Geographical variation in inorganic arsenic in paddy field samples and commercial rice from the Iberian Peninsula

Antonio J. Signes-Pastor[a,*], Manus Carey[a], Angel A. Carbonell-Barrachina[b], Eduardo Moreno-Jiménez[c], Andy J. Green[d], Andrew A. Meharg[a]

[a]Institute for Global Food Security, Queen's University Belfast, David Keir Building, Malone Road, Belfast BT9 5BN, Northern Ireland, United Kingdom. [b]AgroFood Technology Department, Miguel Hernández University, Carretera de Beniel, km 3.2, 03312 Orihuela, Alicante, Spain. [c]Department of Agricultural Chemistry, Universidad Autónoma de Madrid, 28049 Madrid, Spain. [d]Doñana Biological Station, EBD-CSIC, Amarico Vespucio Rd. 41092, Seville, Spain

*Corresponding author. E-mail address: a.signes-pastor@qub.ac.uk (A.J. Signes-Pastor).

Abstract

This study investigated total arsenic and arsenic speciation in rice using ion chromatography with mass spectrometric detection (IC-ICP-MS), covering the main rice-growing regions of the Iberian Peninsula in Europe. The main arsenic species found were inorganic and dimethylarsinic acid. Samples surveyed were soil, shoots, and field-collected rice grain. From this information soil to plant arsenic transfer was investigated plus the distribution of arsenic in rice across the geographical regions of Spain and Portugal. Commercial polished rice was also obtained from each region and tested for arsenic speciation, showing a positive correlation with field-obtained rice grain. Commercial polished rice had the lowest i-As content in Andalucia, Murcia and Valencia while Extremadura had the highest concentrations. About 26% of commercial rice samples exceeded the permissible concentration for infant food production as governed by the European Commission. Some cadmium data is also presented, available with ICP-MS analyses, and show low concentration in rice samples.

Keywords: Inorganic arsenic; Cadmium; Rice Soil Shoots; Iberian Peninsula; Arsenic speciation

Introduction

The greatest arsenic (As) toxicity is attributed to inorganic arsenic (i-As), a non-threshold class 1 human carcinogen (IARC, 2004). Other than cancers, human exposure to i-As has been associated with diverse health problems, including genotoxic effects (Banerjee et al., 2013). Rice and rice-based products have been identified as prevalent sources of i-As to human diet, particularly in South-East Asia where rice is the staple food (CAC, 2014; EFSA, 2009; IARC, 2004; Mondal & Polya, 2008). A strong correlation between urinary As and rice consumption has been reported (Banerjee et al., 2013; Cascio et al., 2011; Melkonian et al., 2013). Several sub-populations have elevated i-As intakes from rice consumption, notably babies and young children who have 3-fold higher consumption based on body weight. In addition, adults who have gluten allergies or are lactose-intolerant tend to consume more rice, thereby exposing themselves to higher As contamination (EFSA, 2009; IARC, 2004; Meharg, Deacon, et al., 2008; Signes-Pastor, Carey, & Meharg, 2016). The UN FAO/WHO has proposed the maximum level of 0.200 mg/kg i-As in polished rice (CAC, 2014). The European Commission (EC) has recently legislated the maximum limit thresholds of i-As in rice. These are 0.200mg/kg i-As for polished rice, 0.250 mg/kg i-As for parboiled rice and husked rice, 0.300 mg/kg i-As for rice waffles, rice wafers, rice crackers and rice cakes, and 0.100 mg/kg i-As for rice destined for the production of food for infants and young children (EC, 2015). However, there is still much debate that these guidelines and standards are set too high to protect people's health (Schmidt, 2015).

Arsenic is ubiquitous in the environment and flooding of soils, as in paddy cultivation, creates anaerobism, which mobilizes i-As into porewater (Lu et al., 2009). This leads to rice being much more efficient at assimilating As into its grain than other cereals (Williams et al., 2007). Rice grown under aerobic conditions, such as for upland rice production, has much lower i-As in its grain. However, aerobic cultivation leads to ~10-fold increase in cadmium (Cd) concentration in rice grain (Xu, McGrath, Meharg, & Zhao, 2008). Cadmium, like i-As is a carcinogen and renal toxicant with a half-life in the human body of ~40 years (Meharg & Zhao, 2012).

Unlike other foods such as fish, As species in rice are dominated by i-As and the organic compound dimethylarsinic acid (DMA) (EFSA, 2009; Meharg et al., 2009; Williams et al., 2005). The i-As content in rice and rice-based products have been widely-surveyed and variation in the concentration has been reported between countries (Carbonell-Barrachina et al., 2012; Meharg et al., 2009) and specific differences in i-As concentration in rice have also been reported within countries (Sommella et al., 2013; Williams, Raab, Feldmann, & Meharg, 2007). If low-As rice grain can be sourced and marketed this could potentially be sold as a premium product (Norton et al., 2009).

The rice growing regions in Portugal and Spain are distributed throughout the Iberian Peninsula and represent about 36% of the total rice production in Europe (Ferrero & Nguyen, 2004). Belgium, United Kingdom, Germany, and France are the main EU countries where Spanish rice is imported (Sainz, Sanz, Aguado, & Martín-Cerdeño, 2014).

In the current study, total arsenic (t-As) was determined in 40 soils samples from the Iberian Peninsula, and As speciation was determined in 40 shoots and 20 field-collected rice grains. In addition 144 commercial rice samples were categorized for As species, including polished rice, parboiled rice and brown rice. The relationships between t-As in soils and As species in shoot and rice, both field-collected and commercial, were explored. Likewise, the differences in As speciation of field-collected and commercial rice were studied. The paddy field regions that produce low i-As were identified, and to assess further potential toxicological risks, Cd concentration in field-collected and commercial rice grain was also analyzed.

Material and methods

Reagents and equipment

Arsenic speciation in shoots and rice, both field-collected and commercial, was performed using a Thermo Scientific IC5000 Ion Chromatography system, with a Thermo AS7, 2 250 mm column with a Thermo AG7, 2 50 mm guard column interfaced with a Thermo ICAP Q ICP-MS.

Total As in soil and Cd analyses in field-collected and commercial rice samples were carried out with the Thermo ICAP Q ICP-MS in direct solution acquisition mode using a Cetac ASX-520 Auto Sampler.

Other equipment used included a freeze dryer Christ Alpha 1-4 LD Plus, a Retch PM100 rotary ball-mill with a zirconium oxide lined vessel and zirconium oxide grinding balls, a OHAUS-Discovery digital weighing scale with 5 decimals, a CEM MARS 6 1800 W microwave digestor, and a Sorvall Legend RT centrifuge. For As speciation and Cd analyses 50 ml polypropylene centrifuge tubes, conical, with purple HDPE flat screw were used for sample preparation. Teflon pressure vessels (CEM, MARS 6) were used to digest soil samples to determine t-As.

