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EVALUACIÓN DEL CONTENIDO DE ARSÉNICO DEL ARROZ URUGUAYO Y LAS VARIABLES PARA MINIMIZAR SU CONTENIDO EN GRANO

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TABLA DE CONTENIDOS

RESUMEN

Los principales objetivos de este trabajo de investigación fueron a) cuantificar los niveles y rango de distribución de arsénico inorgánico (iAs) y total (tAs) en el grano de arroz uruguayo y b) identificar manejos de riego y fertilización fosfatada que permitieran reducir al mínimo la concentración de arsénico inorgánico en el grano de arroz. Para cumplir el primer objetivo se realizó un muestreo representativo de los cultivares sembrados a nivel nacional y su distribución en las diferentes regiones arroceras (n = 150) para el análisis de contenido de AsI y AsT en grano de arroz blanco pulido, con el objetivo de detectar patrones espaciales y la variabilidad asociada a los materiales genéticos. Para alcanzar el segundo objetivo se llevaron a cabo dos ensayos parcelarios durante las zafras 2018-2019 y 2019-2020 en Paso de la Laguna, INIA Treinta y Tres con la variedad Índica INIA Merín. Se probaron 5 tratamientos de riego, un testigo de inundación continua y 4 tratamientos de riego alternativos que combinaron uno o dos secados de suelo de baja severidad en diferentes estadios fenológicos del cultivo, y 2 tratamientos de fertilización con P (0 y 50 unidades de P_2O_5). Se analizaron las variables contenido de iAs (mg kg^{-1}) en grano y rendimiento (kg ha^{-1}). El muestreo nacional arrojó que los valores de iAs tuvieron una media de 0,06 (0,005-0,195 mg kg^{-1}), sin superar el nivel máximo de 0,2 mg kg^{-1} (CODEX Alimentarius). El contenido promedio de tAs fue 0,178 (0,015-0,629 mg kg^{-1}) encontrándose 85 % de las muestras por debajo de 0,3 mg kg^{-1}. El contenido de iAs en muestras de chacras localizadas sobre rocas ígneas fue 41,8 % menor que sobre rocas sedimentarias (0,039 vs. 0,067 mg kg^{-1}). El contenido de tAs tuvo similar tendencia, siendo 57,6 % menor en rocas ígneas vs. sedimentarias (0,084 vs. 0,198 mg kg^{-1}). Las variedades de la subespecie Japónica presentaron valores promedio de iAs 47 % menores que las Índica (0,035 vs. 0,066 mg kg^{-1}), y 34,6% menor tAs (0,123 vs. 0,188 mg kg^{-1}). En los ensayos parcelarios, los secados de suelo no afectaron el rendimiento en grano. El contenido de iAs fue reducido 22 % aplicando la combinación de dos secados de suelo en primordio y plena floración respecto al tratamiento de inundación continua (0,067 vs. 0,086 mg kg^{-1}).

Palabras clave: arroz, arsénico, subespecie, riego, fertilización fosfatada

EVALUATION OF THE ARSENIC CONTENT OF URUGUAYAN RICE AND THE VARIABLES TO MINIMIZE ITS GRAIN CONTENT

SUMMARY

The main objectives of this research work were a) to quantify the levels and distribution range of inorganic arsenic (iAs) and total (tAs) in the Uruguayan rice grain and b) to identify irrigation and P fertilization management to minimize iAs arsenic levels in rice grain. To meet the first objective, a representative sampling of the cultivars planted at the national level and their distribution in the different rice-producing regions (n = 150) was carried out for the analysis of iAs and tAs arsenic content in polished rice grain, in order to detect spatial patterns and variability associated with genetic materials. To achieve the second objective, two field trials were carried out during the 2018-2019 and 2019-2020 seasons in Paso de la Laguna, INIA Treinta y Tres, with the Indica variety INIA Merín. Five irrigation treatments were tested, a continuous flood control and four alternative irrigation treatments that combined one or two low severity soil drying at different crop stages, and two P fertilization treatments (0 and 50 units of P_2O_5). Analyzed variables were: iAs content (mg kg^{-1}) in grain and yield (kg ha $^{-1}$). The national sampling showed that the iAs values had a mean of 0,06 (0,005-0,195 mg kg^{-1}), not exceeding the maximum level of 0,2 mg kg^{-1} (CODEX Alimentarius). The average content of tAs was 0.178 (0.015-0.629 mg kg^{-1}) being 85 % of the samples below 0.3 mg kg^{-1}. The content of iAs in samples of crops located over igneous rocks was 41,8 % lower than on sedimentary rocks (0.039 vs. 0.067 mg kg^{-1}). The content of tAs had a similar trend, being 57.6 % lower in igneous vs. sedimentary rocks (0.084 vs. 0.198 mg kg^{-1}). The varieties of the Japonica subspecies presented average iAs values 47% lower than the Indica (0.035 vs. 0.066 mg kg^{-1}), and 34.6% lower tAs (0.123 vs. 0.188 mg kg^{-1}). In field trials, soil drying did not affect grain yield. The content of iAs was reduced by 22 % applying the combination of two dryings of soil at panicle initiation and full flowering compared to the continuous flood treatment (0.067 vs. 0.086 mg kg^{-1}).

Keywords: rice, arsenic, subspecies, irrigation, phosphate fertilization

1. INTRODUCCIÓN

1.1. PROBLEMÁTICA DE LA PRESENCIA DE ARSÉNICO EN EL GRANO DE ARROZ

El arroz es uno de los alimentos más importantes para la humanidad y constituye la principal fuente de carbohidratos y energía para más de la mitad de la población mundial (Islam et al., 2016, Seyffert et al., 2018). El cultivo es sembrado en casi todos los continentes, con una producción mundial de 756.7 millones de toneladas en una superficie de 164.2 millones de ha (FAOSTAT, 2021). La producción de este grano tiene como fin exclusivo la alimentación humana. Posee, además, gran relevancia en cuanto a que existe un mercado internacional que mueve 3.230.900 toneladas y el equivalente a 1.2 mil millones de dólares (datos tomados del año 2020).

Asimismo, este grano posee propiedades alimenticias como, por ejemplo, ser altamente hipoalergénico, lo cual lo hace muy adecuado para la elaboración de alimentos de infantes (Meharg et al., 2008, Meharg y Zhao, 2012). También es libre de gluten, por lo cual es apto para el consumo por parte de personas celíacas (Kraehmer at al., 2017).

En nuestro país se sembraron en la zafra 2020-2021 unas 140.257 ha con una distribución de 71,8 %, 18,9 % y 9,3 % en las zonas este, norte y centro, respectivamente, proporción que se mantiene relativamente constante, con un porcentaje del área superior al 92 % de variedades de la subespecie Índica y menos de un 8 % de variedades de la subespecie Japónica. Dentro de las primeras es de destacar el predominio de la variedad INIA Merín con un 35 % del área sembrada seguida por INTA Guri CL e INIA Olimar con un 18 % y un 17 % del área, respectivamente. Dentro de las variedades de la subespecie Japónica, la variedad que cuenta con mayor parte del área es INIA Tacuarí. Es un rubro de gran importancia para nuestro país ya que el 95 % de la producción es exportada, generando ingresos por aproximadamente 500 millones de dólares anuales y unos 30.000 puestos de trabajo directos e indirectos (DIEA MGAP, 2021).

El arsénico es un elemento químico presente en la naturaleza que es dañino para los seres vivos. Ha sido clasificado como cancerígeno clase I, siendo las especies inorgánicas más tóxicas para la salud humana que las orgánicas. (Befani et al., 2011, Martinez et al., 2011, Azam et al., 2016, Menon et al., 2020).

Las especies inorgánicas de este elemento han sido relacionadas con el desarrollo de diversos tipos de cáncer como son el cáncer de pulmón, vejiga y piel.

También se ha relacionado a este elemento con otros tipos de patologías en el ser humano como hipertensión, diabetes y nacimientos prematuros (NRC, 2001, WHO, 2004).

Entre las principales rutas de exposición de los humanos al arsénico se encuentra el consumo de aguas contaminadas con este elemento y algunos componentes de la dieta, particularmente el grano de arroz (Meacher et al., 2002, Li et al., 2011, Fu et al., 2011, Meharg y Zhao, 2012, Zhao et al., 2020, Uphadhyay, 2020)

El grano de arroz puede presentar niveles diez veces superiores de arsénico en grano cuando es comparado con otros cultivos de grano destinados al consumo humano como puede ser la cebada o el trigo, aun cuando sea cultivado en los mismos suelos. Esto se debe a características propias de la planta, que absorbe silicio y fósforo en grandes cantidades y justamente es a través de estas rutas que ingresa el As en la planta de arroz; pero también a causas relacionadas con el sistema de producción, particularmente cuando es producido bajo condiciones de inundación del suelo, que reducen el elemento aumentando su solubilidad en la solución del suelo y, consecuentemente, su disponibilidad para las plantas (Das et al., 2004, Williams et al., 2007, Mondal y Polya, 2008, Su et al., 2010).

Dada la relevancia del comercio mundial de arroz y la preocupación de los consumidores tanto a nivel internacional como local por adquirir alimentos de calidad que garanticen su inocuidad, el estudio y la investigación de la presencia de este elemento en el grano de arroz y el desarrollo de estrategias que permitan minimizar su contenido en grano se han convertido en un tema de gran relevancia (Meharg y Zhao, 2012).

Por otra parte, al ser el arroz uno de los principales productos de exportación de nuestro país resulta imprescindible revisar y actualizar los estándares de calidad y las exigencias de inocuidad vigentes a nivel nacional e internacional.

La legislación internacional del Codex Alimentarious vigente establece un nivel máximo de arsénico inorgánico en grano de arroz blanco pulido de 0,2 mg kg^{-1} (FAO y OMS, 2019). También existen otros niveles máximos fijados por organismos nacionales que son referencia cuando se produce arroz con fines específicos. Por ejemplo, la legislación de los Estados Unidos (FDA, 2020) o la Unión Europea (European Union Comission Regulation, 2021) que establecen un nivel máximo de arsénico inorgánico de 0,1 mg kg^{-1} de arroz blanco pulido cuando el destino del grano es para la producción de alimentos para infantes.

1.2. ORIGEN DEL ARSÉNICO PRESENTE EN LOS AMBIENTES ARROCEROS

El arsénico puede encontrarse en los suelos por causas de origen natural o antropogénico. Este elemento es un componente de los sistemas naturales y es por ello que es posible encontrarlo en los minerales primarios de la corteza terrestre: suelos, sedimentos, agua, aire y aun en los organismos vivos, incluso antes de que ocurriera la intervención humana. En la mayor parte de las rocas, su rango de concentración se encuentra entre 0,5 y 2,5 mg kg^{-1}, con una media de 2 mg kg^{-1} (Meharg y Zhao, 2012), siendo más abundantes en rocas de tipo sedimentario, particularmente aquellos sedimentos arcillosos y fosforitas, y en aquellos minerales que sean ricos en hidróxidos de hierro. Mediante la acción de procesos de meteorización y posteriores procesos de edafización el As puede pasar a formar parte de los suelos, encontrándose en mayor concentración que en los minerales primarios. El valor promedio de As en los suelos del mundo sin contaminación es de 5 mg kg^{-1} (Koljonen et al., 1989) con un rango de entre 0 y 40 mg kg^{-1}. Su concentración es en general menor en suelos arenosos o derivados de rocas ígneas y mayor en suelos orgánicos o aluviales a donde puede ser arrastrado en sedimentos o diluido (Baker y Chesnin, 1975). El enriquecimiento de As de origen geológico se encuentra asociado a algunos materiales como esquistos orgánicos negros, sedimentos aluviales del período holoceno, zonas ricas en minerales, zonas con intensa actividad volcánica o termal, piletas cerradas en regiones de clima árido o semiárido, o acuíferos con condiciones de extrema reducción y baja concentración de sulfatos (WHO, 2004).

Por otra parte, el As de los suelos también puede tener origen en la actividad humana. De acuerdo a Saxe et al. (1964), el uso de As por parte del hombre data de hace más de 5000 mil años. Algunos de los usos conocidos de este elemento son la producción de herramientas, pigmentos y decoraciones, la fabricación de espejos y cristales, curtiembres, la fabricación de pinturas y la conservación de madera, además de otros usos vinculados a la medicina y a la fabricación de pesticidas.

Entre las principales fuentes de origen antropogénico desde las cuales se han contaminado suelos agrícolas se reportan: efluentes de minería, industriales o urbanos ricos en As, el uso de fertilizantes o enmiendas contaminadas, la aplicación de pesticidas arsenicales y el uso de aguas de riego contaminadas (Meharg y Zhao, 2012).

En muchos casos el As puede llegar a los suelos o aguas de los agroecosistemas mediante contaminación puntual, cuando los centros urbanos, industrias o actividades mineras se concentran en las cercanías de los sistemas de producción agrícolas. En estos casos es posible rastrear y comprobar cuál es la fuente de contaminación y explicarse el transporte del As por ríos, arroyos, canales u otras fuentes de agua que se usan para el riego de los cultivos. En otros casos el As puede llegar por contaminación difusa a través de la atmósfera, lo cual es más difícil de rastrear y comprobar.

El arsénico puede estar presente en algunos fertilizantes químicos, lo cual tras sucesivas aplicaciones puede enriquecer los suelos agrícolas, sobre todo cuando son aplicados en dosis elevadas. De acuerdo con Charter et al. (1995), la concentración de As de los fertilizantes fosfatados se encuentra entre 3 y 30 mg kg^{-1}.

En la región de Bengala, en el sudeste asiático, el uso de excremento vacuno alimentado con paja de arroz rica en As ha generado problemas de contaminación de suelos (Pal et al., 2009). Punshon et al. (2017), en los Estados Unidos, pudo comprobar que hasta 2015, los residuos provenientes de la faena de pollos y pavos podían tener concentraciones de As por encima de 40 mg kg^{-1}, debido al uso de antibióticos arsenicales en el alimento de las aves (en ese año fueron prohibidos).

Otros autores también han reportado que el uso de algas como enmienda orgánica de suelos puede hacer un importante aporte de As al suelo, que se encuentra principalmente integrando arsenoazúcares y cuyo arsénico es rápidamente degradado en formas inorgánicas (Castlehouse et al., 2003).

