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Irrigation management and variety effects on rice grain arsenic levels in Uruguay

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ABSTRACT

Rice is the most important staple component of the human diet worldwide. The higher amounts of arsenic accumulation in its grain in relation to other crops, determines a potential toxicity risk to humans. This research project aimed to determine the inorganic arsenic accumulation in rice grain (iAs) in two contrasting soil sites, Paso Farias-Artigas (PF) and Paso de la Laguna-Treinta y Tres (PdL), with two different mitigation practices, in Uruguay. These being firstly irrigation management techniques and secondly the use of different varieties. Five experiments were conducted with a split plot design with four blocks over three rice growing seasons from 2014 until 2017. The experimental sites included two irrigation treatments: continuous flooded (C) and alternate wetting and drying (AWD). The split plots included different varieties: *Indicas* and *Japonicas.* Average iAs accumulated in rice grain were 0.07 mg kg^{-1}, well below international limits, even under the C irrigation technique. It was found that iAs accumulation in rice grain can be further reduced by the implementation of AWD in certain soil types. *Japonica* varieties had a lower accumulation of iAs in rice grain, in comparison with *Indicas* at both sites.

1. Introduction

Growing demand for food around the world is expected to expand rice production by 1.1% to almost 510 million tons in 2018/19 [1]. Rice is the most important staple component of the human diet worldwide with an average consumption of 54 kg of grain per person [1]. Arsenic content in rice presents a risk to human health; 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 and inorganic and both constitutes the “total arsenic” content. The inorganic 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) [2,3]. The major component species of total arsenic in rice grain is inorganic arsenic ((As^{III} and As^{V})), which are associated with negative health impacts like cancers [4], hypertension, diabetes, and premature births [5]. Arsenic levels in food are concerning as they are frequently associated with high risk factors in food nutritional safety [6,7].

Rice has naturally higher levels of As [8] as plants have a greater ability to absorb and accumulate it in the grain in relation to other staple food crops [9]. Arsenic (As) absorption by rice plants occurs through different transporters depending on arsenic speciation. As^{V} uptake occurs mainly through phosphate transporters, while As^{III} and methylated forms of As uptake occurs through non-specific aquaporins, mainly responsible of silicic acid uptake [10]. Soil characteristics are very important to determine As content and its availability for plants, but As availability also depends on: pH, redox potential, organic matter content, cation exchange capacity, and concentration of iron oxides [11]. When redox potential reaches high levels (200–500 mV), the predominant arsenic specie is As^{V} which has lower water solubility and, thus, generally reduced bioavailability. Solubility rises when an alkaline pH or high reductive conditions promotes the reduction of As^{V} into As^{III}. In an intermediate condition when redox potential is between 0 and 100 mV, Arsenic solubility depends on dissolution of iron oxides. At high redox potential, Fe^{+2} is oxidized to Fe^{+3}, precipitating as iron oxides or hydroxides, forming an iron plaque [12]. The iron plaque acts to adsorb As and reduces the absorption of As by plants [13]. Organic matter also can reduce the mobilization of As in soils. In India, composted municipal waste successfully reduced native soil As mobilization in the rhizosphere by acting as a binding mediator [14].

Arsenic is a natural component in primary minerals, therefore it is

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also found naturally in soils. The As concentration in uncontaminated soils of the world varies from 5 – 10 mg kg^{-1} [15,16]. When this chemical element is partitioned into the aqueous soil phase rather than the solid phase, it has the potential to be uptaken by plants and can be a problem from a health perspective [17]. The levels of As and their forms in rice grain have previously been found to be affected by irrigation, varieties, fertilization and natural presence in air, soils and waters [7,18,19]. Traditional (i.e., continuous) rice flood management can increase the bioavailability and absorption of As by plants. Under anaerobic soil conditions, arsenate is reduced to arsenite which is much more mobile in soil solution and more easily absorbed by rice roots [8]. Additionally, many bacteria are induced to use Mn or Fe oxides as electron acceptors leading to their dissolution, increasing As displacement in the aqueous phase [17].

Several studies have shown that continuous flooded irrigation results in the highest absorption of As by rice crops. AWD (alternate wetting and drying) is an irrigation technique that allows soil water to subside until the soil reaches an aerobic state in unsaturated soil conditions. According to IRRI (http://www.knowledgebank.irri.org/) AWD is a water-saving technology where irrigation water is applied a few days after the disappearance of the ponded water. Hence, the field gets alternately flooded and non-flooded. This technique allows a reduction in water used without penalizing rice grain yield when water depth dropped to no more than 15 cm below soil surface (safe AWD) and field is re-flooded to a water layer of 5 cm. An increase in oxygen concentration in the rhizosphere may increase redox potential, limiting As mobilization [20]. Several studies have reported that AWD could lead to a reduction in the accumulation of As in grain [21–23], thereby contributing positively to food safety while lowering the environmental impact of rice crops and reducing greenhouse gas emissions [19,24]. In relation to rice yield response to AWD, there is a high degree of variation depending on timing, duration and severity during the drying event of this technique. Previous experiments conducted in Uruguay, reported a yield loss of 15% with the AWD treatment tested that allowed a 50% depletion of available water, relative to continuously flooded management [25]. It was also reported by other authors that rice yield can be reduced when soil moisture was below saturation [26–30]. However, some studies reported no significant impact on rice grain yield with safer AWD techniques [21,22,31–35].

Differences within varieties have been reported in the As levels accumulated in root tillers and grain [36]. Accumulation of As in grain was found to be higher in *Indica* rice varieties compared to *Japonicas* [37]. More than 95% of the As absorbed remains in the roots and only 1% is accumulated in the grain [38].

Arsenic levels in food are strongly regulated and international standards are being continuously debated and revised. Recommended inorganic arsenic (iAs) levels for polished and brown rice in the CODEX are 0.2 and 0.35 mg kg^{-1}, respectively [39]. 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} [40]. The 0.30 mg kg^{-1} is the maximum total 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 [41].

Given the permanent review of international standards in terms of safety, it is important to have local information on cultivated rice varieties and management alternatives for reducing the levels of As to promote food safety, consumer health, sustainability and competitiveness of the rice sector in Uruguay.

Rice is the largest irrigated crop in Uruguay with 164500 ha cultivated annually [42]. National total rice production is 1.4 million tons of paddy rice per year, of which more than 90% is exported worldwide. As such, Uruguay ranks seventh in terms of global rice exports and is one of the main exporters in South America [1]. Continuous flooding is the main irrigation technique implemented by farmers and the most planted varieties are *Indicas*, to secure the highest yields, which were shown by several authors to maximize As uptake in rice grain yields. The Uruguayan rice sector is divided in three regions: East (118391 ha), North (33 448 ha) and Central (12 618 ha) representing 72%, 20% and 8% of total annually rice planted area [42]. Dams built for irrigation purposes that capture rainfall water are the main water source (54%) especially in the North and Central region while in the East the main water sources are rivers, lagoons and dams on a smaller proportion [43]. These water sources have reported low arsenic values [44–46] which were below the limits of 0.050 mg L^{-1} (Class2a) and 0.0050 mg L^{-1} (Class 3) for irrigation surface water [47]. High levels of As in groundwater have been reported in some wells located in the south-west region of Uruguay [46], with 52% of the monitored wells above the limit recommended for drinking water of 0.010 mg L^{-1} [48] while only 27% were higher than the national limit in Uruguay of 0.020 mg L^{-1} [49. Falchi et al. [45], reported in the main rice region of Uruguay (East) lower groundwater As levels in average of 0.0063 mg L^{-1} (0.0022–0.0095 mg L^{-1}) which were below local and the international limits [48,49]. This is unlikely to be an issue as rice is not cultivated in the south-west region and currently no underground water from aquifers is pumped for irrigation purposes in the rice sector in Uruguay.