Deionised water from a Milli-Q Integral 3 system was used for the preparation of reagents and standards. All chemicals were, at least, pro analysis quality. Commercial DMA and monomethylarsonic acid (MMA) from Supelco Analytical, arsenite and arsenate from Sigma–Aldrich and arsenobetaine BCR n626 were used to prepare As speciation standards. A commercial Multi-Element Solution 2 in 5% HNO3 (SPEX CLMS-2) and a Multi-Element Solution 4 in water/Tr-HF (SPEX CLMS-4) were used to prepare the standards for t-As and Cd analyses. In addition, commercial rhodium from Fluka Analytical was used as internal ICP-MS standard. BDH Prolabo Aristar 69% nitric acid and BDH Prolabo Analar Normapur 30% hydrogen peroxide were used for extraction/digestion and to convert any arsenite to arsenate.

Sample sourcing

Samples of soil, rice shoots and grain were collected in September 2014 from the eight main rice-producing regions of the Iberian Peninsula as shown in **Figure 1**. From each paddy field region 5 replicate samples of topsoil from 0 to 15 cm deep (n = 40), shoots (n = 40) and mature rice grain (n = 20), when possible, were collected. The mature rice grain samples were collected when available from Andalucía (n = 3), Catalunya (n = 5), Extremadura (n = 1), Murcia (n = 5), Portugal (n = 1) and Valencia (n = 5). The five sampling sites were distributed throughout each region (5 sampling sites per region). At each site, ~200 g of topsoil, ~200 g of shoots and ~200 g of grain sub-samples were gathered within a sampling area of 5 m 5 m and mixed together in different plastic bags for soil, shoots and rice grain, respectively. A market basket survey was also carried out and commercial rice samples produced in the main rice production regions of the Iberian Peninsula were purchased from supermarkets and local shops (n = 144). This set of samples included brown (n = 20), parboiled (n = 11) and polished (n = 113) rice. The rice-growing region was reported for a subset of 107 polished rice samples (Andalucía (n = 20), Aragón (n = 6), Catalunya (n = 14), Extremadura (n = 3), Murcia (n = 11), Navarra (n = 4), Portugal (n = 20) and Valencia (n = 29)).

Arsenic speciation analysis

Sample preparation for shoots, dehusked field-collected and commercial rice grain

All samples were freeze-dried until complete dryness, and then powdered using a rotary ball-mill. The powdered samples were weighed accurately to a weight of 0.1 g into 50 ml polypropylene centrifuge tubes. Then, 10 ml of 1% concentrated nitric acid were added and left to stand overnight. Then samples were microwave digested. The temperature was raised to 55°C in 5 min. and held for 10 min. and then to 75°C in 5 min and held for 10 min. Finally, the digest was taken up to 95°C in 5 min. and maintained at this temperature for 30 min. Samples were cooled to room temperature. The digestate was centrifuged at 4500g for 20 min. and a 1 ml aliquot was transferred to a 2 ml polypropylene vial and 10 µl of analytical grade hydrogen peroxide was added to convert any arsenite to arsenate to facilitate subsequent chromatographic detection.

QA/QC procedures

Each batch of samples included 2–3 blanks and 2–3 replicate samples of rice certified reference material (CRM) were included (NIST 1568b Rice flour). The rice CRM has t-As and the As species, DMA, MMA and i-As concentrations certified (0.285 ± 0.014, 0.182 ± 0.012, 0.012 ± 0.003 and 0.092 ± 0.010 mg/kg, respectively).

Chromatography

Arsenic speciation was carried out using ion chromatography with mass spectrometric detection (IC-ICP-MS). A gradient mobile phase including A: 20 mM ammonium carbonate and B: 200 mM ammonium carbonate, starting at 100% A, changing to 100% B, in a linear gradient over 15 min. was used. The ICP-MS monitored m/z+ 75 using He gas in collision cell mode. The resulting chromatogram was compared with that for authentic standards: arsenobetaine, DMA, MMA, tetramethyl arsonium and i-As. Arsenic present under each chromatographic peak was calibrated using a DMA concentration series. The arsenobetaine, MMA and tetramethyl arsonium concentrations in shoot and field-collected and commercial rice grain samples were below the LOD.

Total arsenic and cadmium analyses

Sample preparation for dehusked field-collected and commercial rice grain

Samples were freeze-dried until complete dryness, and then powdered. The powdered samples were weighed accurately to a weight of 0.1 g into 50 ml polypropylene centrifuge tubes. Then, 2 ml of concentrated nitric acid and 2 ml hydrogen peroxide were added into the 50 ml polypropylene centrifuge tubes and left to stand overnight. Then samples were microwave digested. The temperature was raised to 95°C in 5 min. and held for 10 min. and then to 135°C in 5 min. and held for 10 min. Finally, the digest was taken up to 180°C in 5 min. and maintained for 30 min. Samples were cooled to room temperature. An internal standard (30 µl of 10 mg/kg rhodium) was added to the digestate and then accurately diluted to 30 ml with deionized distilled water. Lastly samples were analysed for Cd concentration.

Sample preparation for soil

Soil samples were oven dried, and 1.00 mm sieved. Soil samples were weighed accurately to a weight of 0.1 g of soil into Teflon pressure vessels. Then, 2 ml of concentrated nitric acid and 2 ml hydrogen peroxide were added into the Teflon pressure vessels and left to stand overnight. Samples were microwave digested as described earlier. The internal standard was added to the digestate and then accurately diluted to 30ml with deionized distilled water. Finally, samples were analysed for t-As.

QA/QC procedures

Each batch of samples included 2–3 blanks and 2–3 replicate samples of rice CRM (NIST 1568b Rice flour) or 2–3 replicate samples of soil CRMs (ISE 921 and NCS ZC73001). The rice CRM has the Cd concentration certified (0.022 ± 0.001 mg/kg) and the soil CRMs have the t-As concentration certified (29.9 ± 1.78 and 18.0 ± 2.0 mg/kg for ISE 921 and NCS ZC73001, respectively).

Statistics

The As and Cd concentrations in the Iberian Peninsula samples did not follow a normal distribution. Therefore, the rank-based non-parametric test Kruskal–Wallace was used to carry out inference statistical analyses. These statistical analyses and plots were performed using the R Statistical Software (R Core Team, 2014). The limit of detection (LOD) was calculated as the mean of blank concentrations plus three times the standard deviation of the blank concentrations multiplied by the dilution factor. When samples were below the LOD a value of ½ LOD was assigned for statistical analyses of the data.

Results

Analytical recoveries, arsenic species analyses

The mean (±SE) recovery of rice CRM flour NIST-1568b As species was 95±3%, 90±2% and 95±4% for DMA, MMA and i-As, respectively, based on n = 8. The LOD for As speciation, calculated from DMA calibration and based on n = 5 was 0.002 mg/kg.