En los inicios del siglo XX, en el centro-oeste de los Estados Unidos, fueron utilizados pesticidas en huertos, campos de golf y cultivos agrícolas para el control de insectos, que contenían arsenato de plomo y arsenato de calcio (Alden, 1983, Welch et al., 2000). Debido a la baja movilidad del arsénico en el suelo, al día de hoy aún se reportan elevados niveles de As en suelo. A fines del siglo XX, todavía se seguían utilizando algunos compuestos arsenicales como herbicidas de forma localizada, sobre todo en la zona sur de los Estados Unidos, en los cultivos de algodón y campos de golf, los cuales contenían hasta un 50 % de arsénico en peso.

Otro uso de compuestos arsenicales muy difundido durante los inicios del 1900 fue el de baños de inmersión para ganado con el objetivo de controlar garrapatas. De acuerdo con Thomas (1998), más de 3400 instalaciones de baños de ganado existían sólo en Florida, las cuales se llenaban con, aproximadamente, 5600 litros de soluciones arsenicales.

La minería es una de las principales actividades humanas que se asocia a problemas de contaminación con As. El arsénico forma parte de minerales, en especial la arsenopirita. Este mineral es rico en plata y cobre. A su vez, el As se encuentra formando parte de otros minerales primarios asociado a metales como oro, estaño, tungsteno y plomo. Cuando se extraen estos metales de los minerales primarios, se genera un gran volumen de rocas de desecho que son acumuladas en piletas o como material de relleno. En algunas zonas del oeste de los Estados Unidos, esto ha generado problemas de elevados niveles de As en suelo, particularmente en terrenos sobre los que se han extendido áreas residenciales (Saxe et al., 1964). Este es un problema importante también en algunos países asiáticos. Zhu et al. (2008) reportan concentraciones de tAs en grano en el rango de 1,23-2,05 mg kg^{-1} en cultivos de arroz creciendo sobre suelos afectados por la actividad minera en Hunan, China. La actividad minera también puede contaminar el agua que es utilizada para regar cultivos de arroz. Por ejemplo, en Bangladesh se han reportado niveles de 1,23-2,05 mg kg^{-1} en el grano de arroz regado con aguas contaminadas con As (Meharg y Rahman, 2003, Williams et al., 2005).

En el sudeste asiático se ha reportado la ocurrencia de elevados niveles de arsénico en suelo como consecuencia del riego de cultivos de arroz con aguas subterráneas de acuíferos que se encuentran ubicados en materiales geológicos con elevados contenidos de As (Burgess et al., 2010, Neumann et al., 2010).

Durante los años 30 se desarrolló el tratamiento de maderas con compuestos arsenicales. Su uso industrial se difundió sobre fines de la década y el uso residencial fue muy importante durante los años 70. El CCA o arsenato de cobre cromado es un compuesto que mediante un tratamiento de presión es aplicado a las maderas para protegerlas de la acción de hongos, termitas y otros organismos que perforan la madera, aumentando su durabilidad. Esto compuestos pueden ser lavados desde maderas tratadas hacia el suelo, generando contaminación. De acuerdo a Stilwell y Gorny (1997), en suelos que se encuentran debajo de estructuras de madera tratadas con CCA se pueden encontrar niveles de arsénico de hasta 350 mg kg^{-1}.

Incluso en aquellas regiones productoras de arroz donde el arsénico se encuentra en los suelos por causas naturales debido a procesos de formación de suelos a partir de materiales geológicos ricos en arsénico, este elemento puede encontrarse en niveles elevados en los granos de arroz y ser un problema para la salud de sus consumidores, como ocurre en algunos países del sudeste asiático.

1.3. ESPECIACIÓN DEL ARSÉNICO EN EL SUELO

En el suelo existen diversas formas en las que se encuentra el arsénico, que pueden ser inorgánicas u orgánicas. Las principales formas de arsénico inorgánicas son bajo su estado de oxidación + 3 o + 5, arsenito (As^{III}) y arsenato (As^{V}). Las principales formas orgánicas metiladas son ácido monometilarsónico (MMA) y ácido dimetilarsínico (DMA. Las formas inorgánicas son más tóxicas para el ser humano que las orgánicas. Lo contrario ocurre en cuanto a la toxicidad para los vegetales, presentando mayor toxicidad las formas orgánicas. Estas cuatro especies de arsénico pueden ser absorbidas por las raíces de las plantas y pueden ser transportadas hasta depositarse en los granos siguiendo diferentes rutas de transporte. En particular, el As^{V} sigue las rutas de transporte del fosfato debido a sus similitudes químicas, mientras que el As^{III}, MMA y DMA son transportados a través de acuaporinas de la membrana celular y comparten las rutas de transporte con su análogo químico: el ácido silícico

(Meharg y Zhao, 2012, Bolan et al., 2013, Meharg y Meharg, 2015, Islam et al., 2016, Seyffert et al., 2016, Suriyagoda et al., 2018).

Las formas inorgánicas As^{III} y As^{V} son sensibles a las condiciones de oxidorreducción. Estas reacciones pueden ser alteradas por las condiciones químicas del suelo (pH y Eh), así como también por la acción enzimática de las comunidades de microorganismos que se encuentran en el suelo. Ciertos microorganismos pueden usar el arsenato como aceptor de electrones produciéndose su reducción a arsenito (Heimann et al., 2007), mientras que otros microorganismos pueden oxidarlo utilizándolo como fuente de energía (Rhine et al., 2006). Por otra parte, el arsenito puede ser metilado para transformarlo en formas orgánicas, bajo condiciones aeróbicas o anaeróbicas, en procesos mediados por microorganismos o totalmente mineralizado (Cullen y Reimer 1989, Gao y Burau, 1997, Huang et al., 2007). Cuando el suelo se encuentra en condiciones aeróbicas u oxidativas, la forma estable predominante es el As^{V}, mientras que en suelos inundados, en condiciones anaeróbicas predomina el arsénico en forma de As^{III} (Masscheleyn et al., 1991, Takahashi et al., 2004, Zhao et al., 2010).

Cuando la forma predominante es el As^{V}, bajo condiciones oxidativas este puede permanecer adsorbido a minerales del suelo como son los oxihidróxidos de hierro y manganeso, arcillas y materia orgánica, manteniéndose relativamente inmóvil en el suelo y minimizando su disponibilidad para las plantas. Por otra parte, bajo condiciones de reducción como las que se producen en la etapa en la que el cultivo permanece inundado, la dilución de estos oxihidróxidos de hierro y manganeso, así como la reducción del arsenato en arsenito que tiene menos afinidad por las cargas de la fase sólida del suelo, hacen que su movilidad en el suelo y su disponibilidad para las plantas en el suelo aumenten.

Otro factor que afecta la movilidad del As en el suelo es el pH de su. En suelos con pH levemente ácido o cercano a la neutralidad, la movilidad del As es limitada en un rango de pH entre 4 y 8. Esta se incrementa cuando el suelo tiene un pH alcalino (Saxe et al., 1964, Masscheleyn et al., 1991)

El cultivo de arroz en nuestro país se desarrolla bajo condiciones de inundación del suelo, como en gran parte del mundo. La inundación del suelo durante gran parte del ciclo del cultivo afecta las condiciones de oxidorreducción y pH de este, haciendo que el potencial redox de la solución del suelo, que inicialmente se encuentra en valores cercanos a 300 mV, alcance valores negativos en torno a – 100 mV y valores de pH cercanos a la neutralidad luego dos o tres semanas de haber sido inundado, lo cual favorecería la reducción del arsénico, aumentando la concentración de especies más reducidas que tienen mayor movilidad en el suelo y son más biodisponibles para las plantas (Masscheleyn et al., 1991, Williams et al., 2007, Xu et al., 2008).

1.4. ALTERNATIVAS DE MITIGACIÓN DE LA ACUMULACIÓN DE ARSÉNICO EN EL GRANO DE ARROZ

1.4.1. Manejo de riego

En primer lugar, en aquellas partes del mundo donde se utiliza agua de riego con elevados contenidos de As, ya sea de origen antropogénico o natural, la primera medida a tomar sería buscar fuentes de agua alternativas (Meharg y Zaho, 2012, Zhao y Wang, 2020).

Por otra parte, tal como fue desarrollado en el capítulo anterior, inducir condiciones aeróbicas al suelo para evitar la reducción del As y aumentar su adsorción y retención en el suelo favorecería una menor acumulación del elemento en las plantas y grano de arroz. De acuerdo con Li et al. (2009), la correlación entre la caída del potencial redox del suelo y el aumento de la disponibilidad de As tiene un $r^2 = 0.87$. Los mismos autores afirman que plantas de arroz que crecieron bajo inundación continua tuvieron 63, 26 y 20 veces mayor contenido de As en la paja, cáscara y grano, respectivamente, comparado con plantas que crecieron en condiciones de suelo aeróbicas.

Varios investigadores a nivel internacional han implementado estrategias de riego denominadas AWD (*alternate wetting and drying*) que consisten en alternar períodos de inundación con períodos en los que se permite el secado del suelo para inducir condiciones aeróbicas. Según Linquist et al. (2015), es posible lograr una reducción de 58 % en el contenido de arsénico en grano mediante la aplicación de este tipo de técnicas de riego. A nivel nacional, Carracelas et al. (2019) alcanzó una reducción de la acumulación de iAs en grano del 40% en un estudio desarrollado durante dos años en el norte del país, en la localidad de Paso Farías, aplicando AWD durante la etapa vegetativa del cultivo.

Existen otras alternativas al riego por inundación que podrían reducir el contenido de As en grano, que van desde el desarrollo de cultivos de arroz en condiciones semiaeróbicas sembrado sobre camellones (Duxbury y Panaullah, 2007) hasta la producción de arroz en condiciones completamente aeróbicas con la aplicación de riego sólo cuando las lluvias no sean suficientes para mantener una productividad aceptable del cultivo (Bouman et al., 2007).

1.4.2. Mejoramiento genético

La identificación de líneas de programas de mejoramiento que permitan una menor acumulación de As en grano sería una alternativa muy importante en aquellas regiones del mundo en las que existen graves problemas de exposición al As a través del consumo de arroz. Norton et al. (2009) detectaron una variabilidad de entre 4 y 4,6 veces en acumulación de tAs en el grano de arroz de poblaciones de mejoramiento en Bangladesh, determinando, además, que las diferencias genéticas eran estables testeando 76 genotipos diferentes en dos sitios experimentales. Por otra parte, al repetir los ensayos en el sur de China, Bengala e India se pudieron detectar diferencias importantes en el ranking que estarían explicadas por la interacción de los genotipos con el ambiente (tipos de suelo, manejo de agua, fuente de contaminación, entre otros).

Existen varios estudios a nivel internacional que se han enfocado en detectar estas diferencias entre genotipos con el objetivo de utilizarlos en programas de mejoramiento, como, por ejemplo, los desarrollados por Pillai et al. (2010), Ahmed et al. (2011) o Kuramata et al. (2011).

De acuerdo con Meharg y Zhao (2012), las diferencias en acumulación de As en grano podrían estar explicadas por diferencias en el largo del ciclo, que podrían mantener durante más tiempo expuesto el cultivo a la presencia de As en el suelo, variaciones genéticas en los transportadores de fósforo y acuaporinas de membranas a través de las cuales ingresa el As a la planta, o también por una mayor liberación de oxígeno por parte de las raíces hacia la rizosfera, manteniendo un mayor potencial redox, favoreciendo la oxidación de arsenito en arsenato y un mayor desarrollo de óxidos de hierro en la periferia de las raíces manteniendo retenido al As y reduciendo su disponibilidad para las plantas.

1.4.3. Fertilización y enmiendas de suelo

De acuerdo con Li et al. (2009), el agregado de Si al suelo podría contribuir en reducir el contenido de arsénico en la paja y el grano de arroz en un 78 y un 16 %, respectivamente. Este efecto se debe a la similitud química entre el ácido silícico con el As^{III} o arsenito, el cual competiría por ingresar a la planta a través de la proteína carrier de membrana Lsi2. Por otra parte, estos autores afirman que el agregado de silicio tiene poco o nulo efecto sobre la acumulación en planta y grano de las formas orgánicas MMA o DMA, ya que estas ingresan a la planta a través de las acuaporinas Lsi1. Además de la reducción en la absorción de As por parte de la planta, Meharg y Zhao (2012) y Meharg y Meharg (2015) afirman que el agregado de silicio sería beneficioso para mitigar otros efectos de estrés biótico en la planta.

El agregado de fosfato al suelo podría actuar compitiendo por los sitios de absorción a través de los cuales ingresa el As en forma de As^{IV} o arsenato en la planta por a ser análogos en su estructura química. La limitante es que en condiciones de inundación en que es el arroz mayormente producido, el arsenato es reducido a arsenito durante la etapa de riego del cultivo, por lo tanto, no sería eficaz el efecto competitivo del agregado de fosfato. Incluso podría actuar incrementando la disponibilidad de arsénico para las plantas, ya que es más fuertemente retenido por los sitios de intercambio del suelo que el arsenato, por lo que podría incrementar la liberación de este último a la solución del suelo (Hossain et al., 2009, Talukder et al., 2011, Wu et al., 2011).

Finalmente, algunos esfuerzos se han realizado para probar el efecto del agregado de hierro al suelo. De acuerdo con Hossain et al. (2009), se podría favorecer el incremento de la formación de una importante placa de hierro en la rizosfera que actuaría como fosa, reteniendo aquel As del suelo que se encontrara en la cercanía de las raíces. Estos autores obtuvieron poco efecto del agregado de sulfato de hierro al suelo, argumentando que la placa de hierro podría actuar concentrando el arsénico en la rizosfera.

2. REGIONAL VARIABILITY OF ARSENIC CONTENT IN URUGUAYAN POLISHED RICE

Á. Roel, F. Campos, M. Verger, R. Huertas , G. Carracelas

2.1. RESUMEN

Caracterizar la variabilidad interna del país respecto a la acumulación de arsénico (As) en el grano de arroz es muy importante, así como lo es para todas aquellas regiones productoras de arroz, de forma de verificar el cumplimiento de los límites de la legislación internacional y regional. En este estudio se diseñó un robusto esquema de muestreo (n = 150 muestras) con el fin de determinar los niveles de arsénico total (tAs) e inorgánico (iAs) del grano de arroz pulido, cubriendo todas las regiones productoras de arroz del país, durante dos zafras arroceras.

