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Arsenic and selenium levels in rice fields from south-west of Spain: influence of the years of monoculture

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ABSTRACT

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There is a lack of information regarding the arsenic (As) and selenium (Se) concentrations in Spanish rice (*Oryza sativa* L.) fields and how soil conditions affect such concentration, especially those derived from the typical monoculture practiced in the studied area. To clarify these aspects, 76 soil samples and 95 grain samples were collected from 19 rice fields along the Vegas Altas area, the most important rice growing area of south-west of Spain. The results suggested a significant increase in the soil total As and Se concentrations as the number of monoculture years increased. While As concentration reached toxic levels in 12 out of the 19 locations, Se concentration in all the analysed fields could be considered as deficient. An increase of the As and Se concentration in soil produced a subsequent increase of the concentration of both elements in the rice grain. Therefore, it might be extremely important to control both levels. It would be necessary to establish different actions, including rotations with other crops, in order to remediate As accumulation and to increase Se intake.

Keywords: paddy field; As-Se interaction; biofortification; crop rotation; soil contamination

Arsenic (As) is a class-one carcinogen occurring ubiquitously in the environment from both anthropogenic and geogenic sources. Selenium (Se) is an essential micronutrient for humans and animals, the deficiency of which can cause numerous health disorders as well as increased risk of cancers (Rayman 2012, Sun et al. 2014). Although chemically similar, As and Se exert antagonistic effects in human, animals (Zeng et al. 2005) and in some plants (Feng et al. 2009). It has been found that Se can be used to decrease the toxicity of As or other metalloids (Sun et al. 2014). The maximum daily intake for As should be lower than 0.3 mg/kg but the recommended dietary allowance (RDA) for Se is about 55–70 μg/kg (Elmadfa 2009, WHO 2010). Based on clinical trials, As intake should be reduced as much as possible because of its long-life, while a regular dose of 200 μg Se/day is recommended to reduce the incidence of certain cancers, cardiomyopathies and other diseases (Reid et al. 2008). According to Díaz-Alarcón et al. (1996), Spanish Se intake is about 33 μg/day and it should be increased to reach the recommended values.

Food consumption provides the principal route for As and Se intake for most of the population. Among cereals, rice (*Oryza sativa* L.) has a great importance in Mediterranean areas, commonly used for human food. Within Spain, the Vegas Altas region of Extremadura has the oldest and most important rice fields of Spain, with about 20 000 ha, with a grain production of about 140 000 t (MARM 2014). Total Se and As concentrations in rice grains range between 40 μg Se/kg (Ventura et al. 2007) and 67 μg Se/kg (Matos-Reyes et al. 2010) in Portugal and south-east of Spain, while the As concentrations found are about 105 μg As/kg (Matos-Reyes et al. 2010).

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The concentration of As and Se in plant-based food is directly related to their concentrations in soil where the plants were grown. Values of total As range between 600–1200 μg/kg (Menjivar et al. 2009) and total Se between 134–172 μg/kg (Poblaciones et al. 2014) in the South of Spain. These values are slightly higher than the established threshold of 10 000 μg As/kg (IARC 2004) but much lower than the lower threshold for providing crops with enough Se, set in 300 μg Se/kg (Hawkesford and Zhao 2007). However, these concentrations might be quite different in rice fields. The special soil characteristics in flooded conditions may affect their availabilities for plant uptake and its later accumulation. In such conditions, arsenite is the predominant form, which is more toxic, soluble and therefore more mobile than arsenate (Bogdan and Schenk 2008). Moreover, it could suppose a cumulative impact when flooded conditions are repeated for years. This fact could be a serious problem in Vegas Altas where rice monoculture under flooded conditions is the traditional management, and several fields account for more than 30 years of monoculture. Hence, the general aim of this study was to evaluate the effect of soil characteristics and number of monoculture years on As and Se concentrations in soil and rice grain.