Analytical recoveries, total arsenic and cadmium analyses

The mean (±SE) recovery of rice CRM flour NIST-1568b Cd was 90 ± 10% based on n = 3. The mean (±SE) recovery of soil CRM ISE 921 and NCS ZC73001 t-As were 105 ± 1% and 110 ± 2% based on n = 3, respectively. The LOD for t-As and Cd analysis based on n = 5 was 0.009 mg/kg.

Soil

The t-As concentration in soil ranged from 2.3 to 17 mg/kg with a median concentration of 8.7 mg/kg across all regions. Soil from Portugal had the highest t-As concentration (median of 15 mg/ kg). Similar median values were found for Catalunya (11 mg/kg), Andalucía (10 mg/kg), Aragón (9.5 mg/kg), Navarra (8.8 mg/kg) and Valencia (8.2 mg/kg), while lower median t-As concentrations were found for Murcia (5.4 mg/kg) and Extremadura (4.2 mg/kg); P = 0.005.

Shoots

The i-As was predominant in rice shoots and DMA only represented a small percentage of the sum of As species (∑As). The i-As concentration ranged from 0.257 to 17.1 with a median concentration of 2.7 mg/kg for all shoot samples. It was found that the highest median i-As concentrations were for Extremadura (11.0 mg/kg), Portugal (9.8 mg/kg) and Catalunya (8.5 mg/kg), whereas lower median i-As concentrations were for Valencia (2.9 mg/kg), Andalucía (2.5 mg/kg), Aragón (1.8 mg/kg), Navarra (1.5 mg/kg) and Murcia (1.4 mg/kg); p = 0.026. A positive correlation factor was found between soil t-As concentration and shoots i-As concentration (R = 0.15) (**Figure 2**). The DMA concentration ranged from 0.002 to 0.162 with a median concentration of 0.006 mg/kg for all shoot samples. The median DMA concentrations in shoots from Portugal (0.042mg/kg) and Extremadura (0.031 mg/kg) were higher than that from Aragón (0.011 mg/kg), Catalunya (0.008 mg/kg), Andalucía (0.005 mg/kg), Navarra (0.004 mg/kg), Valencia (0.004 mg/kg) and Murcia (0.003 mg/kg); P = 0.014.

Dehusked field-collected rice grain

The predominant As species in field-collected rice grain was i-As. The i-As percentage, and i-As and DMA concentrations had the following median and range across all the field rice grain samples: 85, 41–97%, 0.088, 0.052–0.161mg/kg and 0.017, 0.003– 0.073 mg/kg, respectively. The relationship between soil t-As concentration and field-collected rice grain i-As concentration tended to describe a hyperbolic pattern, approaching a maximum of approximately over 0.100 mg/kg (**Figure 2**). A positive correlation was found between field-collected ∑As and commercial rice grain ∑As concentration (R = 0.98), and between field-collected i-As and commercial rice grain i-As concentration (R = 0.76) (**Figure 3**). The i-As content in the field-collected rice grain samples used to evaluate the relationship with commercial rice had the following median and range concentration according to region: 0.100, 0.061–0.130 mg/kg (Andalucía), 0.120, 0.074–0.150 mg/kg (Catalunya), 0.075, 0.063–0.161 mg/kg (Murcia) and 0.093, 0.063–0.097 mg/kg (Valencia) (**Figures 2** and **3**).

Commercial rice

Brown rice had the highest i-As concentration, $P < 0.001$; and polished rice had the lowest ∑As concentration; $P = 0.001$ (**Table 1**). The median and range of i-As concentration for the entire polished rice dataset was 0.071 and 0.027–0.175 mg/kg, respectively. It was found that the highest median i-As concentration was for Extremadura/Portugal (0.087 mg/kg), whereas the lowest median i-As contents were for Andalucía (0.054 mg/kg), Valencia (0.063 mg/kg) and Murcia (0.057 mg/kg); $P < 0.001$. The percentage of t-As represented by i-As in polished rice had a median of 57% and varied from 14% to 95%. Polished rice from Murcia had the highest median i-As percentage (87%), while similar values were found for Valencia, Catalunya, Aragón/Navarra and Andalucía (ranging from 51% to 62%), and that for Extremadura/Portugal was much lower (41%); $P < 0.001$. The DMA concentration in polished rice had a median concentration of 0.055mg/kg and ranged from 0.003 to 0.291mg/kg across all regions. It was found that the highest median DMA concentration was for Extremadura/Portugal (0.139 mg/kg), while the lowest median DMA content was for Murcia (0.009mg/kg); $P<0.001$ (**Table 1** and **Figure 4**).

The Cd concentration was consistently low for all the rice grain samples and was close to the LOD (**Table 1**).

The regression analysis of i-As against ∑As concentration in the entire dataset of polished rice had a slope of 0.186 and R2 equal to 0.38. The regression analysis of i-As against ∑As concentration between regions showed that the slopes for Murcia, Catalunya, and Aragón/Navarra, ranging from 0.445 to 0.969, were higher than that for Valencia, Andalucía and Extremadura/Portugal ranging from 0.091 to 0.232. The slope and R2 of DMA against ∑As concentration for all polished rice was 0.799 and 0.92, respectively. Analysis of DMA against ∑As concentration between regions established that Extremadura/Portugal, Andalucía and Valencia had similar slopes (0.896, 0.787 and 0.765) and higher than that for the other regions ranging from 0.150 to 0.539 (**Table 2**). A negative correlation factor was found in polished rice between ∑As concentration and i-As percentage (R = 0.73) (**Figure 4**).

Discussion

The Iberian Peninsula paddy fields soil As has a predominantly geogenic or mining-derived origin (Ramos-Miras et al., 2014). The paddy field soils analysed here had a low to moderate t-As concentration according to previous study (Khan, Stroud, Zhu, McGrath, & Zhao, 2010).

Paddy soil in Portugal had 3-fold higher median As content than Murcia and Extremadura, where soil t-As was the lowest. Williams et al. (2011) monitored As soil bioavailability to rice by analysing soil porewater dynamics and applying the dynamic sampling technique diffusive gradients in thin films (DGT). They found that paddy soils, even at baseline t-As concentration had As elevated grain due to large labile As reservoirs that resupplied flux from the soil into the porewater (Williams et al., 2011). Khan et al. (2010) also evaluated As bioavailability to rice and reported higher As bioavailability in paddy soils irrigated with contaminated water, from which As is likely to bear Fe oxides and absorb to soil minerals, than paddy soil with geogenic or miningderived As, from which As may be largely present in more insoluble and non-labile forms (Khan et al., 2010).