The general objective of this paper was to determine the iAs accumulation in rice grain in two contrasting soils sites, Paso Farias - Artigas (PF) located in the North region and Paso de la Laguna - Treinta y Tres (PdL) in the East region, commonly used for rice production in Uruguay. This research project also aimed to identify alternative irrigation management techniques to traditional flooding that could be used to limit or reduce the iAs accumulation in grain and to determine differences in iAs levels within the two most commonly planted rice varieties in Uruguay.

The specific aims of this research were to: 1. determine if continuous flooded conditions can increase the bio-availability of As in soils, resulting in a higher accumulation of As in grain in relation to the alternative irrigation technique AWD, 2. determine if *Indica* varieties promote higher levels of absorption and accumulation of As in the grain in relation to *Japonicas* and 3. investigate if soil types have an influence on the levels of As accumulation in rice grain.

2. Methods

2.1. Study site description

Experiments were conducted in two experimental units located in Paso Farias, Artigas department in the Northern region (PF: Lat: −30.50S, Long: −57.12W) and in Paso de la Laguna, Treinta y Tres department, in the Eastern region (PdL: Lat: −33.27S, Long: −54.17W) of Uruguay (Fig. 1).

This study was conducted throughout the rice growing seasons of 2014/15–2015/16 - 2016/17 in PdL and during season 2014/15–2016/17 in PF. These study sites have soils which are typical of the rice growing regions in Uruguay.

2.2. Field management

For all years, the planting date ranged from 29 September to 03 October and 08 October to 14 October for the PF and PdL locations, respectively.

Land preparation consisted of a minimum tillage performed in the summer, approximately six to nine months prior to rice planting. Disc plowing was used to control weeds and incorporate previous pasture residues. Additionally, one landplane operation was done and contour levees of 20–30 cms height were constructed. Tillage operations, sowing, pre, post-emergence weed controls and first Nitrogen application were all done on dry soils before permanent flooding. Typical rotation in the experimental sites consisted of one year of rice followed by two to three years of perennial pastures (mixes of grasses and legumes). Soil property information for each field site was determined at the INIA soil laboratory

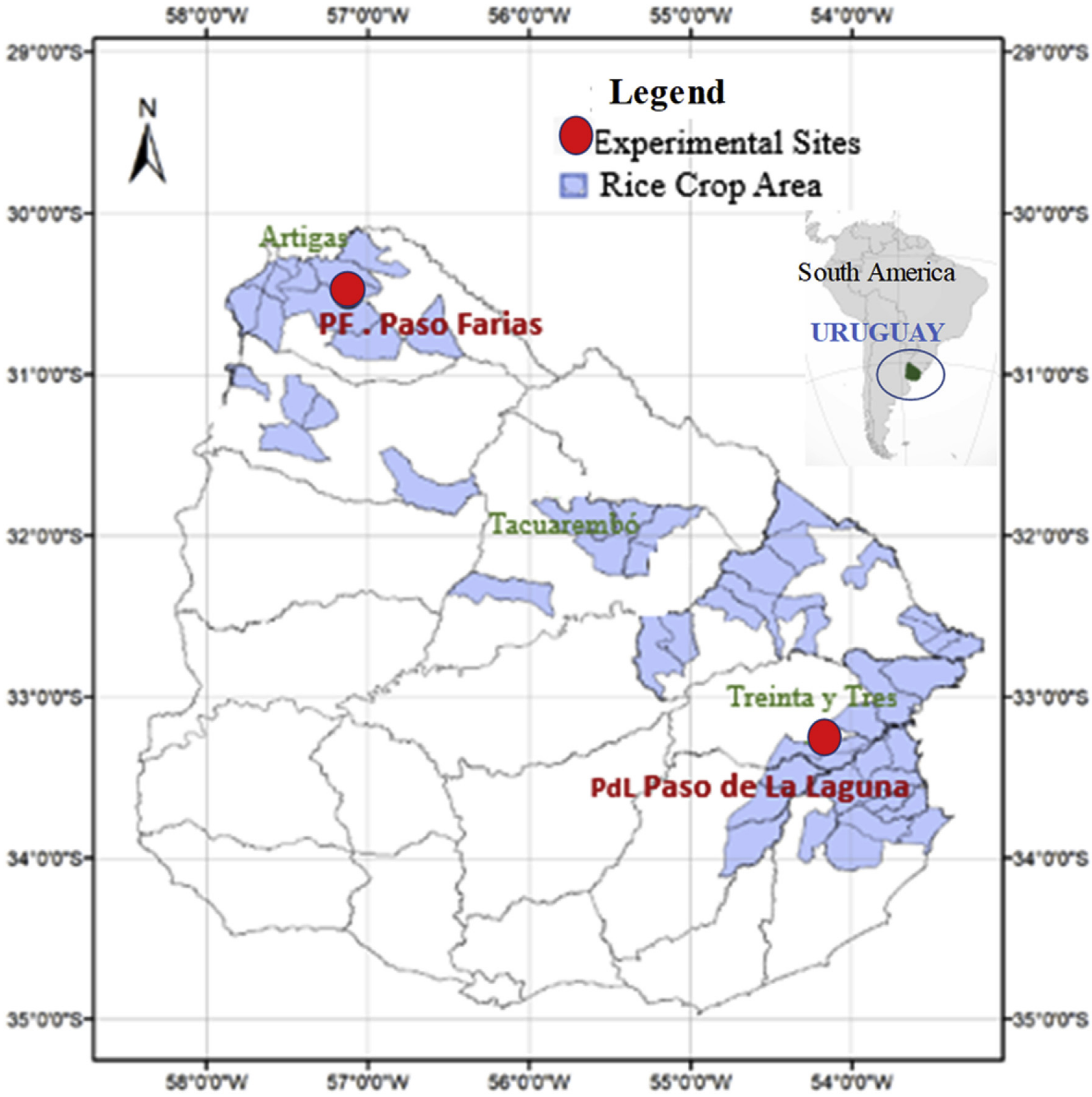


Fig. 1. Location of the rice field experimental sites PF Paso Farias in the North and PdL-Paso de la Laguna in the East (INIA), where experiments were conducted in Uruguay.

(Table 1).

Indica and Japonica type cultivars were planted at both sites (Fig. 2). Direct sowing of rice was performed using a six-row (PF location) or nine-row (PdL location) Semeato brand grain drill (https://www.semeato.com.br/). Row spacings were 17 and 20 cm for the PF and PdL sites, respectively. Sowing density ranged from 145 kg ha^{-1} to 165 kg ha^{-1} depending on the variety as the sowing rate was adjusted by germination percentage and weight of seeds to get the target of around 500 viable seeds m^{-2}.

Fertilization of the crop was based on soil analyses for each site. In PF it consisted of a basal application of Nitrogen (18 kg N ha^{-1}), Phosphorus (46 kg P_2O_5 ha^{-1}) and Potassium (36 kg K_2O ha^{-1}) plus two urea applications at tillering, prior to the flooding and panicle initiation (35 kg N ha^{-1} each). In PdL the basal fertilization was at 12 kg N ha^{-1}, 66 kg P_2O_5 ha^{-1} and 45 kg K_2O ha^{-1} while urea fertilization at tillering and panicle initiation was 23 kg N ha^{-1} each at each application.