MATERIAL AND METHODS

Soil and grain samples were collected at the end of the crop growth stage from 19 locations from Vegas Altas del Guadiana, Extremadura region (coordinates from 38°56'N to 39°8'N and 5°41'O to 6°3'O). This area presents Xerofluvents soils and Mediterranean climate. All the used locations were managed following the traditional practices in the area: conventional tillage of 25 cm depth, sowing date between March to May, sowing rate of 160 kg/ha and intermittent irrigation to keep flooded conditions using continuous water flows with a total amount of about 24 000 m^3/ha. Four groups were selected to include at least 4 fields for each group of monoculture years: (i) less than five years; (ii) ranging between 5 and 10 years; (iii) ranging between 10 and 20 years, and (iv) ranging between 20 and 30 years. From each location, four soil samples (0–20 cm) and five rice plants were randomly collected.

The soil samples were mixed and air-dried for 2 days and sieved to < 2 mm in order to carry out chemical soil determinations, such as soil pH (ratio 10 g soil:25 mL deionized H_2O), and As and Se concentration. A portion of each soil was finely ground (< 0.5 mm) using an agate ball mill (Retch PM 400 mill, Retsch, Haan, Germany) to determine the concentration of total and available As and Se in the soil. Total As and Se were determined as follows: samples (1 g) were digested with ultrapure concentrated nitric acid (2 mL) and 30% w/v hydrogen peroxide (2 mL), using a closed-vessel microwave digestion protocol (Mars X, CEM Corp, Matthews, USA), and diluted to 25 mL with ultra-pure water. A blank and a standard were included in each batch of 12 vessels for quality assurance. Mineral concentrations were determined by using an Inductively-Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent 7500ce, Agilent Technologies, Palo Alto, USA) operating in the hydrogen gas mode. Likewise, Se and As extracts were obtained by using KH_2PO_4 (0.016 mmol, pH 4.8) (ratio 10 g dry weight soil: 30 mL KH_2PO_4 w/v), and the concentration of extractable Se and As was determined by the ICP-MS as described above.

Regarding plant material, grains were separated from inflorescence, watered with deionized water, handy polished to remove the external husks, oven-dried at 60°C until constant weight and ground to fine powder (< 0.05 mm) using a mechanical grinder. One gram of rice grain was digested and analysed for total As and Se concentrations as described above for soil samples. A blank and a standard (in this case with tomato leaf material, NIST 1573a) were included in each batch of samples for quality assurance.

Data were subjected to ANOVA considering the group of monoculture years as a factor. When significant differences were found, means were compared using the Fisher's protected least significant difference (*LSD*) test at $P \leq 0.05$. Pearson correlation tests were performed between total and available As and Se in soil and total As and Se concentrations in the grain. All analyses were performed using Statistix v. 8.10 for Windows software (Analytical Software, Tallahassee, USA).

RESULTS AND DISCUSSION

The pH of the sites ranged from 4.7 to 7.8, with an average concentration of 6.2 ± 0.3, which are normal values in rice fields of this area. Total and extractable As concentration ranged from 2100 to

Table 1. Maximum and minimum levels of pH, total arsenic (As) and selenium (Se) and available As and Se in soil samples and in the polish grain depending on the number of years of monoculture (*n* – number of samples)

Parameter		Years of monoculture			
		< 5 (*n* = 5)	5–10 (*n* = 4)	10–20 (*n* = 5)	20–30 (*n* = 5)
Soil	pH	5.5–6.8	4.7–6.2	5.1–6.8	5.0–7.8
	As total (µg/kg)	2100–7800	4300–9182	2266–12 469	5600–18 812
	available As (µg/kg)	73–168	150–232	60–253	194–460
	Se total (µg/kg)	74–91	112–143	80–188	127–225
	available Se (µg/kg)	1.7–2.3	2.7–3.7	2.1–5.2	3.4–6.3
Grain	As concentration (µg/kg)	100–820	210–740	120–300	70–300
	Se concentration (µg/kg)	1.1–20.4	4.1–10.1	0.7–9.3	1.0–17.0