El valor promedio y la mediana de concentración de tAs en grano fue de 0,178 mg kg^{-1} y 0,147 mg kg^{-1}, con un valor mínimo y máximo de 0,015 mg kg-^{1} y 0,629 mg kg^{-1}, respectivamente, y un coeficiente de variación de 63,6 %. La concentración media y mediana de iAs fue de 0,062 mg kg^{-1} y 0,055 mg kg^{-1}, respectivamente, variando desde 0,005 mg kg^{-1} hasta un máximo de 0,195 mg kg^{-1}, con un coeficiente de variación de 51,5 %. Se obtuvo una correlación moderada entre iAs y tAs. Los niveles de iAs en todas las muestras estuvieron por debajo de los límites máximos internacionales de 0,2 mg kg^{-1} para no presentar un riesgo para la salud humana según el Codex Alimentarius (FAO y OMS, 2019). Las chacras que fueron cultivadas en suelos originados a partir de material geológico ígneo reportaron menores niveles de arsénico acumulado en el grano de arroz en relación a aquellas chacras que fueron cultivadas sobre suelos originados de materiales geológicos de tipo sedimentario. Los cultivares de la subespecie Japónica presentaron concentraciones significativamente más bajas de tAs e iAs que los de Índica ($p = 0,0121$ y $p < 0,0001$; respectivamente).

El consumo de arroz por parte de hombres y mujeres adultos en Uruguay es seguro según su nivel de consumo anual y con base en los niveles medios de iAs determinados en este estudio.

Palabras clave: arsénico, arroz, cultivares, riesgo sanitario, material geológico

2.2. 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. *Japónica* cultivars presented significantly lower tAs and iAs concentrations than *Índica* 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.

Keywords: arsenic, rice, cultivars, health risk, geological material

2.3. 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 (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 constitute 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 and Zhao., 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 and Banik., 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 and Zhao., 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 and Zhao, 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). 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 (EU, 2015). 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 and Zhao., 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 and Zhao, 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 +/- 0.026 mg kg^{-1}, respectively. Mondal et al. (2020) reported in Peru average total As concentration in rice was 0.168 ± 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, to investigate and identify factors that are associated and to 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.

2.4. METHODS

2.4.1. 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 carry out the representative sampling, the first step consisted in determining 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 the 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 geocoordinates 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 they are presented in Figure 2a and 2b, respectively. A complete geological description from each sampling site was obtained by joining layers attributes using data management tools. A list of all rice varieties, cultivar type (*Indica* or *Japonica*) and the number of samples in both growing seasons are presented in Table 1.

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° 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 Merín	*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

2.4.2. 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 the 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. 1 g 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 (5 µ, 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.

2.4.3. <u>Statistical Analyses</u>

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.

2.5. RESULTS

2.5.1. 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 Figure 1a.

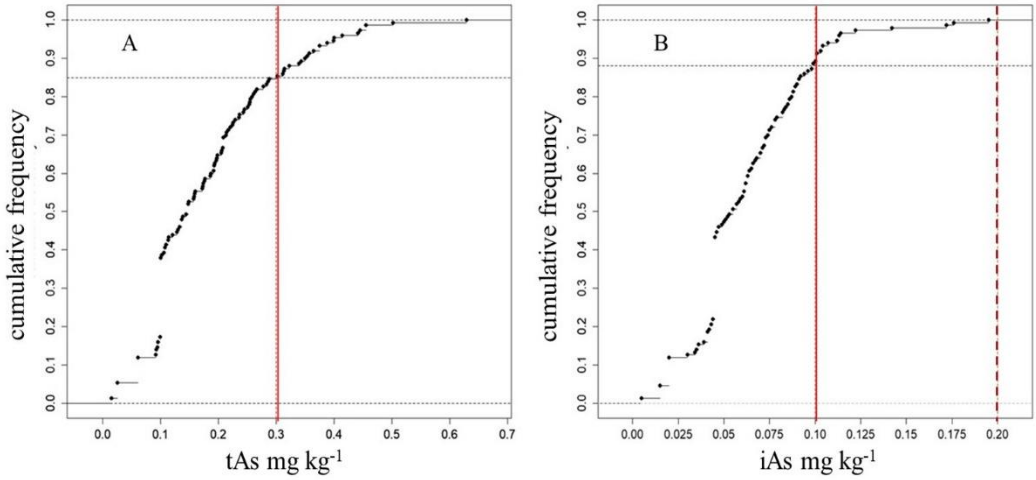

Figure 1. A. Cumulative frequency of total arsenic (tAs mg kg^{-1}) accumulated in polished rice grain in Uruguay. Vertical red line is indicating the regional tAs limit of 0.3 mg kg^{-1} (Mercosur, 2011). B**.** Cumulative frequency of inorganic arsenic (iAs mg kg^{-1}) accumulated in polished rice grain in Uruguay. Vertical lines are indicating the arsenic reference levels for infant food products (red) of 0.10 mg kg^{-1} (EU, 2015; FDA, 2020) and 0.20 mg kg^{-1} (red dashed) for Codex Alimentarious (FAO and WHO, 2019).

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 Figure 2a.

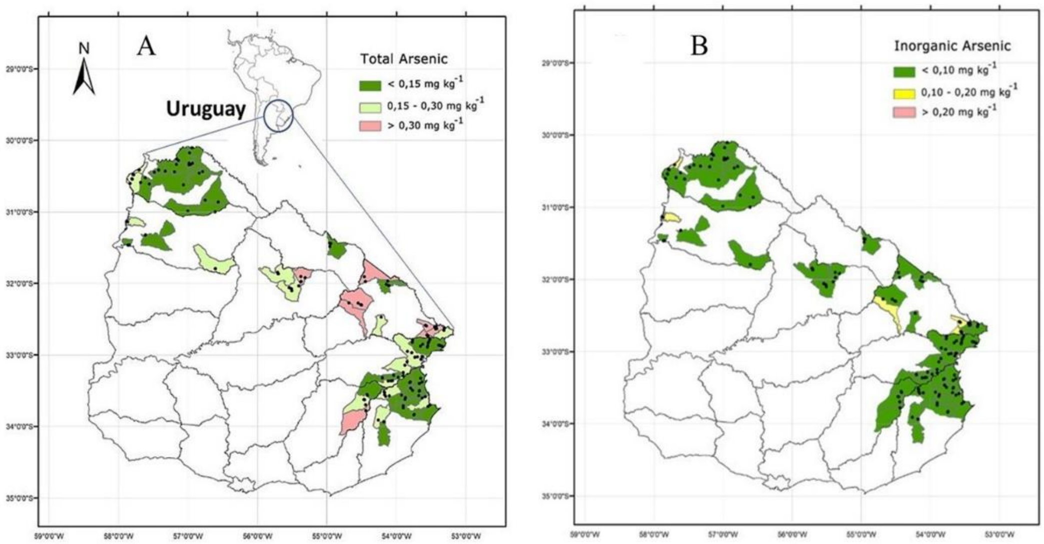

Figure 2. A. Total Arsenic (mg kg^{-1}) levels recorded by sampling site (black dots). Different colors are indicating the range of all samples included in each rice-producing areas of Uruguay (NA). (green: < 0.15, yellow: 0.15 - 0.30 and red: > 0.30 mg kg^{-1}). **B.** Inorganic Arsenic (iAs mg kg^{-1}) 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}).

2.5.2. 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 Figure 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 Figure 2b.

2.5.3. 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. (Figure 3)**.**

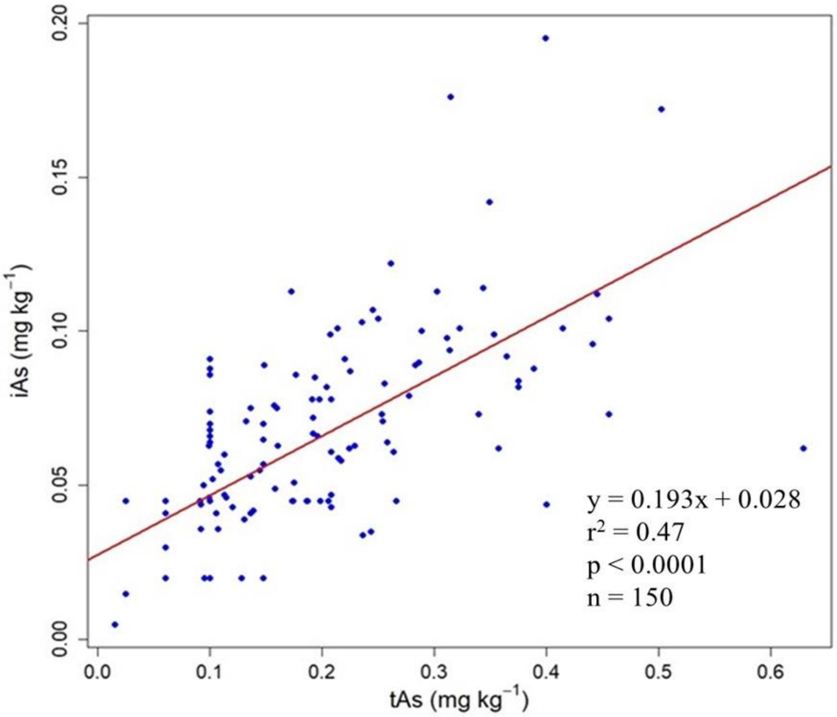

Figure 3. Correlation between tAs and iAs species (mg kg^{-1}) in 150 samples of polished rice grain.

2.5.4. 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.

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 parentheses 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 Merín (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

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.

2.5.5. 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.

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.

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.

2.6. 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 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 the European Union (EU, 2015).

2.6.1. 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} (Figure 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 (Figure 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 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 (Figure 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} (EU, 2015; FDA, 2020).

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

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 and Zhao (2012) ($r^2 = 0.768$).

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 than what was found in other rice regions of the world. Proportionality of iAs to tAs varies regionally (Zhao et al., 2010; Meharg and Zhao., 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 and Banik, 2019)

2.6.2. 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; Fachi 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 adn 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, and 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 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 on 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).

2.6.3. 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 and Zhao., 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 and Zhao (2012). INIA Merín 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.

2.6.4. 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 research developed in Hainan Island, 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}).

These results 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 in 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 affirm 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 also be taken into consideration in these results and described associations due to the imbalanced 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 (Figure 2a). Soil types and field characteristics of higher slope at the north could favor a reduction of the anoxic saturated conditions periods and potentially reduce As soil availability. In contrast, nonspecific regional trend (Figure 2b) of iAs can be observed along regions.

2.7. CONCLUSIONS

Total As levels were in average 0.178 mg kg^{-1} with a range of 0.015 to 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 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.

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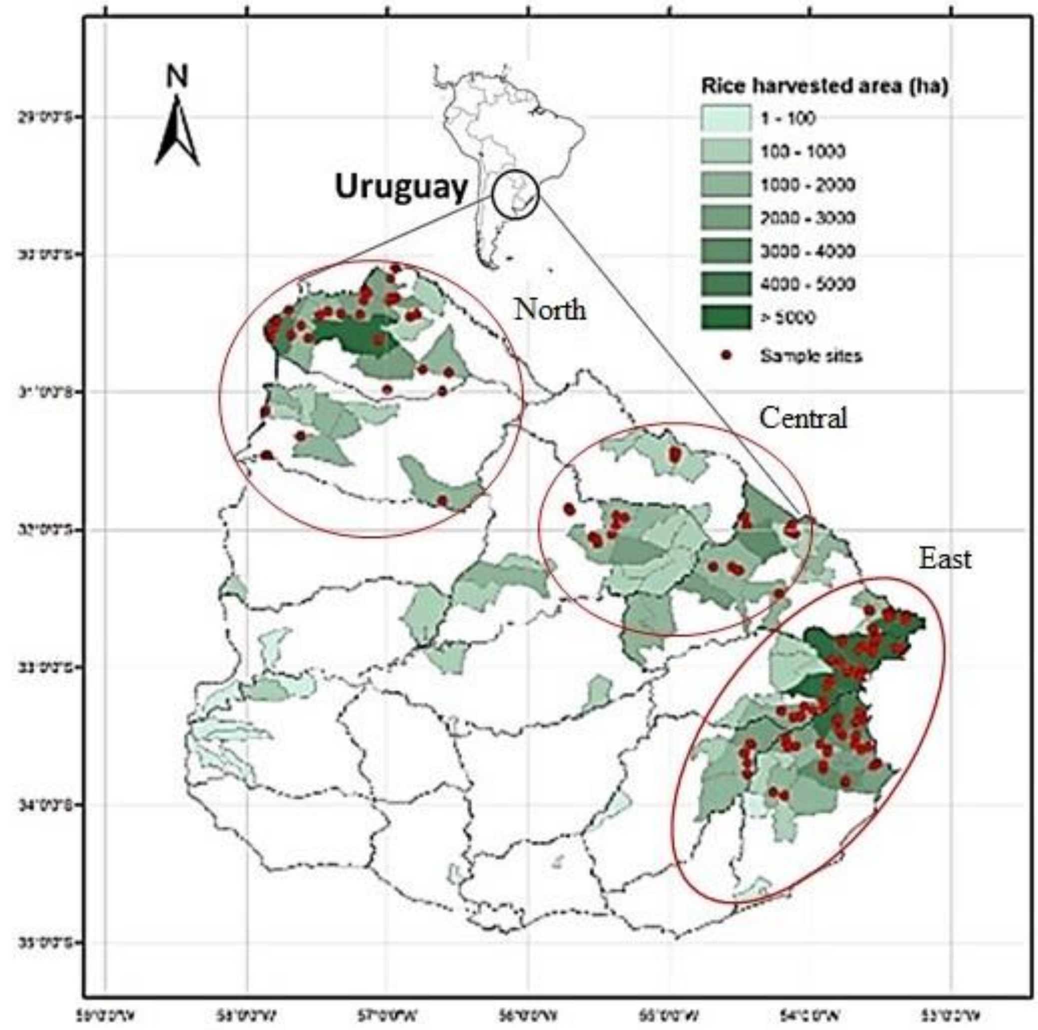
2.10 SUPPLEMENTARY INFORMATION

2.10.1. SUPPLEMENTARY FIGURE 1



Supplementary Figure 1. Sampling sites (red dots) across rice-producing areas (green) in Uruguay according to the Census MGAP information (DIEA MGAP, 2011).