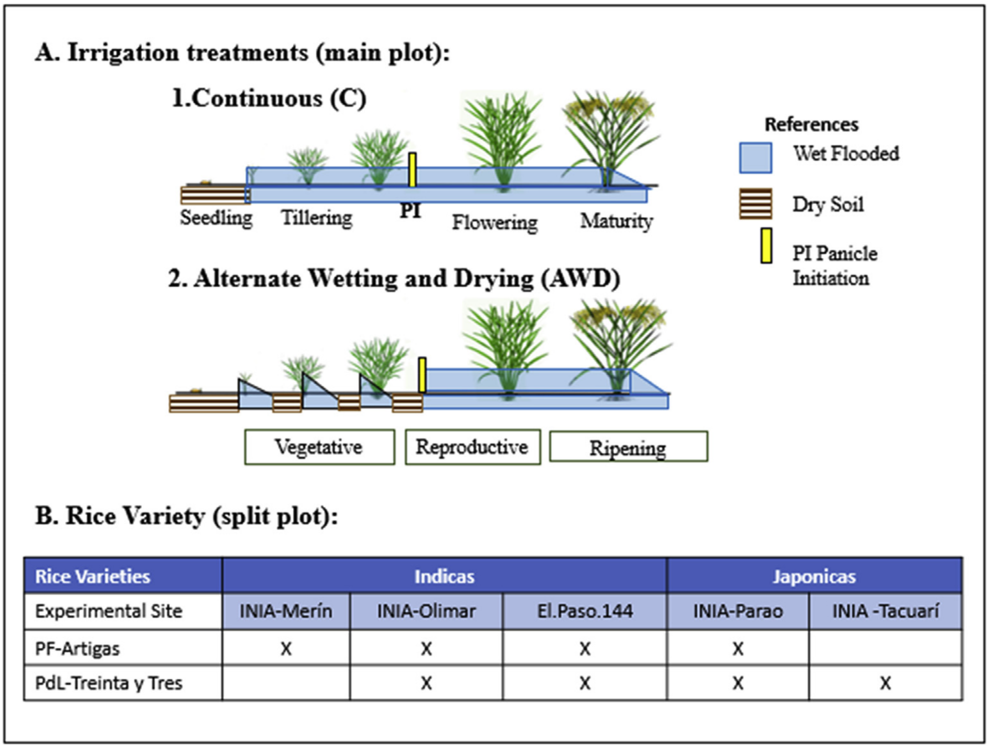
Weed controls varied accordingly to the type of weeds and their degree of incidence across sites and seasons, as per INIA's recommendations. In PdL the chemical products used to control weeds were: glyphosate, propanil, quinclorac, clomazone, exocet, cibelcol and ciperof. In PF: glyphosate, clomazone pyrazosulfuron, metsulfuron and penoxsulam at the standard recommended doses and rates was used. Applications of fungicides to control diseases were not necessary.

Table 1
Soil parameters information determined in INIA soil laboratory. Soil texture information for the first horizon (0–30 cms) Source: SIGRAS, webpage.

Soil Parameter	Experimental Site	
	Paso Farias, PF	Paso de la Laguna, PdL
pH (water)	7.1	5.9
Organic Matter %	4.7	2.1
P Citric Acid (ppm)	4.5	6.9
K (meq/100 g)	0.24	0.18
Texture		
Sand %	10	30
Silt %	38	43
Clay %	52	27
Soil	Vertisol (Itapebí Tres Arboles)	Brunosol (La Charqueada)

2.3. *Treatments and experimental design*

The experimental design was a split plot with 4 blocks in both the PF



Rice Varieties	Indicas			Japonicas	
Experimental Site	INIA-Merín	INIA-Olimar	El.Paso.144	INIA-Parao	INIA -Tacuarí
PF-Artigas	X	X	X	X	
PdL-Treinta y Tres		X	X	X	X

Fig. 2. Irrigation treatments (traditional continuous flooded (C) and alternate wetting and drying (AWD)) and Varieties (*Indicas and Japonicas*) tested at two experimental sites, Paso Farias (PF) and Paso de la Laguna (PdL).

and PdL sites. Main plots consisted of Irrigation treatments while Variety formed the split plot. Four varieties (3 *Indicas* and 1 *Japonica*) were tested in PF: INIA Olimar, ElPaso144, INIA Merin (*Indicas*) and INIA Parao (*Japonica*). Also 4 varieties were evaluated in PdL: INIA Olimar, ElPaso144 (*Indicas*) and INIA Parao, INIA Tacuarí (*Japonica*) (Fig. 2).

Two irrigation management practices were compared at each site. Continuous traditional flooding (C) that represents the most common rice flood management (control) and the alternative irrigation method: alternate wetting and drying (AWD). In treatment C, flooding started 20–30 days after emergence and a water layer of 10 cm above the soil surface was maintained after flooding throughout all the crop cycle. For the AWD treatment, the soil was permitted to dry periodically, allowing a water depletion of 50% of soil available water in the first 20 cm of the soil, which was equivalent to 22–25 mm for the soils at PF and PdL. A water balance was conducted for each site to manage the irrigation in the AWD treatment considering the effective precipitation, crop evapotranspiration and soil water storage capacity. Effective precipitation - EP (mm) was calculated considering the rainfall and surface runoff water according to the precipitation index method and is available at http://www.inia.uy/gras/Monitoreo-Ambiental/. The evapotranspiration was retrieved from INIA weather stations (http://www.inia.uy/). The available water storage capacity for the soils was determined by the difference between the volumetric moisture at field capacity and the volumetric moisture at permanent wilting point. Both parameters were obtained from the tension-humidity curve obtained using the Richards method (Richards, 1948). Additionally, moisture content in the soil was determined in the AWD treatment in the PdL site. The methods used were gravimetric, with weekly measurements at a depth of 0–15 and 15–30 cm, and by capacitance probes FDR (Decagon Devices, EC-5) with continuous measurements, installed at a depth of 0–10 cm.

This management technique (AWD) resulted in oxic and anoxic conditions in the soil (saturated and unsaturated), until panicle initiation. After this stage the crop was continuously flooded and managed as per the control treatment C (Fig. 2).

The source of irrigation water was different between sites. In PF, irrigation water source was from a reservoir (gravity irrigation) while in PdL, the irrigation water was pumped from the local river (Olimar).

2.4. Chemicals and crop parameters measured

The parameters measured were: 1. In the Soil: Total arsenic (tAs) and Bioavailable arsenic (bioAs) at sowing, sampled at two depths: 0–15 cm and 15–30 cm. Bioavailable As at the end of the crop cycle (harvest) was also measured at the same two soil depths. 2. In the Water: Arsenic (As), at 5 and 6 periods during the flooding for AWD and C treatments, respectively. 3. In the crop: Inorganic arsenic in polished grain. All samples were analyzed in the Technological Laboratory of Uruguay - LATU. Additionally, pH and redox potential were measured in irrigation water at each sampling moment (0, 5, 10, 30, 45, 60 days from the start of flooding in C and 0, 5, 10, 30, 45 days in AWD). Also, the crop was harvested to determine rice yield for each treatment.

2.4.1. Arsenic in soil

Bioavailable Arsenic (bioAs) and total Arsenic (tAs) were determined at two soil depths at two stages throughout the growing season: sowing (Initial) and harvesting (End). bioAs represents specifically-sorbed As in soils that may be potentially mobilized due to changes in pH or P addition [50]. Soil samples were made to pass through a 2 mm sieve, dried until constant weight and homogenized in a porcelain mortar [51]. For tAs analysis 1 g of dried soil was digested with 10 mL of nitric acid in a microwave oven (Millestone, Ethos One, Italy) and the digests were diluted up to 30 mL with deionized water [52]. Inductively coupled plasma-Optical emission Spectrometry was used to determine total arsenic in soil samples [53] (ISO, 2007). bioAs was extracted using 0,05 M NH4H2PO4 [50,54]. Five grams of soil was mixed with 25 mL of 0.05 M NH4H2PO4 and shaken at room temperature for 16 h in an orbital shaker (GLF 3016, Deutschland). The samples were centrifuged at 3000 g for 15 min and the supernatants were filtered through a 0.45-mm membrane filter. Graphite furnace atomic absorption spectrometry was used to determine bioavailable As in soil samples. 10 μg of palladium nitrate (Pd(NO3)2) and 6 μg of magnesium nitrate (Mg(NO3)2) were used as regular modifier in a transversally heated graphite furnace with Zeeman correction (PerkinElmer, AA 800, USA).