In our Iberian study here i-As in shoots increased with soil t-As, demonstrating a positive correlation. Yet, shoots from Extremadura had high i-As concentration, while low soil t-As content, suggesting that a greater proportion of soil t-As from Extremadura paddy soil was mobilized to be bioavailable for plant uptake compared to the soils from the other regions. As bioavailability strongly depends on environmental factors (Zhao, Ma, Meharg, & McGrath, 2009), suggesting that in Extremadura soil As is likely to be in more labile forms, bearing Fe oxides and absorb to soil minerals (Khan et al., 2010). A high proportion of soil t-As was also transferred to shoots from Catalunya and Portugal soils compared to those from Murcia, Valencia, Aragón, and Navarra. In these latter regions, soil As is expected to be present in more insoluble and less labile forms, and probably more influenced by the DOC dynamics as previous studies have suggested (Khan et al., 2010; Williams et al., 2011). The main mechanisms that affect i-As bioavailability in the Iberian Peninsula paddy fields deserve further investigation.

The As speciation in shoots here varied geographically and more than 95% of shoot As was in the i-As form, in keeping with other studies (Abedin, Cresser, Meharg, Feldmann, & Cotter-Howells, 2002; Norton et al., 2010). This is understandable since i-As is predominant in porewater (Khan et al., 2010; Williams et al., 2011) and arsenite, which is the dominant species of As in reducing environment, is readily assimilated by rice plant via silicic acid pathway through aquaporin channels (Abedin, Feldmann, & Meharg, 2002; Ma et al., 2008; Zhao et al., 2009). The As levels in rice shoots from Andalucía have been reported previously: median of 2.6 and 1.2mg/kg in rice shoots from Doñana and Cádiz, respectively (Williams et al., 2007). Similar values were found in shoots from Valencia, Andalucía, Aragón, Navarra and Murcia. However, an increase of up to 7–9-fold in the i-As content was found in shoot samples from Extremadura, Portugal and Catalunya compared to that previously reported in Cádiz (Williams et al., 2007).

In the present study i-As and DMA dominated As speciation in field rice grain, in agreement with earlier studies (Meharg et al., 2009; Williams et al., 2005; Zhao, Zhu, & Meharg, 2013). Yet, DMA showed more efficient above ground translocation than i-As, which may be due to its poor -SH coordination as suggested by previous studies (Norton et al., 2010; Raab, Ferreira, Meharg, & Feldmann, 2007). DMA concentration in both shoots and grain were in the same range, suggesting that DMA was unloaded at a similar rate into these two compartments. In contrast, i-As in rice grain was two orders of magnitude lower than in shoots, suggesting a much less efficient grain unloading of i-As. The relationship between soil t-As and field-collected rice grain i-As suggested a hyperbolic trend, describing a moderation grain i-As concentration at high soil t-As. Similar trend has previously been reported between shoots and grain As concentration due to a decrease translocation efficiency alongside increasing shoot As accumulation (Lu et al., 2009; Norton et al., 2010; Williams et al., 2007).

Graphical analysis of combined i-As and ∑As concentrations in rice grain from the field and the market basket surveys were in agreement, linking As distribution from field-collected to commercial rice grain samples. The As concentration in commercial rice from the main rice-growing regions of the Iberian Peninsula was strongly influenced by the type of rice and the geographical origin. Commercial brown rice type had twice i-As and ∑As concentration than polished rice. This corroborates earlier studies that reported higher As concentration in brown rice, where As is preferentially localized in the bran, than in polished rice, where As is generally dispersed throughout the grain (Meharg, Lombi, et al., 2008; Sun et al., 2008). An earlier survey including polished and brown rice from Spain found a mean i-As concentration of 0.097mg/kg (n = 11) and of 0.154 mg/kg (n = 11), respectively (Torres-Escribano, Leal, Vélez, & Montoro, 2008), which compare well to the values reported here. Geographical As speciation variation has been reported previously and environmental conditions seems to play a major role compared to genetic factors (Zhao et al., 2013). Meharg et al. (2009) analyzed an extensive dataset including 901 polished rice samples from 10 countries and reported significant geographical variation in t-As and i-As concentration. They found similar median i-As concentrations for polished rice from China, Italy and U.S. (0.120, 0.120 and 0.100 mg/kg, respectively), whereas lower median i-As concentration was for India and Bangladesh (0.030 and 0.070 mg/kg) (Meharg et al., 2009). Geographical variation in i-As content of rice has also been reported within a country. Sommella et al. (2013) analysed rice from 4 rice-growing regions in Italy. For Lombardia, Piemonte and Emilia similar i-As concentration was found (mean of ~0.100 mg/kg), while Calabria, located in the south of Italy, had lower i-As content (mean of 0.060 mg/ kg) (Sommella et al., 2013). Prior studies have reported t-As and i-As concentration in rice from Spain (Meharg et al., 2009; Williams et al., 2005). However, only a few studies with a limited number of samples have reported i-As concentration in polished rice according to the rice-growing region of the Iberian Peninsula. The mean i-As concentration has been previously reported for polished rice from Valencia (0.075 mg/kg), Catalunya (0.101 mg/kg) and Andalucía (0.101 mg/kg), which are within the range reported in this study (Torres-Escribano et al., 2008). The mean i-As content of 0.180 mg/kg has been reported for Portuguese polished, which is a higher value than that found here (Tattibayeva et al., 2015). A significant regional i-As concentration variation was found with the lowest concentration in Andalucía, Murcia and Valencia, while the highest was in Extremadura/Portugal. This is consistent with the findings of the field study here. Indeed, Portuguese soil had the highest t-As concentration, and although soil from Extremadura had low t-As, the i-As content in shoots from Extremadura and Portugal was much higher than that for Andalucía, Murcia, and Valencia, where a less efficient i-As transfer from soil to shoot and grain have been suggested.

A wide variation in the relative percentage of i-As and DMA has been reported previously. Williams et al. (2005) compared As speciation in commercial polished rice produced in Bangladesh, India, Europe and the U.S. They found high percentages of i-As (~80%) in Bangladeshi and Indian rice. In comparison, European and U.S. rice had a lower percentage of i-As with a mean of 64% and 42%, respectively, with the corresponding high percentage of DMA (Williams et al., 2005). The mean i-As percentage of 62% have been reported for Spanish rice (Torres-Escribano et al., 2008). Compared to this value, in the study here, polished rice from Murcia had 1.4-fold higher median percentage of i-As concentration, similar to that for Bangladeshi and Indian rice. In contrast, Extremadura/Portugal had 1.5-fold lower median percentage of i-As content, agreeing with that reported for U.S. rice (Williams et al., 2005).

The percentage of i-As decreased with ∑As concentration in all regions describing a clear negative correlation, in keeping with earlier studies (Meharg et al., 2009). This may suggest that a physiological switch occurs enhancing methylated As species plant uptake from the soil micro-flora when reaching i-As critical levels, which concurs with recent evidence suggesting the lack of in planta methylation ability in rice (Lomax et al., 2012; Zhao et al., 2013).