2.10.2. DESCRIPTION OF HEALTH RISK ANALYSIS

Following the same procedure and scenarios used by Menon et al. (2020), a health risk analysis for consumers with an average daily consumption (ADC) of rice of 0.032 kg d^{-1} values reported for Uruguay (FAO, 2018), an average concentration of iAs of 0.062 mg kg^{-1} and also three different average body weights of 83.6, 70.2 and 9 kg for male, female and infant, respectively, was performed. The estimated daily intake (EDI) for males, females and infants were 2.34 x 10^{-5}, 2.78.8 x 10^{-5} and 2.17 x 10^{-4} mg $kg^{-1}d^{-1}$, respectively. In order to compute lifetime cancer risk (LCR), a slope factor (SF) of 1.5 mg kg^{-1} d^{-1} established by the United States Environmental Protection Agency (US EPA, 1977), which assumes daily exposure over an entire lifetime, was used (Supplementary Table 1).

Considering that the acceptable upper limit for LCR set by the US EPA is 1.0 x 10^{-4} mg kg^{-1} d^{-1} and following scenario 1, that assumes a fixed daily consumption, independent to the body weight, of 0.032 kg d^{-1}, the LCR were lower than the upper limit set by the US EPA with values of 3.50 x 10^{-5}, 4.17 x 10^{-5} and 3.26 x 10^{-4} mg kg^{-1} d^{-1} for males, females and one year old infants, respectively.

On the second scenario, the maximum ADC (kg d^{-1}) was calculated for each target population in order to reach the LCR limit of 1 x 10^{-4} mg kg^{-1} d^{-1} showing that male adults, female adults and 1-year old infants daily safe consumption could reach 0.089, 0.069 and 0.010 kg d^{-1}, respectively. These consumption levels correspond to annual equivalents of 32.5 kg, 25.2 kg and 3.65 kg for the same target population groups.

Target hazard quotient (THQ) and the margin of exposure (MoE) also reported in Table 3 are two other aspects to consider in the risk analysis of food consumption (Scenario 3). THQ is calculated as the relation of the EDI to a reference oral dose (RfD) for iAs set up by the US EPA in 0.0003 mg kg^{-1} d^{-1}. MoE is calculated as the relation of a benchmark dose lower confidence limit (BMDL) and EDI. The BMDL is set at 0.0003 mg kg^{-1} d^{-1} for a 0.1 % increased incidence of various cancers. In summary, the THQ is the inverse of the MoE and, hence, THQ values ideally be < 1, whereas the MoE > 1 to avoid iAs health risks (IARC, 2004).

Supplementary Table 1. Lifetime cancer risk (LCR), target hazard quotient (THQ) and margin of exposure (MoE) under different scenarios: scenario 1 is based on current per capital consumption rates of 0.032 kg d^{-1} like values reported in Uruguay; scenario

2 is maximum ADC to avoid LCR and scenario 3 is ADC based on THQ and MoE. Key: AC = Average concentration of iAs in Uruguayan polished rice (mg kg^{-1}); ADC = Average daily consumption rate of rice (kg); BW = Average body weight of the local population; and EDI = Estimated daily intake.

Target population	AC iAs mg kg^{-1}	ADC kg	BW kg	EDI mg kg^{-1} day^{-1}	LCR	THQ	MoE
Scenario 1							
Adult Male	0.062	0.032	83.6	2.34E-05	3.50E-05	0.08	12.84
Adult Female	0.062	0.032	70.2	2.78E-05	4.17E-05	0.09	10.78
1 Year old infant	0.062	0.032	9	2.17E-04	3.26E-04	0.72	1.38
Scenario 2							
Adult Male	0.062	0.089	83.6	6.60E-05	1.00E-04	0.22	4.5
Adult Female	0.062	0.069	70.2	6.60E-05	1.00E-04	0.22	4.5
1 Year old infant	0.062	0.01	9	6.60E-05	1.00E-04	0.22	4.5
Scenario 3							
Adult Male	0.062	0.405	83.6	3.00E-04	4.50E-04	1	1
Adult Female	0.062	0.34	70.2	3.00E-04	4.50E-04	1	1
1 Year old infant	0.062	0.044	9	3.00E-04	4.50E-04	1	1

3. IRRIGATION AND PHOSPHOROUS FERTILIZATION MANAGEMENT TO MINIMIZE RICE GRAIN ARSENIC CONTENT

F. Campos, Á. Roel, G. Carracelas, M. Verger, R. Huertas and C. Perdomo

3.1. RESUMEN

Esta investigación tuvo como objetivo minimizar los niveles de arsénico inorgánico en el grano de arroz pulido mediante el uso de diferentes prácticas de riego y fertilización con fósforo, manteniendo al mismo tiempo el rendimiento de los cultivos y la productividad del agua. Se realizaron dos experimentos durante las temporadas 2018-2019 y 2019-2020 utilizando un diseño de parcelas divididas con tres bloques, cinco tratamientos de riego (parcelas principales) y dos niveles de fósforo (subparcelas). Los tratamientos de riego consistieron en un control tradicional de inundación continua (CF) y cuatro técnicas de riego alternativas con uno o dos eventos de secado durante la etapa de riego del cultivo. Los niveles de fertilización con fósforo investigados fueron un control sin fertilizar (0 kg P_2O_5 ha^{-1}) y el nivel de fertilización recomendado de 50 kg P_2O_5 ha^{-1}. En cada tratamiento se midieron el pH del suelo y los potenciales redox. Los eventos de secado estratégicos de baja severidad fueron efectivos para alcanzar condiciones aeróbicas en el suelo, lo que resultó en valores de Eh superiores a 50 mV. El tratamiento alternativo de riego con dos eventos de secado, implementado durante la etapa de primordio floral y plena floración, fue el más efectivo para reducir el arsénico inorgánico en el grano sin afectar el rendimiento del grano ni la cantidad de agua de riego aplicada. Esta técnica de riego podría ser considerada como una alternativa de manejo a la tradicional inundación continua para minimizar la acumulación de arsénico inorgánico en el grano con el fin de atender estándares especiales de calidad o requerimientos específicos del mercado. El arsénico inorgánico acumulado en grano estuvo por debajo de los niveles máximos internacionales en todas las muestras analizadas, con un valor promedio de 0,084 mg kg^{-1}.

Palabras clave: arroz, arsénico inorgánico, potencial redox, riego

3.2. ABSTRACT

This research sought to minimize inorganic arsenic levels in polished rice grain by using different irrigation and phosphorous fertilization practices while also maintaining crop yield and water productivity. Two experiments were conducted during seasons 2018-2019 and 2019-2020 using a split-plot design with three blocks, five irrigation treatments (main plots) and two phosphorous levels (sub-plots). Irrigation treatments consisted of a traditional continuous flood (CF) control and four alternatives irrigation techniques with one or two drying events during the irrigation cycle. The phosphorous fertilization levels investigated were an unfertilized control (0 kg P_2O_5 ha^{-1}) and the recommended fertilization level of 50 kg P_2O_5 ha^{-1}. Soil pH and redox potentials were measured in each treatment. Strategically-timed, low severity drying events were effective at achieving aerobic soil conditions, resulting in Eh values over 50 mV. The alternative irrigation treatment with two drying events, implemented at panicle initiation and full flowering, was the most effective in reducing inorganic arsenic in grain without affecting grain yield or the amount of irrigation water applied. This irrigation technique could be considered as an alternative management to the traditional continuous flooded to reach minimal inorganic arsenic accumulation in grain in order to attend special quality standards or specific market requirements. Accumulated inorganic arsenic in grain was below international maximum levels in all analyzed samples, with an average value of 0.084 mg kg^{-1}.

Keywords: rice, inorganic arsenic, redox potential, irrigation

3.3. INTRODUCTION

Rice is the most important source of carbohydrates for almost half of the world population. Most of world rice production (75 %) over 93 million ha are under continuous flooding irrigation (Rao et al., 2017). In Uruguay, rice is the main irrigated crop, reaching almost 80 % of total irrigation area in the country, with 140 to 195 thousand ha annually planted (DIEA MGAP, 2020). Rice seeding starts mainly in October, in dry soil conditions, and most crop management operations are done before irrigation is initiated. The most sown varieties are *Indica* type, representing 70 % of total rice production area. Rice is irrigated using a shallow, continuous flood within a contour levee gate (i.e., cascade) system. Irrigation normally begins about 15-25 days after crop emergence when plants have 3-4 leaves and begin tillering (Counce et al., 2000). A 5-10 cm water layer is maintained until 10-20 days before harvest (Carracelas et al., 2019a). Crop yields average 8.6-ton ha^{-1}. National paddy rice production is over 1.2 Mt, and more than 95 % is annually exported worldwide (DIEA MGAP, 2020). Total water consumption under continuous flooding irrigation ranges from 11.000 to 15.000 m^{3} ha^{-1}, being 50 % of total water consumption apported by rainfall irrigation period can last, in average, for 90 days (80 - 100 days) (Böcking et al., 2008; Ricetto et al., 2017; Carracelas et al., 2019a).

Continuous flooding presents some advantages to rice crop system such us better weed control, higher nutrient availability, reduced disease incidence and protection against low temperatures during microspore formation (Humphreys et al., 2006), which are important to ensure high yields. Considering that only 6000-7000 m^{3} ha^{-1} are required by rice evapotranspiration during crop cycle (Blanco et al., 1984; Carracelas et al., 2019a), the interruption of continuous flooding irrigation in short periods at strategic crop stages could lead to a reduction in irrigation water inputs or even to a higher rainfall capture, improving irrigation water-use efficiency (Massey et al., 2014; Avila et al., 2015).

Arsenic is a harmful element for humans and is associated with diverse health problems as cancer, hypertension, diabetes and premature birth (NRC, 2001; WHO, 2004). Drinking water and rice consumption are two of the major dietary sources of arsenic for humans (Meacher et al., 2002; Li et al., 2011; Fu et al., 2011; Meharg and Zhao, 2012; Zhao et al., 2020).

Arsenic in rice grain can be found in inorganic (iAs) and organic (oAs) forms, being the first group more toxic for human health. Main iAs species in rice grain are arsenite (As^{III}), and arsenate (As^{V}), while most relevant oAs compounds are monomethylarsonate (MMA) and dimethylarssinate (DMA). Inorganic As in rice in Uruguay have being reported with levels below international regulation. However, there is a permanent interest from the rice industry to develop techniques to satisfy special quality standards or specific market requirements like the baby food sector. In South America arsenic speciation in rice grain can vary greatly depending on the rice producing region (Roel et al., 2021).

Rice is recognized for having a special ability to accumulate As in the grains due to its inherently ability to take up and translocate As into grain in relation to other crops (Islam et al., 2016). Additionally, anaerobic conditions under traditional flood management result in higher As bioavailability in rice fields (Williams et al., 2007; Su et al., 2010; Meharg et al., 2012). Zhao and Wang (2020) concluded that the concentration of As and cadmium (Cd) in rice grain can vary by three orders of magnitude, depending on bioavailability of these two elements in soil, rice genotype and crop growing conditions. As and Cd bioavailability are both affected by redox potential (Eh, mV) and pH. Lower and even negative values of redox potential that occur under flooding and anaerobic conditions can determine an increase in As bioavailability while Cd bioavailability will decrease. The suspension of flooding irrigation during short periods can induce soil aerobic conditions by increasing redox potential with the objective of reducing As availability for rice plants. Carracelas et al. (2019b) determined that negative Eh (mV) values could be reached after 50 days of soil continuous flooding for two experimental sites in Uruguay. Arsenic absorption by plants depends on the As speciation: the chemical form As^{V} absorption occurs mainly through phosphate transporters due to its similar chemical characteristics; while As^{III} absorption path is through aquaporins responsible of silicic acid uptake. Phosphates plays an important role in As dynamics in soils competing with As for adsorption sites or Fe-plaque via ligand exchange mechanisms, increasing its bioavailability for plants

(Peryea and Kammereck., 1997; Bolan et al., 2013; Wu et al., 2021). On the other hand, when As reaches a critical concentration in soils, As absorption as As^{V} might be reduced by competing for phosphates transporters. Abedin et al. (2002) found that increasing phosphate concentrations in the range from 0.01 to 0.5 mM in the solution of hydroponically grown rice with 0.05 mM of arsenate could reduce As uptake. The decrease in arsenate absorption was higher at higher phosphate concentration. Traditional fertilization of phosphorus in Uruguay consists in the application of 50 units of P at planting. There is a lack of information regarding if this management can affect As grain levels.

The irrigation management technique known as alternate wetting and drying (AWD) applies single or multiple field drying periods, even below saturation, at different crop cycle stages, inducing aerobic conditions to the soil. The increase in oxygen concentration in the rizosphere causes an increase in redox potential, reducing arsenic mobilization (Meharg and Zhao, 2012; Seyfferth et al., 2018). Many benefits related to food safety production and reduction in environmental impacts have been attributed to AWD irrigation techniques, as reduction in As accumulation in rice grain, reduced irrigation water inputs and lower greenhouse gas emissions. However, high variability in results in rice grain yield impact by using AWD has been reported, mainly related with the combination of timing, duration, and severity of soil dryings events when applying this technique (Linquist et al., 2015; Tarlera et al., 2016; Mitra et al., 2017; Yang et al., 2017, Carrijo et al., 2017, Martínez-Eixarch et al., 2021).

Taking into consideration reported variability on rice productivity caused by AWD and the issue of the potential difficulties to implement at large scale rice systems, as in Uruguay, we decided to explore strategic low severity soil drainage at different stages of the crop.