18 812 µg/kg and from 60 to 460 µg/kg, respectively, with average values of 203 ± 121 µg/kg and 7824 ± 1024 µg/kg, the first one for total As and the second for extractable As (Table 1). These As levels were quite higher than those found by Menjivar et al. (2009) in the southwest of Spain, with values ranging between 600 and 12 000 µg/kg. Given the fact that soils are considered as As-contaminated when they present concentrations higher than 10 000 µg/kg (IARC 2004), six locations from this study show hazardous levels of As in soil. Moreover, the correlation between total and extractable As was positive and significant, but only less than 3% of total As was extractable. On the other hand, total Se concentration in soil ranged between 74 and 225 µg/kg, with a mean value of 134 ± 11 µg/kg. These Se levels were quite lower than those found by Díaz-Alarcón et al. (1996) and Poblaciones et al. (2014) in non-flooded agricultural soils of Spain. According to Hawkesford and Zhao (2007) soil classification according to the concentration of total Se, Vegas Altas area could be considered as marginal. Regarding the extractable Se, its correlation with total Se was positive and strongly significant, but only about 2.5% of total Se was extractable, with an average concentration of 3.5 ± 1.2 µg/kg (Table 1). These values were quite lower than those found by Poblaciones et al. (2014) (6.2 µg Se/kg) in a non-flooded agricultural soil of Spain.

Despite the very low extractability of the soil As, the As concentrations in the polished grain ranged from 70 to 820 µg/kg, with a mean concentration of 300 µg/kg (Table 1). These values were considerably higher than the 100 µg/kg found in grain of white rice in Spain (Matos-Reyes et al. 2010) or 140–170 µg/kg in Japan (Sun et al. 2014). The usual total As concentration in the grain obtained from uncontaminated soils ranges from 80 and 200 µg/kg (Zavala and Duxbury 2008), while the upper level was allowed by WHO (2010) as non-toxic level. Consequently, in 12 out of the 19 studied locations, As represented a serious problem to be urgently dealt with, especially in three study fields where As concentration was 3-fold higher than the upper threshold (Table 1). A consumption of 150 g of this polished rice might suppose an intake of 123 µg of As, more than a third of the upper limit for the dietary intake of As in humans, i.e. 300 µg As/kg (WHO 2010).

Regarding Se, the extremely low plant-bioavailable Se in soil resulted in very low Se concentrations in grain (0.7–20.4 µg/kg). Higher values (67 µg/kg) were found by Matos-Reyes et al. (2010) in south-east Spain, or by Ventura et al. (2007) in Portugal (40 µg/kg) in white rice grain. Therefore, a consumption of 150 g of white rice might provide an intake ranging from 5.6 to 12.6 µg Se per day, with a mean value of 10 µg Se per day. All these values are extremely low to achieve the recommended 200 µg Se per day, amount that would help to decrease the incidence of certain cancers (Reid et al. 2008). Under such conditions, it could be very interesting to develop

Table 2. Two-way ANOVA for the measured parameters as affected by the years of monoculture

Soil parameters	*F*	Grain parameters	*F*
Df	17	*df*	17
pH	1.4	Fe concentration	3.4^{*}
Total arsenic	4.4^{*}	Zn concentration	4.4^{*}
Available arsenic	13.8^{***}	As concentration	2.0
Total selenium	10.7^{***}	Se concentration	0.4
Available selenium	7.7^{**}		

df – degree of freedom; *F* – *F*-value according to ANOVA indicating *P*-value ($^{*}P \le 0.05$; $^{**}P \le 0.01$; $^{***}P \le 0.001$)

Table 3. Total and available arsenic (As, μg/kg) and selenium (Se, μg/kg) in soil (mean ± standard error) as affected by the length of monoculture

Years of monoculture	As total	Available As	Se total	Available Se
< 5	4566 ± 1088^{b}	115 ± 19^{b}	84 ± 3^{c}	2.0 ± 0.1^{c}
5–10	6470 ± 1189^{b}	196 ± 19^{b}	128 ± 9^{b}	3.2 ± 0.3^{bc}
10–20	8015 ± 1947^{ab}	133 ± 36^{b}	136 ± 19^{b}	3.3 ± 0.5^{b}
20–30	$12\,244 \pm 2365^{a}$	369 ± 51^{a}	187 ± 18^{a}	4.8 ± 0.6^{a}
Average	7824 ± 1025	203 ± 29	134 ± 11	3.3 ± 0.3

Means in a column with different letters were significantly different ($P \leq 0.05$) according to the Fisher's protected *LSD* (least significant difference) test for the length of the monoculture period

an Se agronomic biofortification program in the rice fields of this area.