2.4.2. Arsenic in water

Graphite furnace atomic absorption spectrometry was used to determine As in water samples [55]. As was measured at 193.7 nm in an Atomic Spectrometer lengthwise heated with deuterium background correction (PerkinElmer, AA 200, USA). 15 μg of palladium nitrate (Pd (NO3)2) and 10 μg of magnesium nitrate (Mg (NO3)2) were used as a regular matrix modifier. Sampling was done at 0, 5, 10, 30, 45 and 60 days after flooding in the continuous irrigation treatment while in AWD treatment sampling was done up to 45 days after flooding only as flooding started after panicle initiation and the duration of this period was shorter in this treatment.

Redox potential and pH were measured in the field using a portable device Horiba model D-52-meter manual platinum electrode [24]. This device allowed the recording of instantaneous measurements at each sampling event (0,5,10,30,45,60) days after flooding in C and 0,5,10,30 and 45 days in AWD. Five replicates measurements were taken between rows at 10 cm depth, in each of the four blocks.

The limits of detection and quantification to determine arsenic in water, soil and grain by the Technological Laboratory of Uruguay (LATU) are presented in Table 2. In order to perform statistical analyses, when a sample was below the analytical detection limit (DL) it was considered as half of the value of DL and when a sample data was higher than the limit of detection but lower than the limit of analytical quantification (QL), the mean value between both analytical limits was used.

2.4.3. 3-inorganic arsenic (iAs) in polished grain

Polished rice grain samples were frozen until grinding and were grinded with a blade mill to pass a 1 mm sieve. 1 gr of milled rice was digested with 10 mL of 0.28 M Nitric Acid (Merck, 65% for analysis) in 50 mL plastic tubes, 90 min at 95 °C in a preheated water bath (GLF 1083, Deutschland). The extracts were diluted with 6.7 mL of deionized water, centrifuged at 3000 rpm for 10 min and filtered with a 0.45 μm nylon syringe filter. The filtrate pH was adjusted to 6–8.5. 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 [56]. Hamilton PRP-X100 anion exchange column (5μ, 4,6 × 150 mm) was used, and 10 mM ammonium phosphate dibasic (99,5% pure, Crystals, Mallinckrodt) at pH of 8.25 (±0,05) was used as mobile phase. As was monitored at *m/z* of 75 with standard cell mode. Calibration curves were prepared with arsenite (998 mg L^{-1}), arsenate (1000 mg L^{-1}) stock standards from Spex Certiprep (USA), Monosodim acid methane arsonate sesquihydrate MMA ($\geq$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; and 7532a, Brown Rice Flour National Metrology Institute of Japan) were included as quality control samples. Certified reference materials (1568b and 7532a) were used to assess the accuracy of total As concentration and As speciation for rice flour.

Table 2
Analytical detection and quantification limits of the methodologies used to determine inorganic arsenic in grain (iAs), soil (tAs and bioAs) and water (As) by the Technological Laboratory of Uruguay.

Analytical methodology limits	Rice Grain	Soils		Water
	Inorganic As (iAs mg kg $^{-1}$)	Total As (tAs mg kg $^{-1}$)	Bioavailable As (bioAs μg L^{-1})	Arsenic (As mg L $^{-1}$)
Detection Limit (DL)	0.03	0.6	10	0.001
Quantification limit (QL)	0.06	3	20	0.003

2.4.4. 4-rice yields (kg ha^{-1})

Harvest was done manually in the middle of experimental treatments plots when grain moisture was lower than 21%. Harvested area was 5.95 m^2 (7rows X 5 m) in PdL and 5.1 m^2 each (10 rows × 3 m) in the PF site. The rice samples were mechanically threshed, and grain yields were normalized to 14% moisture.

2.5. Data analysis

Statistical analyses were all performed in R software [57] using the emmeans [58] and nlme packages [59]. A linear mixed effect model was used to fit each of the response variables. Analyses of variance was followed by means separation using the Tukey test. For iAs in grain and rice yield, the fixed effects considered were: Site, Irrigation, Varieties and their interactions. Block, Irrigation and Season were considered as random effects according to a split-plot experimental design. All other soil and water measured parameters were also analyzed using the linear mixed effect model.

3. Results

3.1. Total arsenic (tAs) and Bioavailable Arsenic (bioAs) in soils

Average initial tAs in the soil at sowing was 2.14 mg kg^{-1} in PF site, while in the soils at PdL site tAs was 69% significantly higher with an average value of 3.62 mg kg^{-1}. Additionally, bioAs was 15.1 mg kg^{-1} (99%) higher in PdL compared to PF (Table 3).

There were no significant differences in tAs and bioAs within soil samples in different soil layers (depths: 0–15 cm and 15–30 cm). Also, the interaction site*soil depth was not significant for these soil parameters ($P < 0.05$).

At both sites, average bioavailable As concentrations increased during the rice growing season. There were no significant differences in the levels of bioAs registered at harvest within sites. Additionally, no significant differences were registered in the bioAs levels, within the two irrigation treatments evaluated, C and AWD (Table 4).

3.2. Arsenic, pH and redox potential (Eh) in water

Average Arsenic levels registered in the irrigation water were 0.00224 mg L^{-1}. Arsenic levels registered in the irrigation water were 55% higher in PdL in relation to the PF site. The AWD treatment resulted in a significant As reduction in irrigation water of 24% in relation to C for

Table 3
Total Arsenic (tAs) and Bioavailable Arsenic (bioAs) determined initially (sowing of rice) in soil samples taken at different soil depths (0–15 cm and 15–30 cm) in two experimental sites: Paso de la Laguna (PdL) and Paso Farias (PF).

Classification criteria	Arsenic in Soils at sowing (Initial)	
Site	Total Arsenic (tAs mg kg^{-1})	Bioavailable Arsenic (bioAs μg L^{-1})
PdL	3.62 a	30.30 a
PF	2.14 b	15.21 b
Average	*2.88*	*22.76*
CV%	*27.56*	*15.40*
P<0.05	***	***
Depth (P<0.05)	*NS*	*NS*
Site* Depth (P<0.05)	*NS*	*NS*

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.

Table 4
Bioavailable arsenic in soils (bioAs μg.L^{-1}) determined at harvest time by irrigation treatments and the interaction with experimental sites, Paso de la Laguna (PdL) in the East and Paso Farias (PF) in the North.

Classification criteria	Bioavailable Arsenic in Soils (bioAs μg.L^{-1}) Final-Harvest
Irrigation	*NS*
Site	*NS*
Irrigation*Site	
Site -PdL	
1.Continuous (C)	27.00 a
2. Alternate Wetting and Drying (AWD)	34.67 a
Site-PF	
1.Continuous (C)	40.88 a
2. Alternate Wetting and Drying (AWD)	29.31 a
Average	*32.96*
CV%	*11.71*
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.

both sites. (Table 5).

Water pH was significantly higher (11%) at the PF site in the North (6.1) relative to the PdL site in the East (5.5). There were no significant differences in the pH registered in irrigation water between C and AWD at either site. The interaction between irrigation and site was not significant for the pH parameter (Table 5).

Average Redox Potential of water was 81.7 with non-significant differences between both experimental sites (PF and PdL). Average pH and Eh evolution registered during two seasons for each irrigation treatment and experimental site are presented in Fig. 3. In C, values were measured right after the establishment of permanent flood (20–30 days after emergence) while in the AWD this measurement started during the flooding period from panicle initiation until 20 days before harvest.