Based on existing information, alternative irrigation techniques (AIT) to continuous flooded treatment have been designed to explore the application and combination of short and low severity soil drying periods at specific stages along the whole crop cycle aiming to avoid grain yield penalty (Carrijo et al., 2019).

The primary objective of this paper was to study the relationship between irrigation management and phosphorous fertilization on iAs accumulation in polished rice grain of a long cycle *Indica* variety (INIA Merín). The main hypothesis tested is that drying the field at certain periods and reducing the application of phosphorous fertilizer would reduce inorganic arsenic (iAs) levels in polished rice grain without affecting grain yield compared to conventional practices.

Specific aims of this research were: 1. to determine if alternative irrigation techniques would be effective at modifying chemical properties of soils to reduce iAs bioavailability and accumulation in rice grain, and 2. to investigate if not applying the traditional phosphorous fertilization management of 50 U of P at planting could affect iAs accumulation in polished rice grain.

3.4. MATERIALS AND METHODS

3.4.1. Site Description

Experiments were conducted in Paso de la Laguna (PdL) at the National Institute for Agricultural Research (INIA) experiment station located in Treinta y Tres, the eastern rice producing region of Uruguay (33°16'11.39"S, 54° 9'58.98"O). (Figure 1).

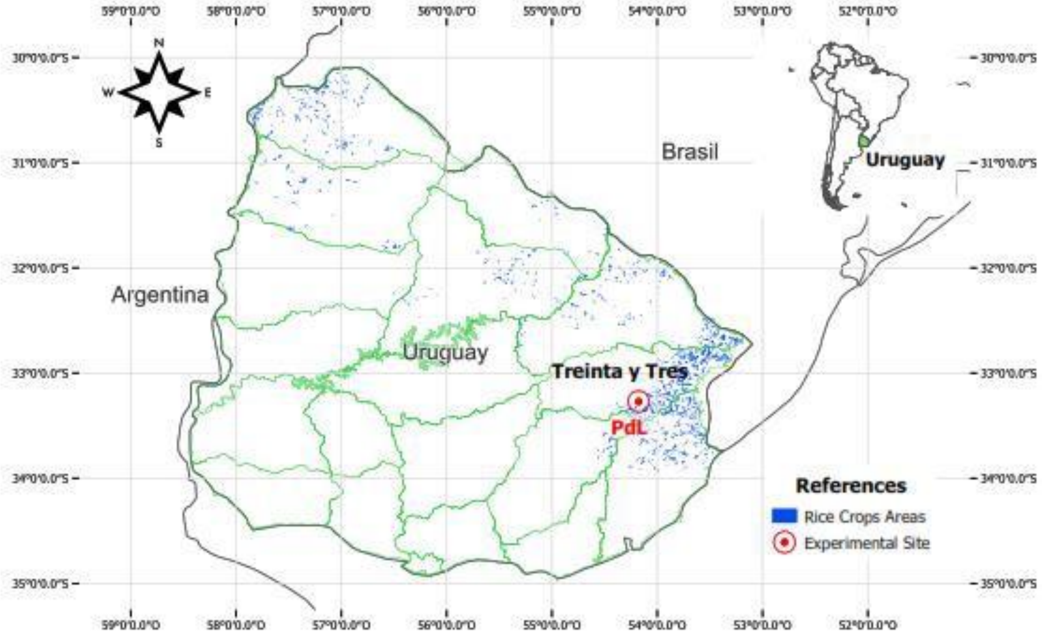


Figure 1. Rice cultivated area in Uruguay and location of the rice field experimental site of Paso de la Laguna (PdL) of the National Institute for Agricultural Research (INIA) on East Region of Uruguay.

Experiments were carried out during 2018 - 2019 and 2019 - 2020 growing seasons on a soil typical of the main rice producing region of Uruguay. The soil, Natraquoll (USDA, 1999), is composed of 13.2 % sand, 61.0 % silt and 26 % clay, CEC of 13.5 mg 100 gr $^{-1}$ and pH of 5.8 and 5.5 in seasons 1 and 2, respectively. The presence of a subsurface soil horizon with high content of clay limits rooting depth to 20 to 30 cm. Organic matter content was 2.24 % for both seasons. Phosphorous levels, determined by citric acid method, were 5 ppm. Potassium content was 0.26 and 0.27 meq 100 g^{-1} for the same seasons, respectively. Irrigation water was obtained from Olimar River, a tributary river of Merín Lagoon.

3.4.2. Field Management

The experiments were planted with a long cycle *Indica* cultivar INIA Merín using a Semeato 249 (https://www.semeato.com.br/) direct drilling seeder with 17 cm of row spacing. Sowing dates were 19th of October in 2018-2019 season and 11th of October for the 2019-2020, which are considered as optimal sowing dates for Uruguayan weather conditions (Tseng et al., 2021). Plant sowing density was adjusted according to the germination percentage and the weight of seeds in order to get 500 viable seeds m^{-2}. Triple superphosphate (46 % P_2O_5) was the phosphorous source applied after sowing in each subplot according to the treatments and experimental design. In the same way, KCl (60 % K_2O) was the potassium fertilizer applied immediately after sowing. Potassium and nitrogen fertilization were defined according to critical levels defined by previous research developed at INIA (Castillo J., 2015) and resumed in Fertiliz-Arr software (INIA's technical recommendation software; www.inia.org.uy). Nitrogen was applied as urea (46 % N) twice during the crop cycle: at tillering and panicle initiation stages. Main management practices are resumed in Table 1. Land preparation, weed control and first nitrogen application were all done on dry soil before flooding. The application of nitrogen fertilizer at panicle initiation was done for all treatments on flooded soils, ensuring the same conditions for all irrigation treatments. In plots being dried during this stage, fertilization was done after reflooding to avoid N losses. Irrigation was terminated two weeks before harvest in all plots.

Table 1. General management practices by season and registered precipitations (pp, mm) during soil drying periods of the alternative irrigation techniques (AIT).

	Season			
	2018-2019		2019-2020	
Sowing	Oct 19th		Oct 11th	
Phosphorous fertilization	Oct 19th		Nov 5th	
Potassium fertilization (rate)	-		Oct 11st	(113 kg ha^{-1} K$_2$O)
Emergence	Nov 6th		Nov 5th	
1st Nitrogen application (rate)	Nov 22nd	(32 kg ha^{-1} N$_2$)	Nov 25th	(28 kg ha^{-1} N$_2$)
Initial flood	Nov 27th		Nov 25th	
Vegetative drying (pp)	Dec 13rd - 26th	(63.6 mm)	Dec 10th - 18th	(58.8 mm)
Panicle initiation Drying (pp)	Jan 2nd - 8th	(89.6 mm)	Dec 26th - 31st	(5.6 mm)
2nd Nitrogen application (rate)	Jan 7th	(43 kg ha^{-1} N$_2$)	Dec 31st	(35 kg ha^{-1} N$_2$)
50 % Flowering	Feb 8th		Feb 9th	
Full Flowering Drying (pp)	Feb 13rd - 19th	(44.6 mm)	Feb 14th-20th	(6.1 mm)
Irrigation ending	Mar 6th		Mar 9th	
Harvest	Mar 18th		Mar 31st	

3.4.3. Experiment Design and Treatments Description

The experimental design consisted of a split plot design with three blocks. In each block, five irrigation treatments were randomized as the main plot factor (46 m^{-2}). Plots were separated by levees and drainage ditches. The main plots were divided into two subplots where phosphorous fertilization treatments were assigned randomly (no P application or 50 units of P). Irrigation was started 20-30 days after crop emergence simultaneously in all the treatment (Figure 2). Continuous flooding (CF, control treatment) and four alternative irrigation techniques (AIT) were tested.

In CF treatment, a 10 cm water layer was kept above the soil surface during the entire irrigation period. In AIT treatments, a 10 cm water layer was kept above soil surface, but plots were drained once or twice at specific crop stages during the season. Vegetative drying treatment (VD) was done 15 days after irrigation started. Panicle initiation drying treatment (PID) plots were dried when the crop was at panicle initiation stage. Vegetative and panicle initiation drying (VPID) treatment plots were dried twice, 15 days after irrigation started and at panicle initiation. Finally, panicle initiation and flowering drying treatments (PIFD) plots were dried twice during crop season, at panicle initiation and full flowering (100 % flowering) stages (***Figure 2***).

Plots were reflooded when a water depletion of 50 % of soil available water was reached in the first 20 cm of soil. Soil hydric parameters such as saturation, field capacity, permanent wilting point and available water content were determined by Richard's method (Richards, L.A., 1948). The targeted volumetric water content (VWC) value for reflooding was 0.376 $m^3 m^{-3}$. According to the targeted water threshold, 18 mm of depletion in the first 20 cm of rooting depth was allowed.

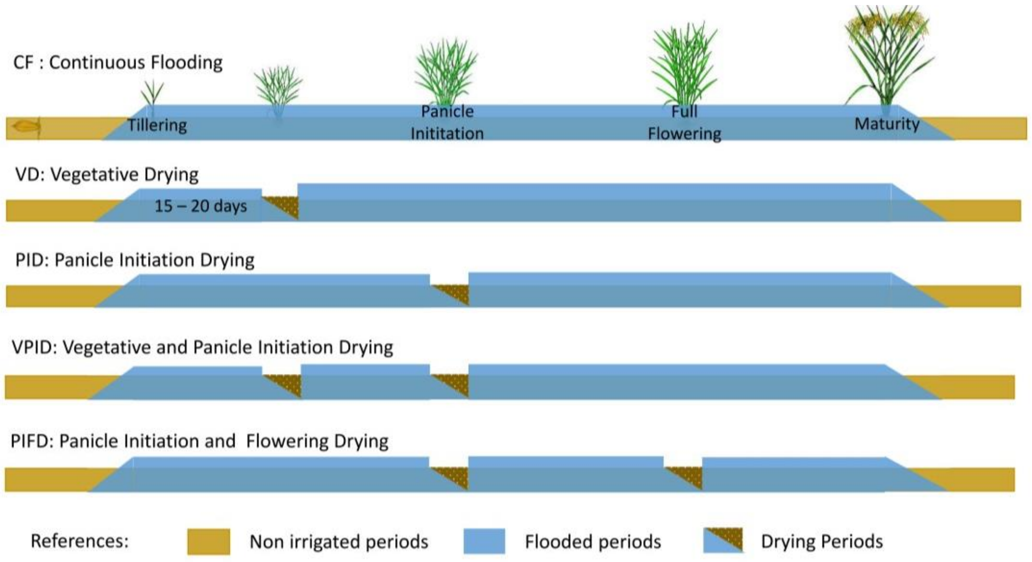


Figure 2. Irrigation treatments evaluated during two crop seasons (2018-2019 and 2019-2020). CF: Continuous flooded, AIT treatments were VD: Vegetative drying,

PID: Panicle initiation drying, VPID: Vegetative and panicle initiation drying, PIFD: Panicle initiation and 100 % flowering drying.

3.4.4. Chemical and Crop Measured Parameters

3.4.4.1. Total and Bioavailable Arsenic in Soils

Bioavailable arsenic (bioAs) was analyzed for both seasons, while total arsenic (tAs) was analyzed only in season 2018-2019. For both analyses, composite soil samples from 0-20 cm were taken from the fields 10-15 days before sowing. Bioavailable and total soil arsenic were determined using microwave digestion and inductively coupled plasma (ICP) optical emission spectroscopy, as described Carracelas et al. (2019b).

3.4.4.2. Redox Potential and pH in Soil

Redox potential (Eh, mV) and pH were measured weekly using a portable Horiba device equipped with a platinum electrode (LAQUA Act model PH120). Five replicates were taken for pH and redox potential, between the second and third rice row, at 10 cm depth irrigation treatment. In those plots there were also installed frequency-domain (FDR) sensors to measure soil moisture content. Additional Eh and pH measurements were taken 24 hours after each reflooding of the plots.

3.4.4.3. Soil Moisture Measurements

Soil moisture was monitored using 10HS (Decagon Devices, Inc., Pullman, WA) FDR sensors connected to EM 50 data loggers (Decagon Devices, Inc., Pullman, WA) which were configured to record soil moisture on an hourly basis. The sensors were installed horizontally at 12.5 cm depth in one plot of each treatment, except for PIFD plot treatment where two sensors were installed at 6 and 18.5 cm to monitor soil moisture when rice plants reached their maximum radical depth. Additionally, gravimetric water content (GWC) was measured at 0-20 cm depth during drying periods. GWC samples were taken 24 hours after each drainage period started, and samples were obtained every 2-3 days to follow soil moisture evolution, being the last sampling done immediately before each reflooding event to determine lowest soil moisture. Soil GWC was determined taking three samples on each plot from 0-20 cm. Each sample was partitioned and pooled into two soil depths, 0-10 cm and 10-20 cm. All samples were dried to 105 °C until constant weight. Soil GWC was then calculated using equation 1, where W = sample wet weight, D = sample dry weight.

Equation. 1

$$GWC = \left(\frac{W - D}{D}\right) \times 100$$

Bulk density was determined using a 4.5 cm diameter soil core from 0-10 cm, 10- 20 cm and 20-30 cm of soil depth. Undisturbed bulk density samples were oven-dried at 105 °C until constant weight. Finally, volumetric water content (VWC) was calculated multiplying GWC and bulk density. Soil available water storage capacity was determined as the difference between VWC at field capacity and VWC at permanent wilt point (Richards, 1948).

3.4.4.4. Inorganic Arsenic in Polished Rice Grain

Determination of iAs in rice grain was done in Technological Laboratory of Uruguay (LATU) following the same procedure described by Roel et al. (2021).

3.4.4.5. Cadmium in Polished Rice Grain

Twelve rice grain samples (two from each of the three blocks) of most contrasting irrigation treatments CF and PIFD (total n = 12) were selected in order to analyze cadmium concentration in both sites in the first season. Polished rice grain samples were ground with a blade mill to pass a 1 mm sieve. Next, 0.3 grams milled rice was digested with 3.0 mL of nitric acid (Merck, 65 % for analysis) and 2.0 mL of hydrogen peroxide (30 % w/v) in a microwave (Milestone, Ethos One, Italy) and the digests were diluted to 50 mL with nitric acid 0.5 % in deionized water. Inductively coupled plasma-mass spectrometry was used to determine Cd (Nex Ion 350 D, Perkin Elmer, USA). Calibration curves were prepared with cadmium (1000 mg L^{-1}) stock standards from Inorganic Venture (USA). Every forth sample, one blank, two fortified samples, and one certified reference standard (Rice Flour, National Institute of Standards and Technology, USA, 1568b) were included as quality control samples. The certified reference material (1568b) was used to assess the accuracy of Cd concentration for rice flour.

