates the presence of low crystallinity and high reactivity Fe species to the alternance of wetting and drying periods causing reductive and oxidizing conditions in soils. Bundschuh et al. (2008), found that the concentration of arsenic in sediments is positively correlated with iron concertation. However, this author affirms that final concentration of As in uncontaminated soils depends on redox potential, having lowland soils more reductive conditions, situation that could explain higher As concentrations. Flooding conditions during rice irrigation could lead to an increasing As availability too, especially in soils originated from sedimentary rocks where inorganic As species are bound to Fe and Mn oxides. In this study soil arsenic availability was not measured from the different rice fields where samples were taken. Measuring the availability of soil As levels will be very important for further research in order to help understand rice grain As content. Particular precaution should be taken also in consideration in these results and described associations due to imbalance number of rice samples from both type of rocks (126 sedimentary and 24 igneous, Table 3).

Similarly with what was reported by Carracelas et al. (2019) rice samples for the north region of the country reported significantly lower tAs levels than in the East part. A trend of relatively higher levels of tAs in the East and Central part of the rice growing regions can be observed in the map (Fig. 2a). Soil types and field characteristics of higher slope at the North, could favors a reduction of the anoxic saturated conditions periods and potentially reduce As soil availability. In contrast nonspecific regional trend (Fig. 2b) of iAs can be observed along regions.

5. Conclusions

Total As levels were in average 0.178 mg kg^{-1} with a range of 0.015–0.629 mg kg^{-1}. Inorganic As levels were in average 0.062 mg kg^{-1} ranging from 0.005 to 0.195 mg kg^{-1}. All rice samples (n = 150) were below the limit of iAs proposed by the Codex international standards of 0.20 mg kg^{-1}.

The average proportion of iAs from tAs in this study was 35%. However, tAs and iAs showed a moderate correlation coefficient (r^2) of 0.47. Therefore, it would be not very accurate to assume a certain fixed percentage of tAs as iAs.

Accumulation of As in rice grain was mainly influenced by the geological material that originated the different soil types where rice was planted and by the cultivar type and rice varieties. Rice cultivated on soils from igneous geological material resulted in significantly lower levels of tAs and iAs in relation to rice planted on soils originated from sedimentary deposits.

Significantly lower As levels (tAs and iAs) were determined in *Japonicas* rice grain in relation to *Indicas* cultivars.

Following the procedure used by Menon et al., 2020, a health risk analysis for Uruguay consumers was done indicating that the consumption of rice by male and female adults is safe according to its level of annual consumption and based on the mean iAs levels determined in this study. The actual level of annual rice consumption is almost three times and two times lower for male and female (32.5 kg vs 11.5 kg and 25.2 kg vs 11.5 kg), respectively than the limit necessary to reach a health risk threshold.

The relatively large number of rice samples analyzed on this study covering all rice regions in the country (n = 150) was suitable for preliminary exploration of associations with other variables. A large variability on the polished rice As levels was observed in all potential exploratory variables, like in most similar studies (Oteiza et al., 2020; Kato et al., 2019; Mondal et al., 2020), indicating that a very complex and unstable interactions may regulate the absorption of this soil element to the plant. Further investigations should be carried out to better determine the level of these factors on affecting rice grain As variability.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.132426.

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