The initial values of redox potential in C were higher in PdL in comparison with PF, following a reduction of these values in both treatments during the flooding period (Fig. 3A). At the final sampling event (60 days after flooding) PdL reached lower negative values while in PF, Eh values were almost zero. In AWD treatments, both sites had very similar Eh trends, with PF having higher values at the first sampling event, while PdL also reached lower negative values at the final sampling date (Fig. 3B).

The pH values were initially lower (acid) in PdL in relation to PF and increased during the flooding period, tending to a value near neutrality (pH 6.0) at both treatments and sites (Fig. 3C and D). At PF, pH values were always higher than levels registered at PdL.

Table 5
Average irrigation water Arsenic levels, pH and Redox Potential, measured during the flooding period by Site and Irrigation management.

Classification criteria	Arsenic in water (As mg L^{-1})	pH	Redox Potential (Eh mV)
Site			
PdL	0.00272 a	5.50 a	79.68 a
PF	0.00176 b	6.09 b	83.72 a
Average	*0.00224*	*5.80*	*81.7*
CV%	*22.72*	*2.17*	54.57
P<0.05	***	***	*
Irrigation			
C	0.00255 a	5.75 a	104.73 a
AWD	0.00193 b	5.84 a	58.67 b
Average	*0.00224*	*5.80*	81.7
CV%	*22.68*	*2.17*	*54.59*
P<0.05	**	*NS*	***
Site* Irrigation	*NS*	*NS*	***

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.

3.3. *Arsenic in grain*

Average Inorganic arsenic values registered in polished grain across both sites were 0.07 mg kg $^{-1}$. This parameter was significantly lower in PF (0.043 mg kg^{-1}) than in PdL.

(0.091 mg kg^{-1}) (Table 6).

Significant differences within varieties were registered while the interaction between irrigation:variety for iAs was not significant. (Table 6). *Japonica* cultivars INIA Parao and INIA Tacuarí resulted in the lowest iAs values in relation to *Indica* type cultivars EP144 and INIA Olimar. Average values were 0.03 mg kg^{-1} lower in *Japonica* cultivars compared to *Indica* varieties (0.05 vs 0.08 mg kg^{-1} respectively). However, no significant differences were registered between INIA Parao and INIA Merín (Fig. 4).

A significant interaction between Irrigation and Site was detected for iAs (Table 6). There were no differences within irrigation treatments in the PdL site (average 0.091 mg kg^{-1}), while AWD determined a significant iAs reduction in grain of 0.02 mg kg^{-1} (39.5%) in relation to the traditional continuous flooding in the PF site (Fig. 5).

3.4. *Rice yield*

Average harvested rice yield in this study was 8567 kg ha $^{-1}$. Values of this parameter reported in the PdL site were 21% higher (1577 kg ha $^{-1}$) than the mean yield recorded for the PF site. Significant differences in rice yield were recorded for region, irrigation management and varieties evaluated (Table 7).

The AWD irrigation treatment resulted in a significant yield reduction of 14% (−1326 kg ha^{-1}) in comparison to the traditional continuous flooding irrigation technique.

The highest rice yields were registered with INIA Merín (10203 kg ha^{-1}) and EP144 (9289 kg ha^{-1}) both *Indica* varieties, followed by INIA Olimar with no significant difference with EP144. The *Japonica* cultivars INIA Parao had a significantly lower rice grain yield in relation to *Indica* cultivars (15% reduction) and INIA Tacuarí reported the lowest rice grain yield (Table 7, Fig. 6).

4. Discussion

4.1. *Arsenic concentration in soils*

In the Brunosol soils at the PdL site, average initial tAs at sowing was significantly higher (69%) in comparison to the Vertisols soils at the PF site. One of the natural sources of Arsenic into paddy rice crops can be derived from the soil type [18], which depends on the sediments that it originated from. The levels of As found in the soils at sowing in the two experimental sites located in the PdL and PF sites (3.62 and 2.14 mg kg^{-1} respectively) were below the reported natural world concentration of As in soils of 5 mg kg^{-1} [16], of 5–10 mg kg^{-1} [15] and well below the Canadian limit for agricultural soils of 12 mg kg^{-1} [60]. Those differences within sites could be associated to lower organic matter (%) and clay percentage registered in the soils at PdL in relation to PF, as reported in previous studies [44]. Studies performed in Entre Rios-Argentina [61], reported an average soil tAs value of 2.9 mg kg^{-1}, ranging from 1.6 mg kg^{-1} in fluvial sediments soils, 3.9 mg kg^{-1} in Vertisols of central-south and 4.1 mg kg^{-1} in wetlands soils of the north.

Arsenic concentration in the soil solution would reflect the bioavailability of As because rice roots absorb As mostly from the soil solution [62]. No differences in the levels of bioAs were registered at different soil depths during both crop stages when measurements were taken (sowing and harvest) and no differences in bioAs was detected within irrigation treatments. The bioAs at sowing in the soils of our study was 15.1 μg L^{-1} (99%) higher in the PdL site compared to the PF site. However, no significant differences were recorded in bioAs levels within regions during

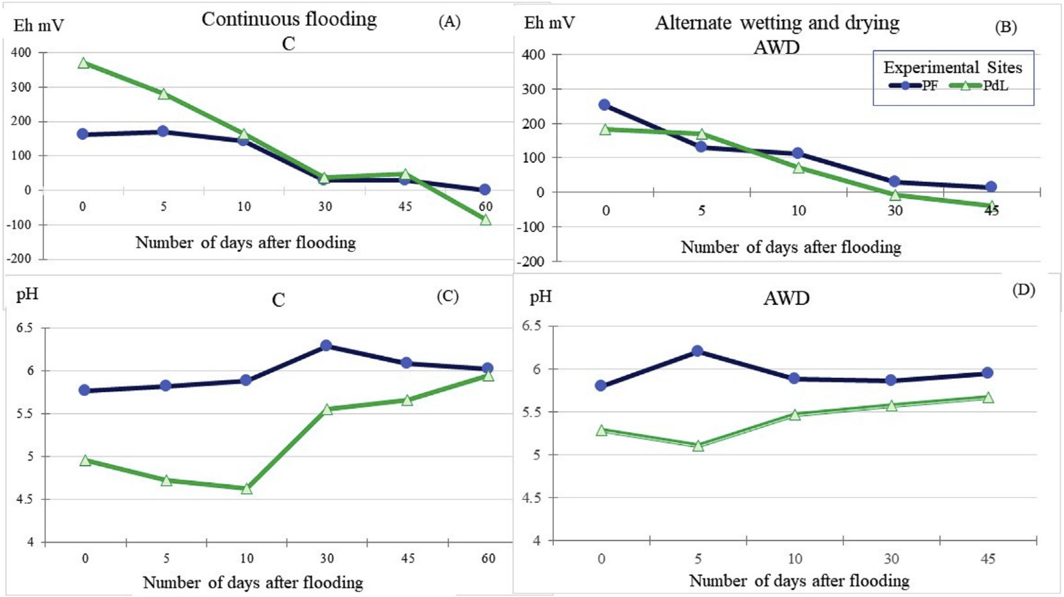


Fig. 3. (A,B) Redox Potential Eh (mV) and (C,D) pH evolution (average) at different days from the start of flooding, for each irrigation treatment, continuous (C) and alternate wetting and drying (AWD) and for each experimental site, Paso de la Laguna (PdL) and Paso Farias (PF).

Table 6
Inorganic Arsenic (iAs mg kg^{-1}) levels accumulated in polished rice grain by sites, irrigation treatments and main varieties cultivated in Uruguay.