3.4.4.6. Irrigation Water Inputs

Irrigation water inputs (WIi) were measured with helicoidal flowmeters (ARAD, WMR50) at the entrance of each plot to allow independent management according to each irrigation treatment. Water was pumped from nearby irrigation channels to ensure full-pipe water flow. Water inputs were then adjusted to m^3 ha^{-1}.

3.4.4.7. Grain Yield

Grain yield was obtained by manual harvest of 4.08 square meters (8 rows x 3 m) from the center of each plot when a grain moisture of 21 % was reached. Samples were mechanically threshed, and grain yields were corrected to 13 % moisture. Harvested samples were meticulously identified and carried to INIA’s grain laboratory where they were dried at 60 °C until 13 % moisture was reached. Grain subsamples identification was codified and As grain content analyses were performed by an independent laboratory (LATU).

3.4.5. Statistical Analysis

All statistical analysis was performed using R software (R Core Team, 2019) in combination with nlme, emmeans, ggplot2 packages. For the response variables yield and iAs, a linear mixed effect model was used. Analyses of variance was performed followed by means separation using Tukey’s test. Fixed effects considered were season, irrigation and phosphorous fertilization treatments and the interaction between season and irrigation treatments. Random effects were block and main plot. For the response variables WIi, the same procedure was performed defining season, irrigation and the interaction between them as fixed effects while block was defined as random effects.

3.5. RESULTS

3.5.1. Soil Moisture

Volumetric water contents measured by the FDR sensors and the VWC values calculated from gravimetric samples taken at the different drying periods and precipitations during irrigation period are represented in Figure 3.

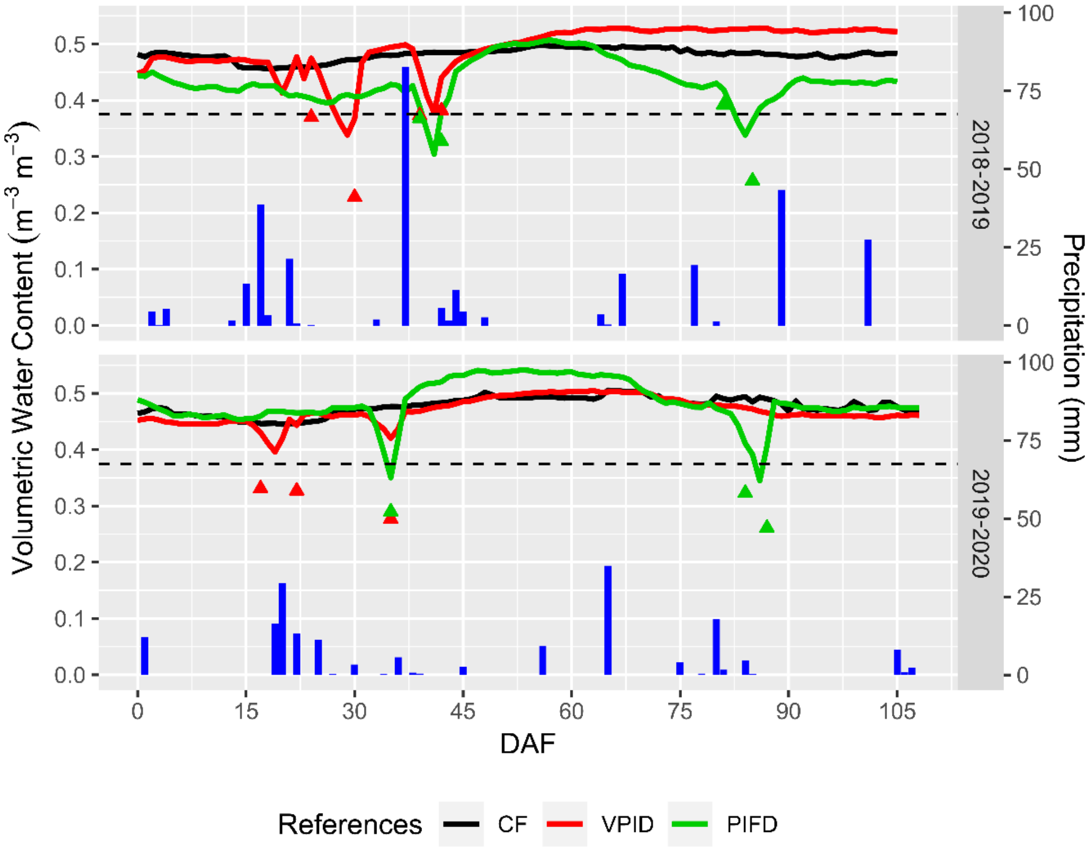


Figure 3. Soil volumetric water content registered along days after flooding (DAF) in PdL experiments for most contrasting irrigation treatments across two crop seasons: 2018-2019 and 2019-2020. Lines represents frequency-domain sensor (FDR) records and triangles represents gravimetric water content for continuous flooded (CF, black), Vegetative and panicle initiation drying (VPID, green) and panicle initiation and full flowering drying (PIFD, red) irrigation treatments. Blue bars represent precipitation events. Black dashed line represents irrigation threshold in order to reflood treatments.

Precipitations records during irrigation period were 282 and 213 mm for season 1 and 2, respectively, and they were lower than the historical average of 392 mm registered during the last 50 years. The duration of each drying period was around 5-7 days when this period was not interrupted by precipitation. Average VWC records from FDR sensors in CF treatment were 0.481 and 0.478 m^3 m^{-3} for first and second season, respectively. VWC in CF treatment never dropped from saturation (Figure 3). Drying periods applied along crop cycle are represented. Vegetative drying period started around 20 DAF in both seasons. Panicle initiation drying period was done 35-40 DAF. Finally, full flowering drying period was done at 80 DAF for both seasons. The targeted irrigation threshold was reached for each of the three drying periods in both seasons.

3.5.2. Redox Potential and pH in Soil

Evolution of redox potential (Eh, mV) for both seasons is represented in Figure 4. Positive initial redox potential values were measured in a range between 100-250 mV for all treatments across seasons. After initial flooding, Eh shows a decreasing trend in both seasons. Control CF treatment reported negative values 15 DAF with a similar behavior in both seasons. Negative values were recorded in this treatment for the rest of the irrigation period. When irrigation was initiated, VPID treatment follows the same decreasing trend until first drying was imposed. Positive Eh values over 150 mV were reached during this drying event. A decreasing Eh trend was observed after reflooding this treatment, followed by an increment up to 150-250 mV at 35-45 DAF when the second drying period was implemented. Similar positive redox potential values were observed at panicle initiation stage in PIFD treatment with later decreasing values as in VPID, followed by a peak observed at 90 DAF immediately after full flowering drying period was applied.

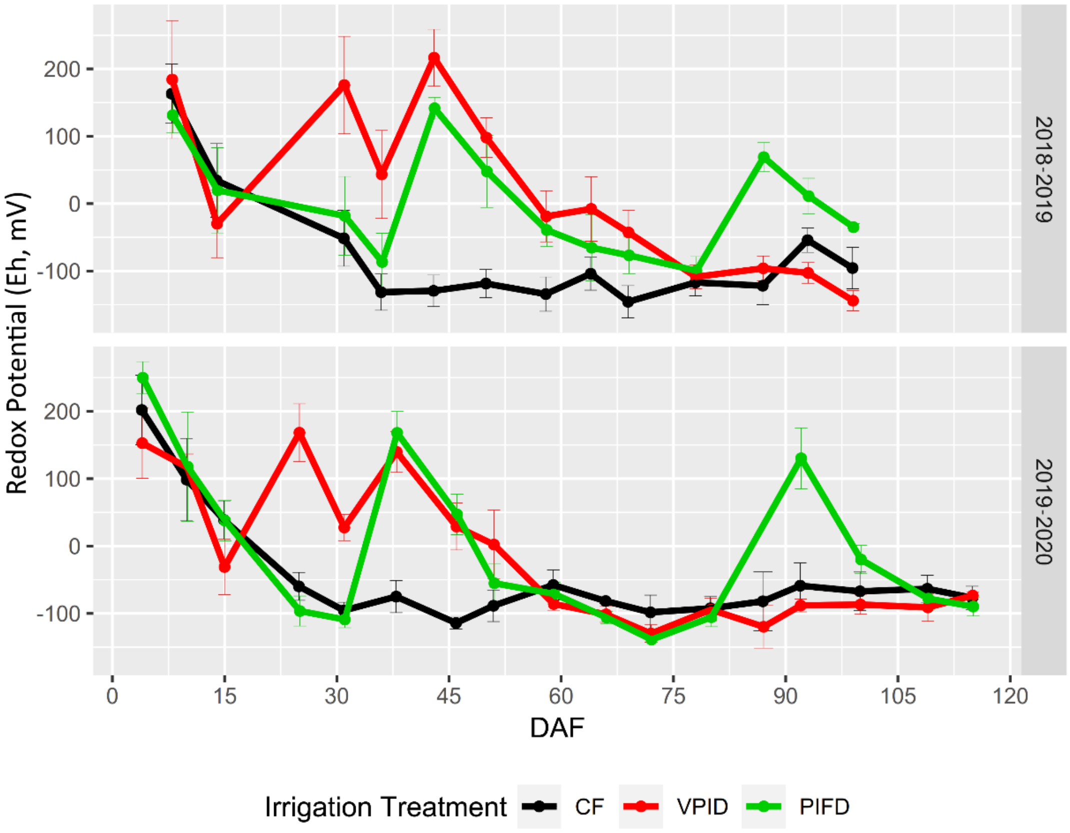


Figure 4. Redox potential (Eh, mV) trend along days after flooding (DAF) for continuous flooding (CF, black line), vegetative and panicle initiation drying (VPID, red line) and panicle initiation and full flowering drying (PIFD, green line) treatments in Paso de la Laguna (PdL) experimental site, across two seasons: 2018-2019 and 2019-2020. Bars represent standard errors of means.

Evolution of pH for both seasons is represented in Figure 5. Initial pH values were between 5 and 6 in both seasons. After initial flood was stablished, pH values were increased in CF tending to neutrality. Around 30 DAF, pH values in CF stayed between 6-7 until harvest. In VPID treatment, the tendency after initial flood was the same as in CF treatment, except for measurements taken immediately after vegetative and panicle initiation drying events when pH tends to decrease and pH values were lower than obtained in CF. In PIFD the initial tendency was to increase pH values until panicle initiation and full flowering drying events were applied. pH measurements taken immediately after these two drying events, (40 and 80 DAF) showed lower pH values than CF, being more evident at panicle initiation drainage.

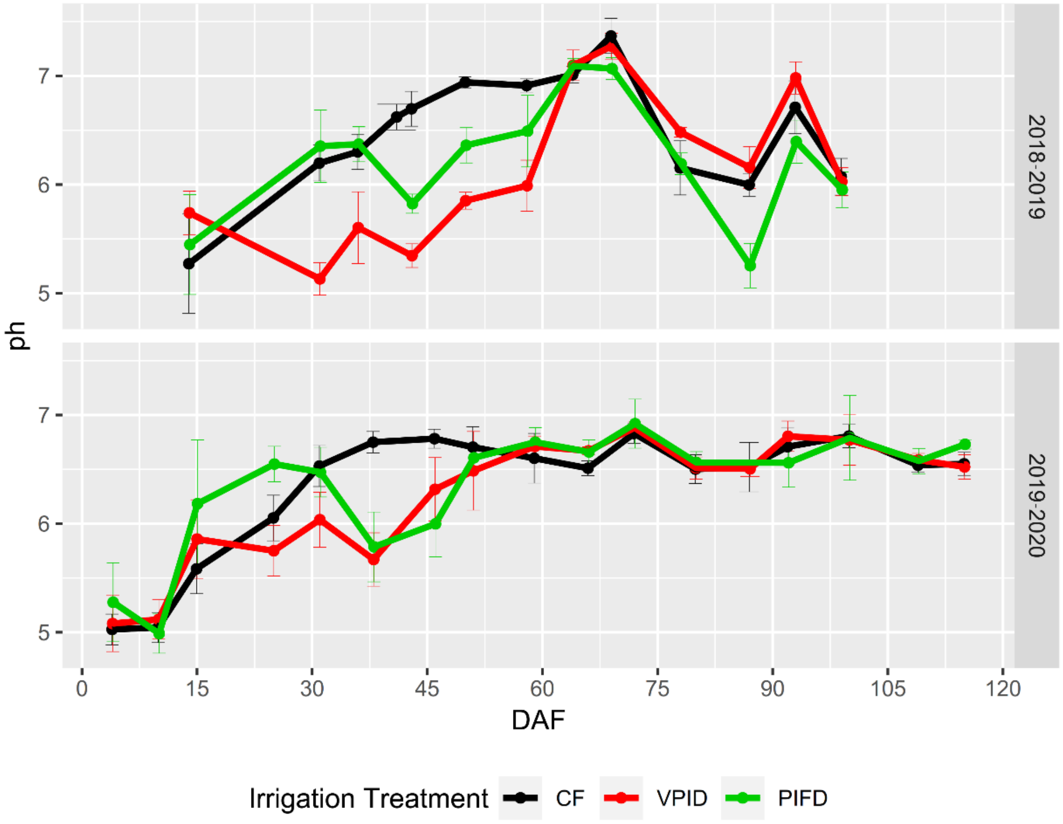


Figure 5. pH evolution along days after flooding (DAF) for continuous flooding (CF, black line), vegetative and panicle initiation drying (VPID, red line) and panicle initiation and full flowering drying (PIFD, green line) treatments in Paso de la Laguna (PdL) experimental site, across two seasons: 2018-2019 and 2019-2020. Bars represent standard errors of means.