Earlier surveys have carried out regression analyses of t-As against i-As and DMA concentration in rice. Meharg et al. (2009) reported that the slope for India and Bangladesh (0.796 and 0.719) were similar, while Chinese and Italian were similar (0.599 and 0.506), and U.S. and Spanish rice much lower (0.275 and 0.193) (Meharg et al., 2009). Zhao et al. (2013) reported a strong linear relationship between t-As and i-As for rice from Asia with a slope of 0.78. In contrast, they reported that the U.S. rice showed a hyperbolic pattern in the relationship, approaching a maximum of approximately 0.15 mg/kg. European rice (Italy, Spain and France) samples appear to be more variable and i-As/t-As relationship exhibits a pattern that was intermediate between those of Asian and U.S. rice (Zhao et al., 2013). The study here shows that regression analyses of ∑As against i-As for polished rice from the Iberian Peninsula had a low slope and was described with an intermediate pattern between the linear and the hyperbolic trend, which agrees with earlier studies (Meharg et al., 2009; Zhao et al., 2013). However, some differences were identified when carrying out regression analysis of ∑As against i-As between regions. Low slopes and similar to that previously reported for Spain and U. S. were found for polished rice from Andalucía, Extremadura/Portugal and Valencia. Aragón/Navarra had a similar slope to that described earlier for Chinese and Italian, whereas Catalunya had a higher slope similar to that reported for India and Bangladesh. Murcia had a much higher regression slope with a high R2, meaning that most of the t-As was i-As. Regression analyses of ∑As and DMA described a strong linear regression with a high slope for polished rice from the Iberian Peninsula and similar to that previously reported for the U.S. (0.817) (Meharg et al., 2009). This trend was consistent across all regions but Catalunya and Murcia, due to DMA data showed wide variability.

Most of the commercial and field rice grain samples had a Cd concentration below the LOD. This corroborates that rice in the Iberian Peninsula is cultivated under flooded conditions, where the Cd bioavailability is low (Arao, Kawasaki, Baba, Mori, & Matsumoto, 2009; Xu et al., 2008). Therefore, rice Cd concentration did not raise any conflict in the Iberian Peninsula growing regions evaluated in the present study here.

The elevated i-As in rice is of concern since 26% of the all our Iberian dataset, which include 144 samples of commercial polished, parboiled and brown rice, and 14% out of 113 commercial polished rice samples, would be illegal for the production of food for infants and young children when the EC regulation is enforced in 2016 (EC, 2015). In addition, there is still much debate that the UN WHO guidelines and EC standards are set too high to protect people's health (Schmidt, 2015). Thus, it has been alternatively suggested that the maximum value be 0.100 mg/kg i-As for all types of rice, and 0.05 mg/kg i-As for products targeted at young children and babies (Schmidt, 2015). The 0.05 mg/kg i-As is a lower value than that obtained for 80% of the polished rice commercial samples included in this study or for even higher percentage when the whole dataset is included in the calculations.

Conclusions

In this study it is shown that i-As and DMA are the main arsenic species in shoots and rice, both field-collected and commercial. Paddy field soils had low to moderate t-As concentrations, which was positively correlated with i-As in shoots and described a hyperbolic trend with i-As in field-collected rice grain. The Extremadura paddy soil suggested higher bioavailability for rice plant uptake. However, further studies regarding i-As paddy soil bioavailability in the Iberian Peninsula are required. The As speciation in commercial rice from the Iberian Peninsula compiled here is the largest dataset reported as yet and highlights that 26% of the rice samples would be illegal for the production of food for infants and young children due to its elevated i-As concentration. On searching for rice with lower i-As concentrations, Andalucía, Murcia and Valencia showed the lowest levels.

Acknowledgement

This work was funded by a Marie Curie Intra-European Fellowship within the 7th European Community Framework Programme (PIEF-GA-2013-622096).

References

Abedin, M. J., Cresser, M. S., Meharg, A. A., Feldmann, J., & Cotter-Howells, J. (2002). Arsenic accumulation and metabolism in rice (Oryza sativa L.). Environmental Science and Technology, 36(5), 962–968. http://dx.doi.org/10.1021/es0101678.

Abedin, M. J., Feldmann, J., & Meharg, A. A. (2002). Uptake kinetics of arsenic species in rice plants. Plant Physiology, 128, 1120–1128. http://dx.doi.org/10.1104/ pp.010733.0.01.

Arao, T., Kawasaki, A., Baba, K., Mori, S., & Matsumoto, S. (2009). Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in Japanese rice. Environmental Science and Technology, 43(24), 9361–9367. http://dx.doi.org/10.1021/es9022738.

Banerjee, M., Banerjee, N., Bhattacharjee, P., Mondal, D., Lythgoe, P. R., Martínez, et al. (2013). High arsenic in rice is associated with elevated genotoxic effects in humans. Scientific Reports, 3, 2195. http://dx.doi.org/10.1038/srep02195.

CAC (Codex Alimentarious Commission). Report of the eighth session of the codex commitee on contaminants in food. (2014) <ftp.fao.org/codex/Reports/Reports_ 2014/REP14_CFe.pdf> Accessed 02.11.15.

Carbonell-Barrachina, A. A., Wu, X., Ramírez-Gandolfo, A., Norton, G. J., Burló, F., Deacon, C., & Meharg, A. A. (2012). Inorganic arsenic contents in rice-based infant foods from Spain, UK, China and USA. Environmental Pollution, 163, 77–83. http://dx.doi.org/10.1016/j.envpol.2011.12.036.

Cascio, C., Raab, A., Jenkins, R. O., Feldmann, J., Meharg, A. A., & Haris, P. I. (2011). The impact of a rice based diet on urinary arsenic. Journal of Environmental Monitoring, 13(2), 257–265. http://dx.doi.org/10.1039/c0em00482k.

EC. Commission Regulation 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs. (2015). <eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:JOL_2015_161_R_0006> Accessed 02.11.15.

EFSA (European Food Safety Authority). Scientific opinion on arsenic in food. (2009). EFSA panel on contaminants in food chain (CONTAM), Vol. 7. <http://www.efsa. europa.eu/sites/default/files/scientific_output/files/main_documents/1351. pdf> Accessed 02.11.15.

Ferrero, A., & Nguyen, N. (2004). Rice is life. The sustainable development of rice-based production systems in Europe. In: Proceeding of the FAO Rice Conference. FAO Rice Conference. <www.fao.org/docrep/008/y5682e/y5682e0g.htm> Accessed 02.11.15.

IARC. (International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans. (2004). Some drinking-water disinfectants and Contaminants, including Arsenic, Vol. 84. <monographs.iarc.fr/ENG/ Monographs/vol84/mono84.pdf> Accessed 02.11.15.

Khan, M. A., Stroud, J. L., Zhu, Y. G., McGrath, S. P., & Zhao, F. J. (2010). Arsenic bioavailability to rice is elevated in Bangladeshi paddy soils. Environmental Science and Technology, 44(22), 8515–8521. http://dx.doi.org/10.1021/ es101952f.

Lomax, C., Liu, W., Wu, L., Xue, K., Xiong, J., Zhou, J., & Steve, P. (2012). Methylated arsenic species in plants originate from soil microorganisms. New Phytologist, 193, 665–672. http://dx.doi.org/10.1111/j.1469-8137.2011.03956.x.