Classification criteria	Inorganic Arsenic in Grain iAs (mg kg^{-1})
Site	
PdL (Paso de la Laguna – Treinta y Tres)	0.091 a
PF (Paso Farias – Artigas)	0.043 b
Average	*0.067*
CV%	*4.524*
P < 0.05	***
Irrigation	
1.Continuous (C)	0.069 a
2. Alternate Wetting and Drying (AWD)	0.064 a
Average	*0.067*
CV%	*4.540*
P < 0.05	*
Variety	
Tacuarí *(Japónica)*	0.046 c
Parao *(Japónica)*	0.057 bc
Merín *(Indica)*	0.076 ab
EP144 *(Indica)*	0.077 a
Olimar *(Indica)*	0.079 a
Average	*0.067*
CV%	*6.651*
P < 0.05	***
Irrigation*Site	
PdL	
1.Continuous (C)	0.086 a
4. Alternate Wetting and Drying (AWD)	0.097 a
PF	
1.Continuous (C)	0.053 a
4. Alternate Wetting and Drying (AWD)	0.032 b
Average	*0.067*
CV%	*6.134*
P < 0.05	***
Irrigation * Variety	
P < 0.05	*NS*

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. (.) = non-estimated.

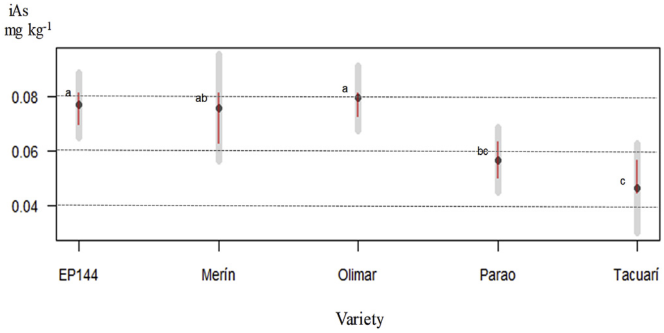


Fig. 4. Inorganic arsenic (iAs) accumulated in polished rice grain (mg kg^{-1}) for the main varieties cultivated in Uruguay. Black dots represent means (least square means), lines indicate confidence interval by Tukey and grey bars are indicating standard errors. Different letters indicate significant differences within treatments for each region with a probability less than 5%.

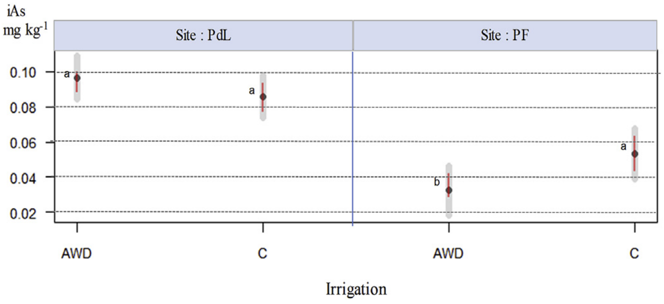


Fig. 5. Inorganic arsenic (mg kg^{-1}) accumulated in polished rice white grain by irrigation management: C: continuous and AWD alternate wetting and drying recorded in different regions. Black dots represent means (least square means), lines indicate confidence interval by Tukey and grey bars are indicating standard errors. Different letters indicate significant differences within treatments for each region with a probability less than 5%.

the final sampling at harvest. Average bioAs levels increased during the cropping cycle from sowing to harvesting (Tables 3 and 4). Arsenic bioavailability has been found to increase under reduced soil conditions, as Fe oxyhydroxides to which As is adsorbed are dissolved and become available to the rice roots [63]. Other authors have reported that arsenic transported through water during irrigation could be another natural source of As into the rice cropping systems [18]. However, the average

Table 7
Rice grain yield (kg ha^{-1}, 14% moisture) registered in two sites of Uruguay, by irrigation treatments and main varieties planted in Uruguay.

Classification criteria	Rice Yield (kg ha^{-1})
Sites	
PdL (Paso de la Laguna – Treinta y Tres)	9500 a
PF (Paso Farias – Artigas)	7635 b
Average	*8567*
CV%	*2.14*
$P < 0.05$	***
Irrigation	
1.Continuous (C)	9230 a
2. Alternate Wetting and Drying (AWD)	7904 b
Average	*8567*
CV%	*2.42*
$P < 0.05$	***
Variety	
Tacuarí	6279 d
Parao	8095 c
Merín	10203 a
EP144	9289 ab
Olimar	8971 b
Average	*8567*
CV%	*2.86*
$P < 0.05$	***
Irrigation*Site	
$P < 0.05$	*NS*
Irrigation * Variety	
$P < 0.05$	*NS*

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.

Arsenic levels registered in the irrigation water in this study, were very low in relation to the limited restriction values for irrigation water [47]. For this reason, the increase in the bioAs during the crop growth period is likely not related to arsenic transported through irrigation water and was associated with the reduced soil conditions. Additionally, tAs in the soil across sites was also very low and below the reported natural values around the world [15,16] and well below the limit for agricultural soils according to the Canadian Environmental Quality Guidelines [60]. While there are other possible sources of As (anthropogenic) such as industrial/urban pollution for paddies downstream of large population centers, contamination of irrigation water, use of fertilizers and pesticides contaminated with arsenic [18], these are generally not relevant in Uruguay rice growing situations. The amount of phosphate fertilizers used in Uruguay and particularly in this study was very low and currently no organic manure is used in Uruguay rice systems, hence this is unlikely to be a source of Arsenic contamination. Therefore, the measured increases in bioAs in the soils over the two experimental sites during the cropping period are likely due to soil/water interactions causing reduced soil conditions and greater bioavailability of As. However, it is important to highlight that bioAs levels were found to be low across both sites and unlikely to be an issue at current levels.

4.2. Arsenic concentration in water, pH and redox potential

The average As levels measured in the irrigation water during this study was 0.00224 mg L^{-1}, which is aligned with values reported in two sites of Ecuador of 0.00142 and 0.00307 mg L^{-1} (Otero et al., 2016) and were below the limited restriction values for irrigation surface water of 0.05 mg L^{-1} (Class 2a) and 0.005 mg L^{-1} (Class3) [47] and well below the limited restriction values for human water consumption of 0.02 mg L^{-1} [49] and 0.01 mg L^{-1} [48]. This information is aligned with the reported average arsenic values of <0.0015 mg L^{-1} in the irrigation surface water collected in lagoons, irrigation channels and rice fields in the East region of Uruguay (from <0.0005 mg L^{-1} to 0.0036 mg L^{-1}) [44,45]. Additionally, a mean arsenic level of 0.00087 mg L^{-1} was reported in a Lagoon [46], which is one of the most important water resources for rice irrigation in the East region in Uruguay. Significant differences where registered between the two sites, with As levels in the water 55% higher at the PdL site (0.00274 mg L^{-1}) compared to the PF site (0.00157 mg L^{-1}). However, As levels in water measured across both sites in this study were very low.

Redox potential (Eh) declined and reached a lower and negative minimum value at the final sampling event, which reflected a more reductive soil condition at PdL in comparison with PF. Meharg and Zhao [18], determined that As liberation into the soil occurs when Eh is below +250 mV at pH = 7. An increase of As availability when Eh decreases it was also reported in other study [64]. Eh values and trend reported at the PdL site were aligned with information reported by Tarlera et al. [24]. According to Masschelyn et al. [12], an increase in solubility of arsenic can occur due to the reduction of iron oxy-hydroxides within the reported range of Eh. When Eh drops below 150 mV at pH = 7, arsenic solubility may increase due to the reduction of Fe^{+3} to Fe^{+2} [65]. Arsenic mobilization in paddy soils can be strongly impacted by soil redox potential. However, this effect can be difficult to quantify using measurements at a single point in time, as fluctuations of soil Eh can be high during the rice growing season [18]. It was found that the increase in As concentration in the soil solution occurs simultaneously with the rise in Fe and Mn concentration [66]. This study affirms that the solubility of As is strongly regulated by Fe reduction in aqueous systems. The slightly more reductive soils conditions registered at the PdL site are likely associated with the higher arsenic water levels found in this site and with the higher inorganic arsenic contents measured in the grain at this site compared to PF.