3.5.3. Total and Bioavailable Arsenic in Soil

Total arsenic (tAs) level in soil at sowing in the first season was 3.506 mg kg^{-1}. Bioavailable As (bioAs) levels in soil at sowing were 0.175 and 0.26 mg kg^{-1} of dry soil in seasons 2018-2019 and 2019-2020, respectively.

3.5.4. Rice Grain Yield

Rice grain yield mean value (13 % moisture) for both seasons was 10921 kg ha^{-1} with a coefficient of variation of 7.5 % (Table 2). Significant differences between seasons were detected, with a mean grain yield of 10490 and 11352 kg ha^{-1} in seasons 1 and 2, respectively. No other statistically significant effects were detected for grain yields. Statistical differences were detected for the interaction between irrigation and phosphorous treatments but mean yield values were not different using Tukey’s test.

Table 2. Rice grain yield (kg ha^{-1}, 13 % moisture) and inorganic arsenic concentrations (mg kg-1) in 2018-2019 and 2019-2020 seasons from Paso de la Laguna (PdL) experimental site in Uruguay, by five irrigation treatments in INIA Merín Variety.

Classification criteria Season	Rice Yield (kg ha^{-1})	iAs (mg kg $^{-1}$)
2018-2019	10490 b	0.075 b
2019-2020	11352 a	0.094 a
Average	10921	0.084
CV %	7.55	18.2
$P < 0.05$	***	***
Irrigation		
Continuous flooding (CF)	10694	0.086 a
Vegetative drainage (VD)	11470	0.089 a
Panicle initiation drainage (PID)	11138	0.090 a
Vegetative and panicle initiation drainage (VPID)	10680	0.090 a
Panicle initiation and flowering drainage (PIFD)	10624	0.067 b
$P < 0.05$	NS	***
Phosphorous fertilization		
0 UP	10845	0.085
50 UP	10997	0.084
$P < 0.05$	NS	NS
Irrigation * Phosphorous fertilization		
$P < 0.05$	*	NS

Means followed by different letters are significantly different with a probability less than 5 % ($P < 0.05$). $P < 0.001$, P < NS: non- significant differences. CV: coefficient of variation.

3.5.5. Inorganic Arsenic in Polished Rice Grain

The average value for iAs in polished rice grain for both seasons was 0.084 mg kg $^{-1}$ with a coefficient of variance of 18.2 % (Table 2***).*** Season and irrigation treatment were significant for iAs while phosphorous fertilization and the interaction between irrigation and phosphorous were not significant. Mean values for iAs levels were 0.075 and 0.094 mg kg^{-1} in seasons 1 and 2, respectively, with the former being significantly lower than the latter. The lowest iAs accumulation was associated with the PIFD treatment with a mean value of 0.067 mg kg^{-1} (Figure 6).

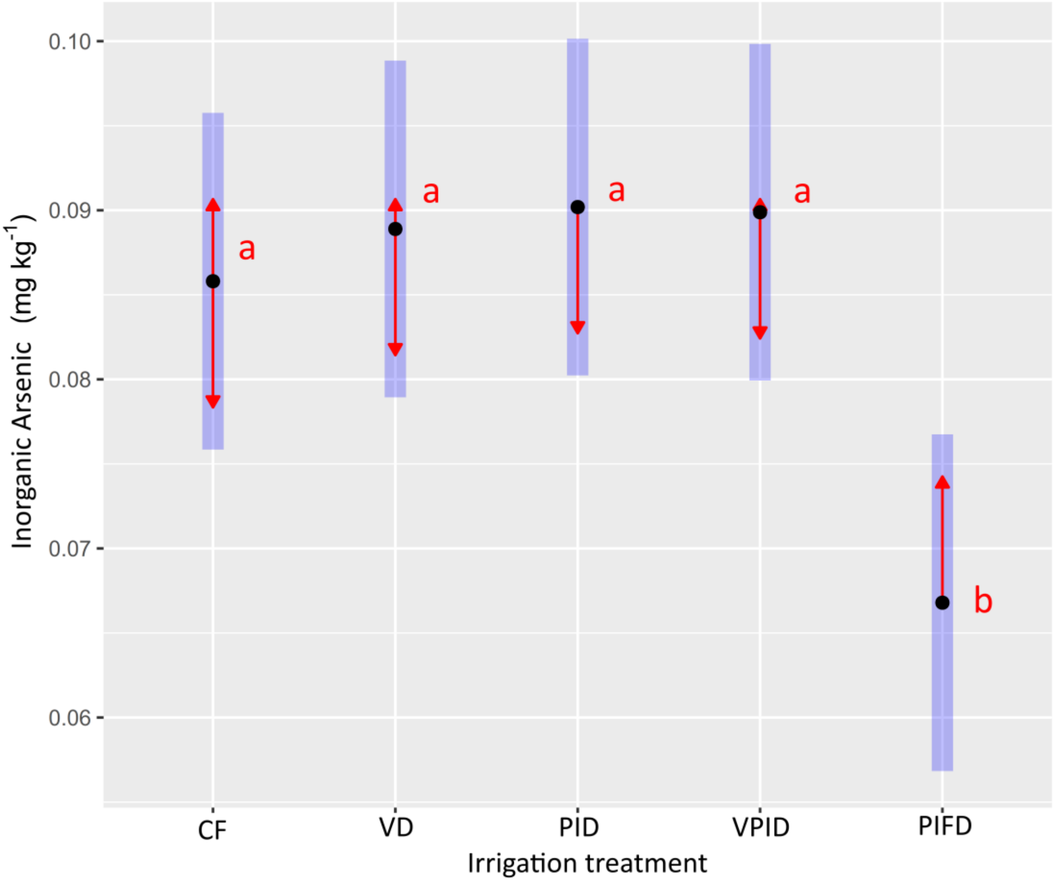


Figure 6, Inorganic arsenic (iAs) content in polished rice grain (mg kg^{-1}) for the different irrigation treatments. CF: Continuous flooded, AIT treatments were VD: vegetative drying, PID: panicle initiation drying, VPID: vegetable and PI drying, PIFD: panicle initiation and full flowering drying in Paso de la Laguna (PdL) experimental site. Black dots represent means, red arrows are indicating confidence intervals by Tukey's test for the estimated marginal means and blue bars indicates standard errors. Different letters indicate significant differences with a probability less than 5 %.

3.5.6. Cadmium in Polished Rice Grain

From the total number of twelve polished rice grain samples analyzed, nine of them presented Cd levels below the method detection level limit of 0.01 mg kg^{-1}. In the three remaining samples, Cd was detected, but its concentration was below the quantification level of 0.03 mg kg^{-1}.

3.5.7. Irrigation Water Inputs

The mean value of total irrigation water was 10423 m^3 ha^{-1} for all irrigation treatments and seasons with a coefficient of variation of 14.3 %. Significant differences were detected between seasons with an average value of 9396 m^3 ha^{-1} for 2018-2019 and 11449 m^3 ha^{-1} for 2019-2020. No interaction between irrigation treatments and seasons was detected.

3.6. DISCUSSION

3.6.1 pH and Redox Potential

pH and redox potential were modified by alternative irrigation techniques compared to continuous flooding (Figure 4 and 5). Negative Eh values were reached 15-20 days after initial flooding, in all treatments, similar to what was reported by Tarlera et al. (2016) and Carracelas et al. (2019b) in Uruguay. According to international research, during that time gap, As that was coprecipitated as Fe oxyhydroxides dissolves and its bioavailability increases when reduction of Fe^{III} to Fe^{II} occurs in the Eh range of 0-100 mV (Masscheleyn et al., 1991; Yamaguchi et al., 2011; Zhao et al., 2020). Honma et al. (2016) demonstrated that dissolved As in soil solution is almost linearly related to dissolved Fe in soil solution. Strategic low severity drying events were effective to turn soil into aerobic conditions reaching positive Eh values over 50 mV, and even over 150 mV in most drying periods, which is expected to reduce As mobility and availability in soils (Figure 4).

At the beginning of irrigation, pH values in CF treatment tend to increase from original acidic values between 5 and 6 to almost reaching the neutrality, being stabilized in a range between 6.5 and 7 around 30 to 45 DAF. In the first season initial increasing tendency was similar, but pH values were more unstable from 45 DAF to irrigation ending. After drying events, pH tends to decrease. Different studies show that As bioavailability is increased by pH values over 6.3 (Masscheleyn et al., 1991; Honma et al., 2016; Zhao and Wang, 2020). This situation generates contrasting soil conditions between irrigation treatments. While in continuous flooding treatment as availability in soil should be increased during the whole irrigation period, in the alternative irrigation techniques treatments a reduction in As availability should be noticed by the combined effect of pH modifications and redox potential, as illustrated in Figures 4 and 5.

3.6.2. Total and Bioavailable Arsenic in Soils

Total As level in soil was measured only in the first season with an average value of 3.51 mg kg^{-1}, similar and slightly lower than the 5 mg kg^{-1} average obtained by Verger. (2015) in a 20 soils sampling of the rice producing regions of Uruguay. Carracelas et al. (2019b) reported tAs levels of 3.62 mg kg^{-1} and 2.14 mg kg^{-1} for two sites in the east and north regions of Uruguay. Quintero et al. (2014), in soils from Entre Ríos province, a rice producing region in Argentina located west from Uruguay river, found tAs levels ranging from 1.6 to 4.1 mg kg^{-1}. The results obtained in actual study are lower than world's average of 5 mg kg^{-1} (Koljonen et al., 1989) and well below Canadian Environmental Quality Guidelines limit (CCME, 2019) of arsenic in soil of 12 mg kg^{-1}.

Bioavailable As in soil was 48.6 % higher in the second season compared to the first one, with values of 0.14 and 0.21 mg kg^{-1}, respectively. The high variability in bioAs might be related to important environmental effect generating such differences within seasons and should be studied deeper. Verger et al. (2015) incubated 10 soils of Uruguayan rice-producing regions under different crop rotations and managements. Maximum As bioavailability was registered between day 5 to 15 after incubation started for all soil samples, and the tendency was to decrease after reaching that peak.

A ten-fold time difference was detected between maximum and minimum bioAs levels during that peak with a range from 0.0175 mg kg^{-1} to 0.1610 mg kg^{-1} of dry soil. After the 5-15 days of incubation peak, bioAs lowered and was stabilized between 0.0120 mg kg^{-1} and 0.0900 mg kg^{-1}. Bioavailable arsenic levels at sowing reported by

Carracelas et al. (2019b) in Uruguay were 0.1515 mg kg^{-1} for Paso de la Laguna and 0.0760 mg kg^{-1} for Paso Farias (north region), which are also in consonance with this study.

3.6.3. Inorganic Arsenic in Polished Rice Grain

The mean and range values of iAs concentration in polished rice grain of 0.084 mg kg^{-1} (0.050-0.113 mg kg^{-1}) are similar to previous studies developed in Uruguay by Carracelas et al. (2019b) and aligned to what was reported by Roel et al. (2021) for this cultivar. These values are below international Codex Alimentarius (FAO and WHO, 2019) maximum levels for inorganic arsenic in polished grain (0.20 mg kg^{-1}) and 76.7 % of samples were below EU (Commisson Regulation 2015/1006) and USA (FDA, 2020) maximum levels allowed for rice used for preparation of infant food (0.10 mg kg^{-1}). Higher average iAs accumulation was found in second season compared to the first one (0.094 vs. 0.075 mg kg^{-1}, respectively), which might be explained by higher bioAs determined in soils (Xu et al., 2008, Carracelas et al., 2019b). Alternative irrigation techniques that applied drying periods during vegetative and panicle initiation were not effective to reduce iAs in grain. However, PIFD treatment that combined two drying periods during panicle initiation and full flowering was effective in reducing grain iAs content by 22.1 % compared to CF treatment. The combination of severity, timing and number of soil drying periods are relevant aspects when designing irrigation management strategies to reduce As accumulation in grain.

According to Carrijo et al. (2019), more than one drying period is necessary to minimize grain As levels when applying low severity soil dryings, which is aligned with the results obtained in this study. Similar irrigation management strategies can reach a reduction in grain arsenic between 16-35 % (Islam et al., 2017; Norton et al., 2017). As iAs is more toxic than organic forms, and the relation between inorganic and total arsenic not responding to a fixed factor, it seems that future studies should be focused on how irrigation management specifically affects As grain speciation and iAs accumulation. Despite iAs levels that were low in both seasons of the present study, results show that it is possible to reduce it even more applying two soil dryings during panicle initiation and full flowering stages as in PIFD treatment. Similar results were reported by Arao et al. (2009) and Zheng et al. (2011). According to these authors, iAs transport and accumulation in grain mainly occurs after flowering stage during grain filling. Other combinations of aerobic cycles during irrigation period, such as alternate wetting and drying irrigation techniques, have been tested in previous studies in Uruguay as an effective strategy to minimize iAs accumulation in grain (Carracelas et al., 2019b) achieving a reduction of 39.6 %, but affecting grain yield in some cases.

Several environmental factors have been reported by the international literature as relevant factors in As absorption by plants and might me explaining the 25 % higher accumulation in 2019-2020 compared to 2018-2019. Arao et al. (2018) studied the relationship between many climatic factors and iAs accumulation in grain, and discovered that average daily mean and average daily minimum air temperature from 2 - 4 weeks after heading were significantly and positively correlated to iAs in grain in a plot study in Japan. In contrast to that study, the average mean temperature in this study for both seasons was 21.6 °C, and the average minimum temperature was lower in season 2019-2020 compared to 2018-2019 (13.72 °C vs. 15.42 °C, respectively). Therefore, specific deeper research should be done to improve the knowledge about environmental and climatic factors effects over As accumulation in grain.

Phosphorous fertilization was not effective as an arsenic accumulation mitigation management alternative. Some authors affirm that higher concentrations of phosphorous in soils can increase As concentration in soil solution when P competes by absorption sites in soils or Fe-plaque, increasing As bioavailability until a critical soil P concentration is reached and competition for uptake paths with arsenate occurs, reducing As uptake (Peryea & Kammereck, 1997; Geng et al., 2005; Bogdan and Schenk, 2009; Meharg and Zhao, 2012; Azam et al., 2016; Mitra et al., 2017). However, a high variability in the response of As uptake under different levels of soil P has been reported. Results of this study are similar to what had been reported by Xu et al. (2008) and Wu et al. (2011). They found no relation between soil P and As uptake since the iAs form absorbed by rice plants under flooding conditions is arsenite. Phosphate addition is expected to reduce arsenate absorption through arsenate transporters, not arsenite.