Lu, Y., Adomako, E. E., Solaiman, A. R. M., Islam, M. R., Deacon, C., Williams, P. N., et al. (2009). Baseline soil variation is a major factor in arsenic accumulation in Bengal delta paddy rice. Environmental Science and Technology, 43(6), 1724–1729. http://dx.doi.org/10.1021/es802794w.

Ma, J. F., Yamaji, N., Mitani, N., Xu, X.-Y., Su, Y.-H., McGrath, S. P., & Zhao, F. J. (2008). Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proceedings of the National Academy of Sciences of the United States of America, 105(29), 9931–9935. http://dx.doi.org/10.1073/pnas.0802361105.

Meharg, A. A., Deacon, C., Campbell, R. C. J., Carey, A.-M., Williams, P. N., Feldmann, J., & Raab, A. (2008). Inorganic arsenic levels in rice milk exceed EU and US drinking water standards. Journal of Environmental Monitoring, 10(4), 428–431. http://dx.doi.org/10.1039/B800981C.

Meharg, A. A., Lombi, E., Williams, P. N., Scheckel, K. G., Feldmann, J., Raab, A., et al. (2008). Speciation and localization of arsenic in white and brown rice grains. Environmental Science & Technology, 42(4), 1051–1057. http://dx.doi.org/ 10.1021/es702212p.

Meharg, A. A., Williams, P. N., Adomako, E., Lawgali, Y. Y., Deacon, C., Villada, A., et al. (2009). Geographical variation in total and inorganic arsenic content of polished (white) rice. Environmental Science & Technology, 43(5), 1612–1617. http://dx.doi.org/10.1021/es802612a.

Meharg, A. A., & Zhao, F. J. (2012). Arsenic & rice. Berlin: Springer-Verlag. Melkonian, S., Argos, M., Hall, M. N., Chen, Y., Parvez, F., Pierce, B., et al. (2013). Urinary and dietary analysis of 18,470 Bangladeshis reveal a correlation of rice consumption with arsenic exposure and toxicity. PLoS ONE, 8(11), 1–10. http://dx.doi.org/10.1371/journal.pone.0080691. Mondal, D., & Polya, D. A. (2008). Rice is a major exposure route for arsenic in

Chakdaha block, Nadia district, West Bengal, India: A probabilistic risk assessment. Applied Geochemistry, 23(11), 2986–2997. http://dx.doi.org/ 10.1016/j.apgeochem.2008.06.025.

Norton, G. J., Duan, G., Dasgupta, T., Islam, M. R., Lei, M., Zhu, Y. G., et al. (2009). Environmental and genetic control of arsenic accumulation and speciation in rice grain: Comparing a range of common cultivars grown in contaminated sites across Bangladesh, China, and India. Environmental Science and Technology, 43 (21), 8381–8386. http://dx.doi.org/10.1021/es901844q.

Norton, G. J., Islam, M. R., Duan, G., Lei, M., Zhu, Y. G., Deacon, C. M., et al. (2010). Arsenic shoot-grain relationships in field grown rice cultivars. Environmental Science and Technology, 44(4), 1471–1477. http://dx.doi.org/10.1021/ es902992d.

R Core Team (2014). R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing. <www.r-project.org/>

Raab, A., Ferreira, K., Meharg, A. A., & Feldmann, J. (2007). Can arsenic-phytochelatin complex formation be used as an indicator for toxicity in Helianthus annuus? Journal of Experimental Botany, 58(6), 1333–1338. http://dx.doi.org/10.1093/jxb/ erl300.

Ramos-Miras, J. J., Díaz-Férnandez, P., SanJosé-Wery, A., Rodríguez-Martin, J. A., Roca, N., Bech, J., et al (2014). Influence of parent material and soil use on arsenic forms in soils: A case study in the Amblés Valley (Castilla-León, Spain). Journal of Geochemical Exploration, 147, 260–267. http://dx.doi.org/10.1016/j. gexplo.2014.09.003.

Sainz, H., Sanz, A., Aguado, J., & Martín-Cerdeño, V. J. (2014). Alimentación en España. Produccion Industria Distribución Consumo. <www.mercasa-ediciones. es/alimentacion_2014/AE2014/index.html#4/z> Accessed 02.11.15.

Schmidt, C. W. (2015). In search of ʹʹjust rightʺ The Challenge of Regulating Arsenic in Rice. 123(1), 16–19. doi:http://dx.doi.org/10.1289/ehp.123-A16.

Signes-Pastor, A. J., Carey, M., & Meharg, A. A. (2016). Inorganic arsenic in rice-based products for infants and young children. Food Chemistry, 191, 128–134. http:// dx.doi.org/10.1016/j.foodchem.2014.11.078.

Sommella, A., Deacon, C., Norton, G. J., Pigna, M., Violante, A., & Meharg, A. A. (2013). Total arsenic, inorganic arsenic, and other elements concentrations in Italian rice grain varies with origin and type. Environmental Pollution, 181, 38–43. http://dx.doi.org/10.1016/j.envpol.2013.05.045.

Sun, G., Williams, P. N., Carey, A. M., Zhu, Y. G., Deacon, C., Raab, A. A., et al. (2008). Inorganic arsenic in rice bran and its products are an order of magnitude higher than in bulk grain. Environmental Science and Technology, 42(19), 7542–7546. http://dx.doi.org/10.1021/es801238p.

Tattibayeva, D., Nebot, C., Miranda, J. M., Cepeda, A., Mateyev, E., Erkebaev, M., & Franco, C. M. (2015). A study on toxic and essential elements in rice from the Republic of Kazakhstan: Comparing the level of contamination in rice from the European Community. Environmental Geochemistry and Health. http://dx.doi. org/10.1007/s10653-015-9687-y.

Torres-Escribano, S., Leal, M., Vélez, D., & Montoro, R. (2008). Total and inorganic arsenic concentrations in rice sold in Spain, effect of cooking, and risk assessments. Environmental Science and Technology, 42(10), 3867–3872.

Williams, P. N., Price, A. H., Raab, A., Hossain, S. A., Feldmann, J., & Meharg, A. A. (2005). Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environmental Science and Technology, 39(15), 5531–5540. http://dx.doi.org/10.1021/es0502324.

Williams, P. N., Raab, A., Feldmann, J., & Meharg, A. A. (2007). Market basket survey shows elevated levels of As in South Central U.S. processed rice compared to California:

Consequences for human dietary exposure. Environmental Science & Technology, 41(7), 2178–2183.

Williams, P. N., Villada, A., Deacon, C., Raab, A., Figuerola, J., Green, A. J., et al. (2007). Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. Environmental Science and Technology, 41 (19), 6854–6859. http://dx.doi.org/10.1021/es070627i.