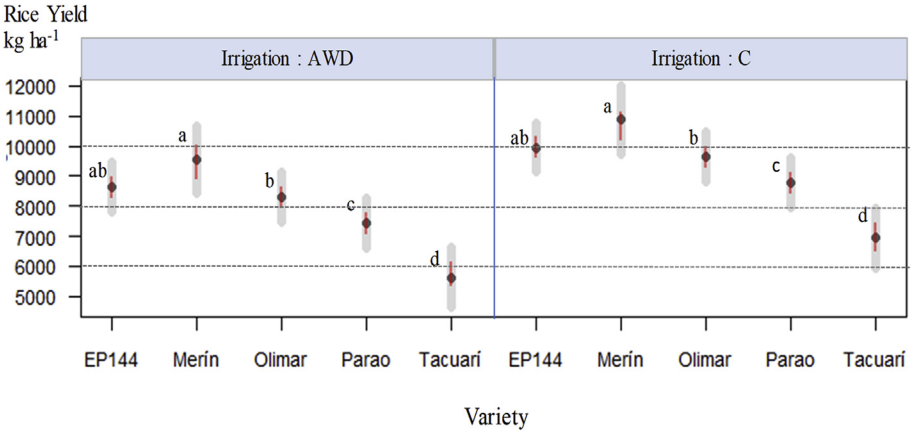


Fig. 6. Rice grain yield (kg ha^{-1}, 14% moisture) for different varieties by irrigation techniques. Black dots represent means (least square means), lines indicate confidence interval by Tukey and grey bars are indicating standard errors. Different letters are significantly different with a probability less than 5%.

According to Honma et al. [67] the recorded trend of redox potential reduction and pH increase found in this study, could potentially correspond to situations where the availability of As can be reduced (Fig. 3). This condition may have further contributed to the very low levels of iAs accumulated in grain found in this study.

4.3. *Arsenic concentration in grain*

Inorganic arsenic values in white polished grain averaged 0.07 mg kg $^{-1}$. across the study sites. Reported values of iAs in this study were below international and regional legislation limits established for human health and food safety: 0.20 mg kg^{-1} for iAs in polished rice grain by the CODEX ALIMENTARIUS [39], and 0.30 mg kg^{-1} for tAs by the MERCOSUR, [40]. Globally, reported values of tAs ranged from 0.05 mg kg^{-1} to 0.38 mg kg^{-1} while reported values of iAs ranged from 0.03 mg kg^{-1} up to 0.25 mg kg^{-1} (Table 8 and Fig. 7). [80]

The measured iAs values in both experimental sites in this study, were generally lower in comparison with reported values by other authors (Fig. 7). Accumulation of iAs in rice grain varies across studies could be explained mainly by the wide range of environments, different varieties, soil types, water sources and differences in cropping systems-management. Amongst these limited studies, results from this study in Uruguay were found to be in the lower range of recorded iAs results in rice grain. Most of the rice producing countries reported mean iAs levels below the international limit established by the CODEX ALIMENTARIUS [39].

In this study, the highest accumulation of iAs in rice grain was found at the PdL site. Similarly, the highest As values were registered in the soils (tAs and bioAs at sowing) and in the water at this site. This information is aligned with Quintero et al. [70], that reported highest As accumulation in grain, in cultivated soils with higher As content such as in wetlands in Northern Argentina.

Although overall iAs accumulated in rice grain were low, it was found that levels can be further reduced by the implementation of alternative irrigation management techniques. The AWD techniques used in this study had a significant reduction in iAs accumulation in grain of 40% at the PF site. This information agrees with information reported by Linquist et al. [19], with a 58% reduction of tAs in polished rice by implementing AWD (from 0.37 to 0.16 mg kg $^{-1}$) in relation to the continuously flooded rice system. Carrijo et al. [22], also determined that grain total tAs concentration decreased by 56 to 68%, in AWD that allowed the soil to dry out from 45 days after sowing until flowering (50% heading) until it reached 25–35% of soil volumetric water content. However, in less severe treatments such as “safe” AWD where the field was reflooded when the water table reached 15 cm below soil surface, the authors didn't find a reduction in total As accumulation in relation to the continuously flooded treatment [22]. Other studies with a higher severity of water stress imposed with AWD have also reported a significantly higher reduction in the accumulation of arsenic in rice grain [78,79].

The lower reduction in iAs measured in this study could be explained by the lower severity of the AWD treatment as it was only implemented until panicle initiation and allowed a water depletion of 50% of the available water. Additionally, at one of the sites (PdL), no differences in inorganic As accumulated in rice grain within irrigation treatments (C and AWD) were found. The soil type and field characteristics of lower slope at the PdL site (Table 1), could favor the anoxic saturated conditions for longer periods in relation to the PF site. Most likely this didn't allow the development of aerobic conditions in the soil for long enough periods to decrease the soil bioavailability of As at the PdL site.

4.4. *Grain yield*

Grain yield was found to be affected significantly by irrigation method. Despite AWD being shown as an alternative irrigation technique that can reduce As accumulation in rice grain under certain conditions, it was found in this study that yield was reduced by 14% in the AWD

Table 8

Inorganic Arsenic (iAs) and total Arsenic (tAs) in rice grain reported in different studies worldwide and international limit established for human health and food safety.

Countries with Reported values	Inorganic Arsenic iAs (mg kg $^{-1}$)	Total Arsenic tAs (mg kg $^{-1}$)	Rice Type	References
Taiwan	0.25	0.38	W	Williams et al., 2005 [69]
Argentina	–	0.34	M	Quintero et al., 2010 [61]
Argentina	0.06	0.33	M	Quintero et al., 2014 [70]
Argentina	0.08	0.30	W	Oteiza et al., 2019 [68]
Argentina	0.10	0.30	*	Farías et al., 2015 [71]
France	–	0.28	W	Meharg et al., 2009 [4]
Australia	0.16	0.28	W	Rahman et al., 2014 [72]
USA	0.11	0.28	W	Meharg et al., 2008 [73]
USA	0.08	0.26	W	Williams et al., 2005 [69]
USA	–	0.26	W	Linquist et al., 2015 [19]
Australia	–	0.26	W	Phuong et al., 1999 [74]
USA	0.10	0.25	W	Meharg et al., 2009 [4]
USA	0.10	0.27	W	Zavala et al., 2008 [75]
China	0.15	0.23	W	Zhu et al., 2008 [36]
Brazil	0.11	0.22	W	Batista et al., 2011 [76]
Italy	0.13	0.22	R	Williams et al., 2005 [69]
Vietnam	–	0.21	W	Phuong et al., 1999 [74]
Spain	–	0.20	W	Meharg et al., 2009 [4]
Japan	–	0.19	W	Meharg et al., 2009 [4]
Spain	0.08	0.17	P	Williams et al., 2005 [69]
Thailand	0.17	0.17	J	Rahman et al., 2014 [72]
Europe	0.08	0.16	*	Williams et al., 2005 [69]
Italy	0.11	0.15	W	Meharg et al., 2009 [4]
China	0.16	0.14	W	Meharg et al., 2009 [4]
Thailand	–	0.14	W	Meharg et al., 2009 [4]
Bangladesh	0.08	0.13	W	Meharg et al., 2009 [4]
Bangladesh	0.08	0.13	W	Williams et al., 2005 [69]
USA	–	<0.12	W	Carrijo et al., 2018 [22]
Thailand	0.08	0.11	J	Williams et al., 2005 [69]
India	0.09	0.10	B	Rahman et al., 2014 [72]
Pakistan	0.08	0.09	B	Rahman et al., 2014 [72]
India	0.03	0.07	W	Meharg et al., 2009 [4]
Canada	0.05	0.07	Wi	Williams et al., 2005 [69]
Egypt	–	0.05	W	Meharg et al., 2009 [4]