The number of years of monoculture practice affected significantly both total and available As and Se in soil (Table 2), such concentrations being higher as the years of monoculture increased (Table 3). In case of total As, differences were significant in up to 10 years of monoculture, while in the case of available As, up to 20 years were necessary to find significantly different values (Table 3). Presumably, the soil anaerobic conditions lead to a reductive dissolution of iron oxides/hydroxides and the consequent release of adsorbed arsenate. The arsenate is then mobilised as arsenite in the water present in the soil pores. Due to the yearly repetition of the anaerobic conditions, the As is accumulated in the soils subjected to this type of management (Xu et al. 2008). However, As concentration in grain was not significantly affected by the years of monoculture in the present study, which might be due to the dry period commonly practiced in the rice fields from this region. According to Zhao and McGrath (2009), traditional rice field areas such as China and Bangladesh have not become As contaminated areas in any case; therefore it would be interesting to highlight the convenience of changing the agricultural practices. Thus, it seem advisable to alternate at least each 8–10 years of rice crop with one or two crops growing aerobically, otherwise it would be compulsory to establish a phytoremediation program in order to decrease As levels in the soils.

Longer periods of monoculture under flooded conditions resulted in higher concentrations of total and extractable Se in soil (Table 3). Maybe flooding could result in a reductive dissolution of iron oxyhydroxides, which are important sorbents of Se species such as selenite (Li et al. 2010). In spite of the higher values of extractable Se concentration in soil, grain Se concentration was not significantly affected by the number of monoculture years. This is likely due to the reduced bioavailability of the forms in which Se accumulates in soil under flooded conditions. Furthermore, even in the higher values of Se recorded in this study, the amount of Se in soil is not high enough to produce crops with sufficient Se for human nutrition.

As expected, both soil available As and Se were highly correlated with its respective total As and Se concentrations. In addition, total and available As was positive and significantly correlated with total and available Se in the soil, but neither the As concentration in grain was correlated with the Se concentration in soil nor the Se concentration

Table 4. Equations of the linear regression and level of significance obtained in the Pearson correlation test performed between total and available arsenic (As) and selenium (Se) in the soil, total As and Se concentration in the grain

	Available As	Total Se	Available Se	Grain As	Grain Se
Total As	$y = 0.02x + 37.2^{***}$	$y = 0.006x + 85.0^{*}$	$y = 0.001x + 2.03^{*}$	ns	ns
Available As	–	$y = 0.24x + 85.0^{**}$	$y = 0.007x + 2.02^{**}$	$y = 0.002x + 0.1^{*}$	ns
Total Se	–	–	$y = 0.03x - 0.19^{***}$	ns	$y = 0.04x - 0.1^{*}$
Available Se	–	–	–	ns	$y = 2.06x\ 0.7^{**}$
Grain As	–	–	–	–	ns

ns – not significant; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$

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in grain was correlated with the As concentration in soil (Table 4). Feng et al. (2009) in *Pteris vittata* L. have found that in low levels of soil Se, the presence of As in soil increases the Se uptake and translocation, but in high levels of Se, the As presence inhibits the Se absorption. Thus, it seems that the uptake of As is suppressed by the addition of Se. However, such study was performed under aerobic hydroponic conditions, and As and Se were added. The lack of correlation found in our study could be related to the flooded conditions. Special attention should be paid to the As concentration in grain, since As has been regarded to be much more toxic and soluble for plants under flooded conditions (Bogdan and Schenk 2008). Total and extractable Se correlated positively with the Se concentration in grain, especially the extractable Se (Table 4). Hence, grain Se concentration could be increased by adding Se fertilizer, especially sodium selenite which seems to be the most efficient Se form under flooded conditions (Li et al. 2010).

The present study showed an increase in soil total As and Se concentrations as the number of monoculture years increased. Twelve out of the 19 studied locations in the Vegas Altas region reached toxic As levels. Conversely, the concentration of Se in the soils of this area can be considered as deficient to provide rice grains with acceptable values for human nutrition. Consequently, it might be advisable to control both As and Se in soil; As by including rotations with other crops, and Se by establishing Se biofortification programs to increase its concentration in the edible part of the crops.

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