Williams, P. N., Zhang, H., Davison, W., Meharg, A. A., Hossain, M., Norton, G. J., et al. (2011). Organic matter-solid phase interactions are critical for predicting arsenic release and plant uptake in Bangladesh paddy soils. Environmental Science and Technology, 45(14), 6080–6087. http://dx.doi.org/10.1021/ es2003765.

Xu, X. Y., McGrath, S. P., Meharg, A. A., & Zhao, F. J. (2008). Growing rice aerobically markedly decreases arsenic accumulation. Environmental Science and Technology, 42(15), 5574–5579. http://dx.doi.org/10.1021/es800324u.

Zhao, F. J., Ma, J. F., Meharg, A. A., & McGrath, S. P. (2009). Arsenic uptake and metabolism in plants. The New Phytologist, 181(4), 777–794. http://dx.doi.org/ 10.1111/j.1469-8137.2008.02716.x.

Zhao, F. J., Zhu, Y. G., & Meharg, A. A. (2013). Methylated arsenic species in rice: Geographical variation, origin, and uptake mechanisms. Environmental Science & Technology, 47(9), 3957–3966. http://dx.doi.org/10.1021/es304295n.

Figure 1. Iberian Peninsula map with the location of the paddy field regions sampled.

Figure 2. Relationship between t-As concentration (mg/kg dry weight) in soil and i-As, DMA and ∑As concentration (mg/kg dry weight) in rice tissues (shoots and field-collected rice grains) according to paddy field region.

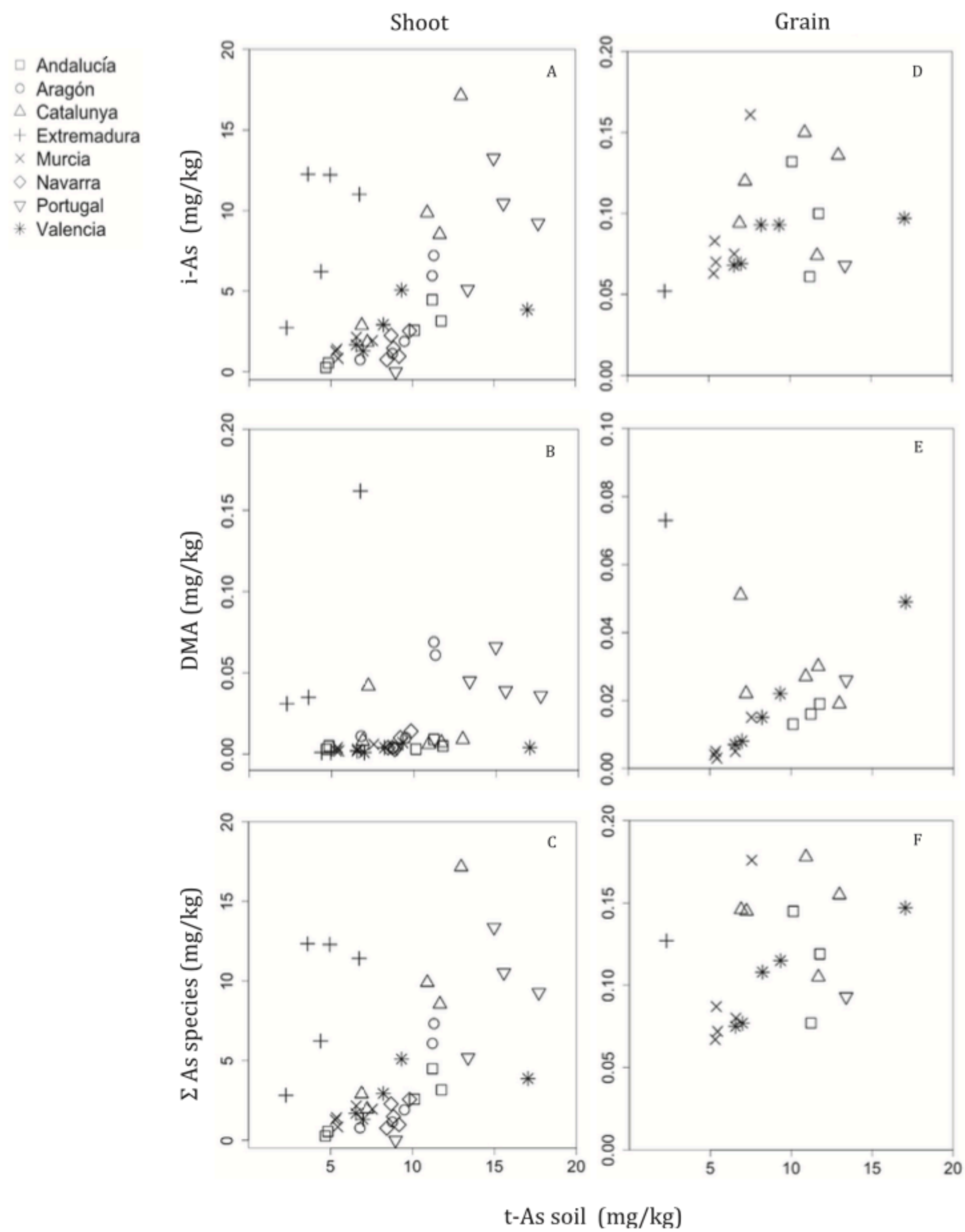

Figure 3. Relationship between polished commercial rice and field-collected rice grain i-As and ∑As concentration (mg/kg dry weight). Each value shows the median and standard error according to paddy field region.

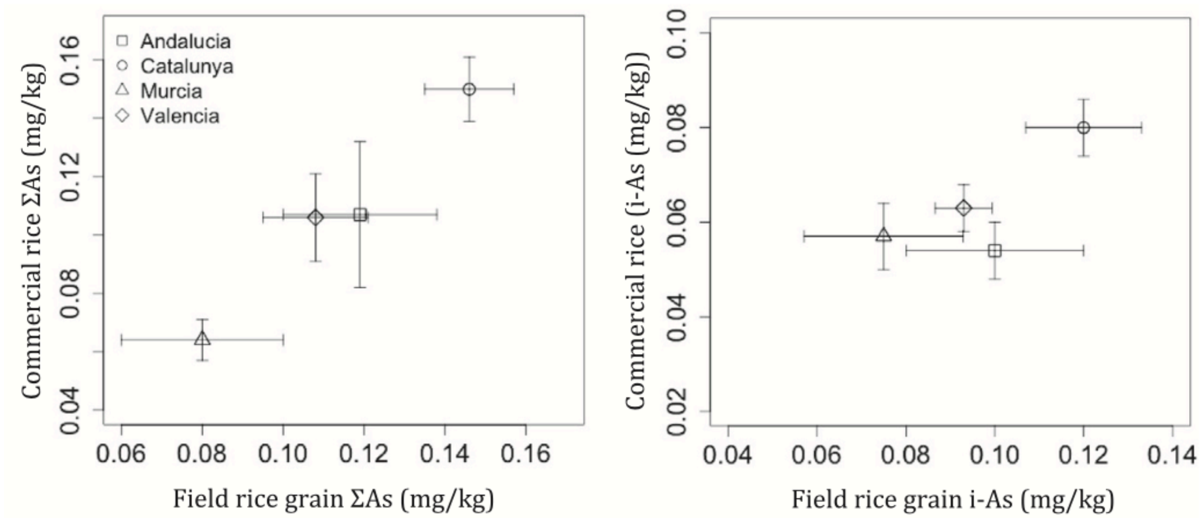

Table 1. Inorganic arsenic (i-As), DMA, ∑As and Cd concentration (mg/kg dry weight) in commercial rice according to type of rice and region.