3.6.4. Cadmium in Polished Rice Grain

Minimizing iAs absorption and accumulation in rice grain applying aerobic cycles during irrigation period could cause a negative effect in Cd accumulation in grain. The increase in Eh and lower pH values could lead to higher Cd availability for plants in soils and higher accumulation in rice grain (Zhao and Wang, 2020). Taking that into account, it was reasonable to analyze Cd concentration in polished rice grain of the most contrasting irrigation treatments. CF treatment as the most anaerobic soil conditions, with lower Eh and more basic pH values in soil solution and PIFD treatment with more aerobic conditions, higher Eh and more acidic pH values. Cd levels in grain were well below Codex Alimentarius maximum levels (0.4 mg kg-1) in all analyzed samples even applying two drying events during crop season. These results are very relevant and encouraging, considering that is possible to apply AIT to obtain minimal iAs content in grain with no significantly Cd increase.

3.6.5. Rice Grain Yield

Average yield of this study was 10921 kg ha^{-1}, with 8.2 % higher yields in season 2019-2020 compared to 2018-2019. These results are consistent with average commercial Uruguayan east rice-producing region yields of 8520 kg ha^{-1} (DIEA, 2020) and 9350 kg ha^{-1} (DIEA, 2021) obtained in seasons 2018-2019 and 2019-2020, respectively. Better climate conditions were registered in season 2019-2020 compared to 2018-2019. According to the information obtained from INIA’s climate station located in PdL, sunshine hours accumulated during crop cycle in 2019-2020 were 344 hours compared to 221 in 2018-2019. Also, higher tank evaporation registers occurred in season 2019-2020 (120 mm vs. 113 mm). Many international studies report contradictory effects on grain yield of alternative irrigation techniques to the traditional continuous flooding system. Linquist et al. (2015), in a two-season study in Arkansas, USA, applied AWD during vegetative stage, with no yield penalty. On the other hand, when AWD was applied during vegetative and reproductive stages, grain yield stability was affected, especially with more severe field dryings. Capurro et al. (2015), in a three-year plot study developed in Paso de la Laguna, Uruguay, found that AWD applied during vegetative stage with an irrigation threshold of 50 % of available water holding capacity affected grain yield stability, obtaining lower yields than CF treatment in one of the three seasons. A yield loss of 15 % was reported by Carracelas et al. (2019a) with a similar type of irrigation treatment.

An important result of this study is the feasibility of AIT without crop yield penalization. Alternative irrigation techniques treatments in actual study were designed with specific low severity soil dryings to minimize iAs accumulation in grain avoiding grain yield affection. Carrijo et al. (2018), in California, USA, found that the appliance of two soil dryings at vegetative and panicle initiation stage, reflooding before 50 % heading had no effect in grain yield, independently of the severity of field dryings. In a later study, Carrijo et al. (2019) determined that the imposition of a single soil drying period within the growing season can mitigate As accumulation in rice grain, but it depends on the severity and timing of the drying period. Drainage during booting and heading were the more effective stages to reduce iAs with no grain yield reduction.

Finally, phosphorous fertilization did not have a significant effect on grain yield. This situation could be explained by phosphorous levels at soils in both seasons that were above critical levels for this crop according to Hernández et al. (2013) for this region.

3.6.6. Irrigation Water Inputs

The only significantly differences in irrigation water inputs detected were associated to the season effect, 9396 m^3 ha^{-1} and 11449 m^3 ha^{-1} average total irrigation water use in all treatments for season 2018-2019 and 2019-2020, respectively. Lower total water amounts registered in season 2018-2019 were associated with higher amounts of precipitation registered in that season. Precipitation during irrigation period 2018-2019 were 32 % higher compared to 2019-2020, with 282 mm and 213 mm for each season, respectively. Different alternative irrigation treatments evaluated did not varied significantly in the total amount of irrigation water used. AIT treatments presented similar amount of water use than the continuous flooding treatment (control), indicating that the drainage and reflooding effects did not alter significantly the total amount of irrigation water required (Supplementary Table 1).

3.7. CONCLUSIONS

Combinations of low severity drainages at different rice growth stages were able to alter soil redox potential and pH behavior compared to the traditional continuous flooding management. Strategic low severity drying events were effective to turn soil into aerobic conditions reaching positive Eh values in most drying periods, which is reported to reduce soil As mobility and availability. Levels of P fertilization had no impact on iAs grain content. The hypothesis that by not applying P will potentially reduce soil As availability was not confirmed. This study shows that there is an alternative water management strategy that consisted in applying two strategic low severity drainages at panicle initiation and full flowering stages that allows a significant reduction of inorganic arsenic content level without penalizing yield.

Similarly, Cd rice grain levels that can potentially increase under this irrigation treatment were well below Codex Alimentarius maximum levels (0.4 mg kg^{-1}) in all analyzed samples. These results fulfill the interest from the rice industry to develop techniques to satisfy special quality standards or specific market requirements like the baby food sector.

Further validation should be done at farmer scale to evaluate the feasibility of the application of this irrigation management alternative. Additionally, the effect of a single full flowering drying event over iAs accumulation in rice grain, not evaluated in this study, should be addressed in future research.

This study addressed only the inorganic content of arsenic in rice, as this is the most toxic component. A relevant aspect that should be also taken in consideration is the organic and total component of this element.

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3.10. SUPPLEMENTARY TABLE

Suplementary Table 1. Irrigation water inputs (m3 ha^{-1}) and irrigation water productivity: kg rice grain per m^3 (kg m^{-3}) in 2018-2019 and 2019-2020 seasons from Paso de la Laguna (PdL) experimental site in Uruguay, by five irrigation treatments in INIA Merín variety.

Classification criteria	Irrigation water inputs (m^3 ha^{-1})	Irrigation water productivity (kg m^{-3})
Season		
2018 - 2019	9396 ^{b}	1.13 ^{a}
2019 - 2020	11449 ^{a}	1.01 ^{b}
Average	10423	1.07
CV %	14.3	12.9
P < 0.05	***	***
Irrigation		
Continuous flooding (CF)	9686	1.13
Vegetative drainage (VD)	10834	1.04
Panicle initiation drainage (PID)	9916	1.11
Vegetative and panicle initiation drainage (VPID)	10722	0.96
Panicle initiation and flowering drainage (PIFD)	10956	1.11
P < 0.05	NS	NS
Season * Irrigation		
P < 0.05	NS	*

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

4. RESULTADOS Y DISCUSIÓN

El presente trabajo de tesis de maestría fue desarrollado en dos componentes principales, cuyas escalas fueron diferentes. Un primer componente, de mayor escala, que hizo foco en el contenido de arsénico de las chacras comerciales de todo el país, muestreando y analizando el contenido de arsénico inorgánico de un total de 150 chacras de todo el país durante dos zafras: 2017-2018 y 2018-2019. Este nos permitió caracterizar el rango de valores de acumulación de arsénico en grano de las principales áreas productoras de arroz a nivel nacional. A su vez, la generación de una base de datos de las chacras muestreadas, con su localización y la información de manejo vinculadas a estas, nos permitió identificar aquellos factores ambientales o de manejo que tuvieron mayor influencia sobre la acumulación de arsénico en grano.

De este componente se desprende que el contenido promedio de arsénico inorgánico a nivel país fue de 0,062 mg kg^{-1}, con un rango de entre 0,005 y 0,195 mg kg^{-1} y un coeficiente de variación del 51,5 %. El 100 % de las muestras analizadas (n = 150) presentaron valores por debajo de 0.2 mg kg^{-1} en arroz blanco pulido, nivel máximo definido por la legislación internacional vigente del Codex Alimentarious (FAO-OMS, 2019). Incluso, el 89 % (n = 133) de las muestras analizadas presentaron valores de arsénico inorgánico por debajo de 0,1 mg kg^{-1}, nivel máximo exigido por la FDA de los Estados Unidos y la Unión Europea para la elaboración de alimentos de niños de primera infancia. En cuanto al contenido de arsénico total, el valor medio para el total de las muestras fue de 0,178 mg kg^{-1}, con un rango de entre 0,015 y 0,629 mg $kg-^{1}$ y un coeficiente de variación del 63,6 %. El 85 % (n = 127) del total de las muestras analizadas presentaron valores de arsénico total por debajo de la legislación del Mercosur, que define un nivel máximo de arsénico total de 0,3 mg kg^{-1} en el grano de arroz blanco pulido. Vale destacar que esta legislación regional está actualmente en proceso de revisión para alinearse a la legislación internacional del Codex Alimentarious pasando a regirse por niveles de arsénico inorgánico debido a la mayor relevancia que presentan estas especies de arsénico desde el punto de vista de la salud humana.

Se detectó una moderada correlación entre el contenido de arsénico inorgánico y el arsénico total en grano ($y = 0,193x + 0,028$, $p < 0,0001$) con un $r^2 = 0,47$. Este coeficiente de correlación es bastante menor al reportado por Meharg et al. (2012) obtenido a partir de una base de datos internacional (r^2 = 0,78), lo cual estaría indicando que no es correcto asumir que un porcentaje fijo del arsénico total corresponde a arsénico inorgánico y reafirmaría el concepto de que no es posible estimar el contenido de arsénico inorgánico en grano a partir del análisis de arsénico total, siendo necesario determinar específicamente el contenido de arsénico inorgánico.

De este componente también se pudo determinar que el material geológico a partir del que se formó el suelo sobre el cual se cultivaron las chacras tuvo un efecto significativo en el contenido de arsénico total e inorgánico en el grano. Aquellas chacras que fueron cultivadas sobre rocas de tipo sedimentario presentaron mayor nivel medio de arsénico total e inorgánico respecto a aquellas que se desarrollaron sobre rocas de tipo ígneo (0,198 mg kg^{-1} AsT y 0,067 mg kg^{-1} AsI vs. 0,084 mg kg^{-1} AsT y 0,039 mg kg^{-1}AsI, respectivamente). También se encontraron diferencias significativas en cuanto al contenido de arsénico total de acuerdo a la región arrocera. La zona norte presentó los menores niveles de AsT, seguida por la zona este y centro, con valores de 0,121 mg kg^{-1}; 0,178 mg kg^{-1} y 0,257 mg kg^{-1}, respectivamente. En cuanto a AsI, la única zona que presentó menor concentración en grano fue la zona norte, sin diferencias significativas entre centro y este, cuyos niveles fueron 0,053 mg kg^{-1}; 0,059 mg kg^{-1} y 0,082 mg kg^{-1}, respectivamente. Esta situación podría explicarse por el predominio de chacras cuyos suelos se desarrollan sobre rocas ígneas en la zona norte y de chacras que se encuentran sobre rocas de tipo sedimentario en las zonas centro y este.

Finalmente, el análisis de la base de datos de chacras comerciales permitió determinar que las variedades de subespecie Índica presentaron mayor acumulación de AsT y AsI respecto a las variedades de la subespecie Japónica, con promedios de 0,188 mg kg^{-1} AsT y 0,066 mg kg^{-1} AsI vs. 0,123 mg kg^{-1} AsT y 0,0035 mg kg^{-1} AsI, respectivamente.

El segundo componente de este estudio se desarrolló a escala parcelaria. Logró identificar un manejo alternativo de riego a la inundación continua que permitiría alcanzar niveles mínimos de iAs en grano, lo cual sería muy relevante para el sector para poder atender mercados que requirieran mínimas concentraciones de iAs en grano. El tratamiento que permitió minimizar el contenido de iAs en grano fue aquel que aplicó dos secados de suelo de baja severidad durante las etapas reproductivas del cultivo de primordio floral y plena floración. Este tratamiento redujo el contenido de iAs de grano un 24,7 % respecto al tratamiento de inundación continua (0,067 mg kg^{-1} vs. 0,089 mg kg^{-1}). A su vez, no existieron diferencias significativas entre los tratamientos respecto a rendimiento, consumo de agua de riego y productividad del agua de riego, lo cual es favorable desde el punto de vista de la adopción de este tipo de manejo de riego. Por otra parte, cabe aclarar que más investigación y la validación de este tipo de tecnologías a mayor escala es necesaria para verificar que no afecte los rendimientos de las chacras ni aumente el consumo de agua de riego, conservando una adecuada eficiencia de uso de agua de riego.

Por otra parte, el manejo de la fertilización fosfatada no fue una estrategia efectiva para reducir la acumulación de iAs en el grano.

5. CONCLUSIONES

La conformación de una base de datos georreferenciada con información de chacras de todo el país resultó una herramienta fundamental para conocer la situación actual del sector arrocero nacional respecto a la concentración de arsénico en el grano de arroz de chacras comerciales y para conocer los factores del ambiente y de manejo que tienen mayor incidencia sobre esta variable.

Del análisis de esta información se desprende que existen alternativas que permiten obtener grano con menor contenido de arsénico inorgánico y total, ya sea a través de la selección de chacras que se hayan cultivadas sobre formaciones geológicas de tipo ígneo predominantes en la zona norte del país o seleccionando chacras de variedades de la subespecie Japónica.

En los ensayos de riego realizados durante dos zafras en la localidad de Paso de la Laguna se pudo comprobar que los secados de suelo de baja severidad aplicados fueron efectivos para modificar las condiciones anaeróbicas del suelo inundado, lo cual quedó evidenciado en el incremento en los valores de potencial redox de la solución del suelo que lograron alcanzar valores positivos y en las lecturas de pH de la solución del suelo, que, luego de evolucionar hacia valores cercanos a la neutralidad al iniciarse el riego, tendieron a volverse más ácidos en las determinaciones realizadas posteriores los secados.

Aun determinándose niveles de arsénico total e inorgánico en grano menores a los valores máximos exigidos por la legislación internacional y regional, se logró detectar un manejo de riego alternativo a la inundación continua del cultivo, que mediante la aplicación de dos secados del suelo combinados en las etapas de primordio y plena floración lograron minimizar la acumulación de As en grano manteniendo la productividad en grano del cultivo.

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