(*continued on next page*)

Table 8 (*continued*)

Countries with Reported values	Inorganic Arsenic iAs (mg kg $^{-1}$)	Total Arsenic tAs (mg kg $^{-1}$)	Rice Type	References
India	0.03	0.05	WB	Williams et al., 2005 [69]
Ecuador	0.12	–	W	Otero et al., 2016 [77]
Uruguay	0.07	–	W	**Current study**
References: W:white, M:mixed; *: not specified; B: basmati; R: risotto; J: jasmine; Wi: wild; P: paella				
International Limits	iAs = 0.2 (polished)- 0.35 (husked).		CODEX (FAO and WHO, 2019)	

*** If the tAs concentration is below or equal to the limit established for iAs, no further testing is required, and the sample is determined to be compliant with the legislation. If the tAs concentration is above the limit for iAs, follow-up testing shall be conducted to determine the iAs (FAO and WHO, 2019).

treatments (7904 kg ha^{-1}) in comparison to continuous flooded treatments C (9230 kg ha^{-1}).This information is in agreement with previous studies reported worldwide by other authors [26–30] and also in Uruguay by Carracelas et al. [25], where AWD resulted in a significant yield loss of 1339 kg ha^{-1} in comparison to the traditional continuous flooded treatment. The yield losses associated with the AWD treatment would likely limit the implementation of this technique in commercial farms. In other studies rice yield was not negatively affected when soils were maintained above saturation or rice plants had access to water using “safe” AWD irrigation techniques [21,22,31–35]. However, under saturated soil conditions and even “safe” AWD practice, no reduction in iAs accumulated in rice grain was reported [22]. The implementation of a mitigation management option, such as AWD, that reduces the crop yield is likely to only be adopted in environments in which arsenic concentrations are an issue.

4.5. Rice variety

Another option to reduce arsenic accumulation in rice grain is by selecting cultivars that accumulate low arsenic levels. *Japonica* cultivars included in this study (Tacuarí and Parao) were found to have on average 35% less accumulation of iAs in rice grain, in comparison to *Indica* cultivars (Olimar and El Paso 144) when grown under the same conditions. However, *Indica* varieties in this study reported significantly higher yields in relation to *Japonicas*, 9488 vs 7187 kg ha^{-1} respectively. Despite yield being reduced on average by 2301 kg ha^{-1} (24%) in *Japonicas* in relation to the *Indica* cultivars, some varieties such as INIA Tacuarí do obtain a price premium related to higher quality that compensate for the lower yields.

In summary, the inorganic arsenic accumulated in rice grain in Uruguay, was found to be very low and below international limits on the two experimental sites monitored in this study. Therefore, the implementation of the mitigation management practices developed in this study are unlikely to be needed for mitigating arsenic uptake in rice, unless arsenic concentrations in areas outside the study sites were significantly different.

5. Conclusions

Inorganic Arsenic accumulated in polished rice grain grown in the Paso Farias (PF) and Paso de la Laguna (PdL) sites were found to be below the regional [40]and international limits [39].Total Arsenic levels in Irrigation water and soils were found to be very low at both sites, which resulted in low levels of iAs accumulated in rice grain at these sites across the monitoring period. The relative higher levels of iAs registered at the PdL site in relation to the PF site can be associated with the higher level of tAs and bioAs in the soil at sowing and with the higher As level in the water, measured at the PdL site.

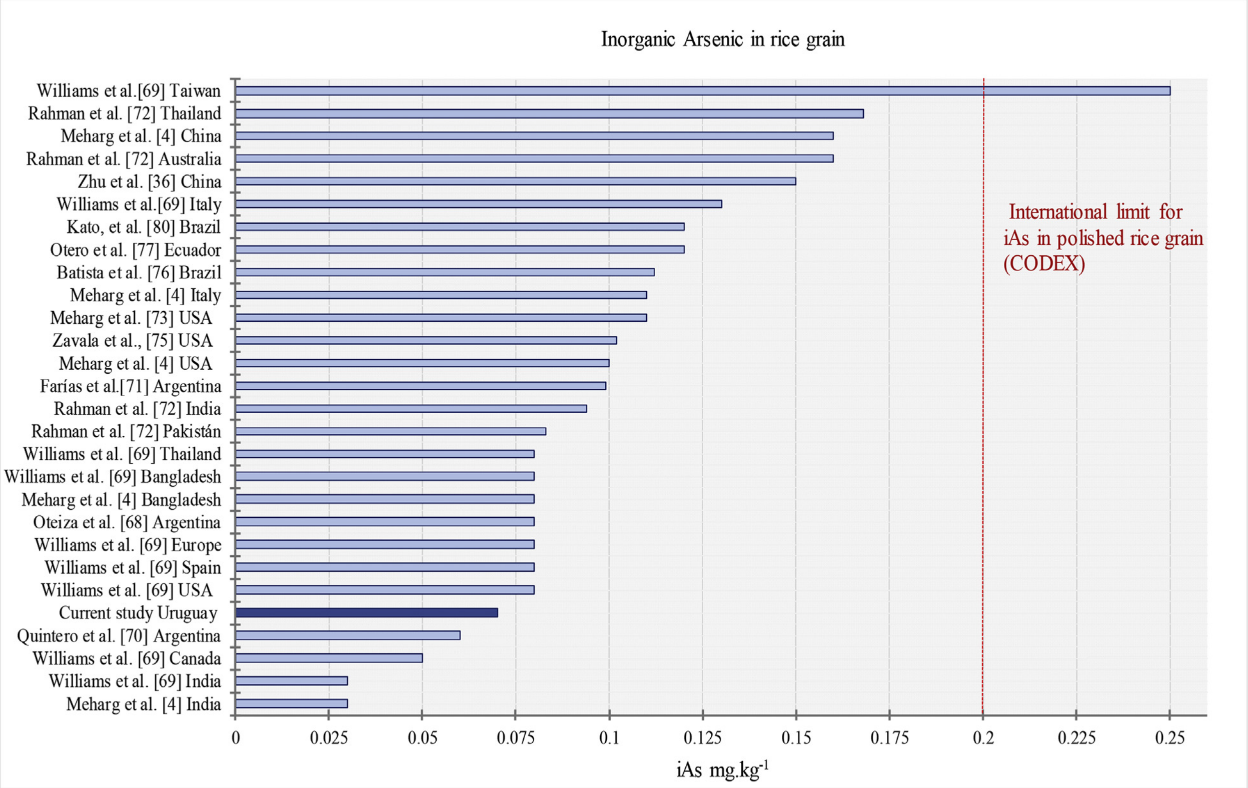


Fig. 7. Inorganic Arsenic accumulated in rice grain reported by country. Red line is indicating the international limit for iAs in polished rice grain established for human health and food safety in the CODEX ALIMENTARIUS [39, 80]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

This study showed that irrigation management and varieties have the potential to affect iAs accumulation in rice grain in Uruguayan growing environments. Even though the levels of iAs accumulated in rice grain were low, this study showed that it was possible to further reduce those levels with irrigation management practices such as AWD on certain soil types and growing conditions. It was also confirmed that *Japonica* varieties accumulate lower amounts of iAs in rice grain in relation to *Indicas* across both experimental sites.

This research was conducted in two specific sites in the rice growing regions in Uruguay, and while these sites are typical for the rice growing regions of Uruguay a more extensive broader study would help provide a comprehensive picture of any likely arsenic issues. Future studies should look to perform regional scale sampling on a wide scale across a large number of rice fields in order to further understand grain iAs levels spatially across the whole rice sector in Uruguay.

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