Origin	Type of rice	n	i-As (mg/kg)	DMA	RAs species	Cd (mg/kg)
				(mg/kg)		
					(mg/kg)	
Portugal & Spain	Brown	20	0.157a	0.084	0.302a	0.004a
			(0.053–0.247)A	(0.006–0.366)	(0.083–0.619)	(0.003–0.035)
	Parboiled	11	0.083b	0.079	0.201ab	0.004a
			(0.022–0.170)	(0.015–0.237)	(0.039–0.407)	(0.003–0.027)
	Polished	113	0.071b	0.055	0.143b	0.003b
			(0.027–0.175)	(0.003–0.333)	(0.037–0.433)	(0.003–0.049)
	P-value		<0.001	0.461	0.001	<0.001
Type of	Origin					
rice						
Polished	Andalucía	20	0.054b	0.055ac	0.107b	0.003
			(0.027–0.130)	(0.019–0.286)	(0.046–0.397)	(0.003–0.009)
	Aragón/	10	0.067ab	0.057ac	0.128ab	0.003
	Navarrra		(0.044–0.154)	(0.029–0.189)	(0.073–0.343)	(0.003–0.003)
	Catalunya	14	0.080ab	0.057ac	0.150ab	0.003
			(0.046–0147)	(0.034–0.237)	(0.121–0.283)	(0.003–0.037)
	Extremadura/	23	0.087a	0.139a	0.224a	0.003
	Portugal		(0.066–0.138)	(0.045–0.291)	(0.118–0.421)	(0.003–0.029)
	Murcia	11	0.057b	0.009b	0.064b	0.004
			(0.039–0.121)	(0.003–0.012)	(0.043–0.133)	(0.003–0.005)
	Valencia	29	0.063b	0.044bc	0.106b	0.003
			(0.028–0.175)	(0.006–0.259)	(0.037–0.362)	(0.003–0.049)
	P-value		<0.001	<0.001	<0.001	0.818

Figure 4. Relationship between ∑As and i-As, DMA concentration (mg/kg dry weight) and i-As (%), and i-As, DMA and ∑As concentration (mg/kg dry weight) in commercial polished rice according to paddy field region.

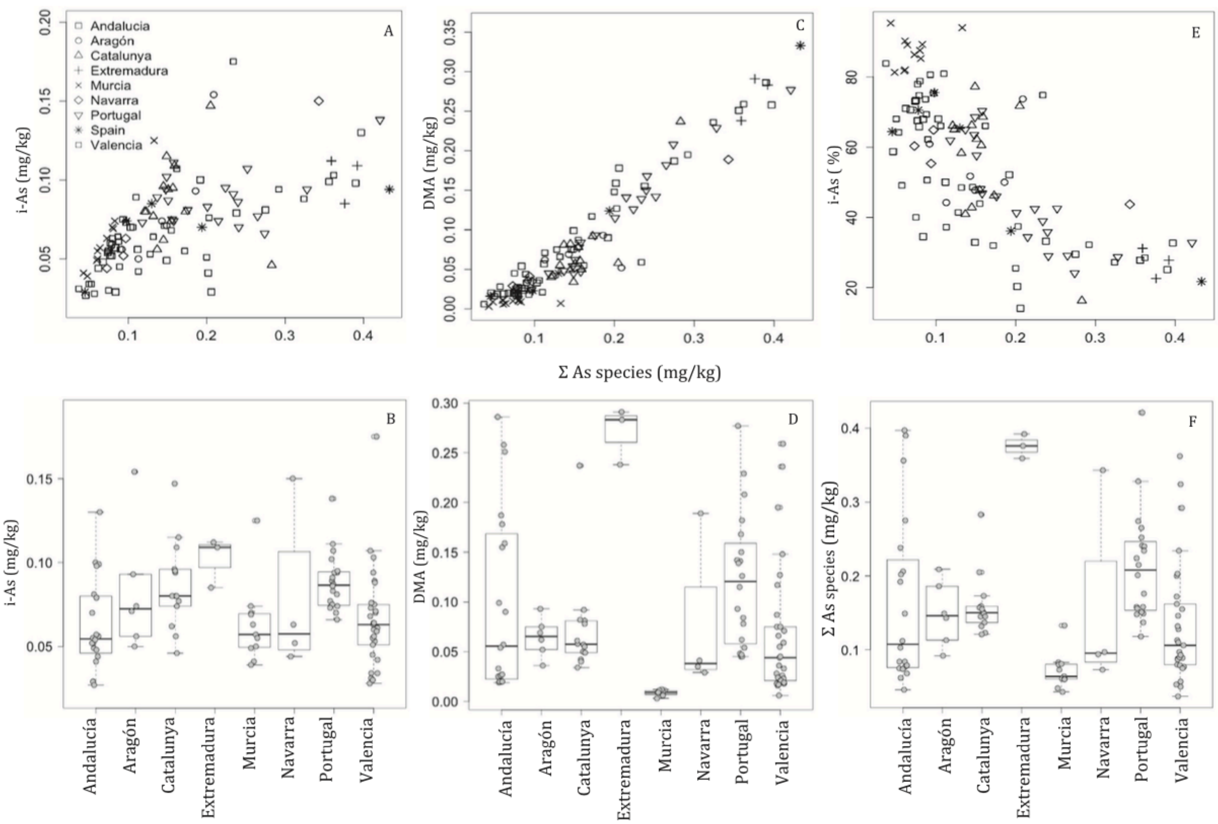

Table 2. Linear regression analysis of ∑As versus As_i and DMA (the intercept is ''*a*'' and the slope is ''*b*'').

Type of rice	Origin	n	i-As			DMA		
			a	b	R2	a	b	R2
Polished	Iberian Peninsula	113	0.044	0.186	0.38	-0.043	0.799	0.92
	Andalucía	20	0.031	0.188	0.61	-0.029	0.787	0.96
	Aragón/Navarrra	10	0.013	0.445	0.8	-0.12	0.539	0.85
	Catalunya	13	-0.022	0.748	0.46	0.021	0.254	0.1
	Extremadura/Portugal	23	0.068	0.091	0.22	-0.068	0.896	0.96
	Murcia	11	-0.006	0.969	0.98	0.007	0.15	0.02
	Valencia	29	0.035	0.232	0.41	-0.035	0.765	0.88