

## Analysis of total arsenic content in purchased rice from Ecuador

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**Abstract:** Natural and anthropogenic sources contribute to arsenic contamination in water and human food chain in Andean countries. Human exposure to arsenic via rice consumption is of great concern in countries where this crop is the dominant staple food, and limited information is available on the arsenic contamination on rice in Ecuador. This work was to contribute to the lack of knowledge analysing total arsenic by hydride generation-atomic absorption spectrometry in the samples of white, brown and parboiled rice purchased in Ecuadorian markets and produced in the two main rice wetlands in Ecuador, Guayas and Los Ríos, were carried out. For the samples from Guayas, arsenic concentration in white, brown and parboiled rice were  $0.174 \pm 0.014$ ,  $0.232 \pm 0.021$ , and  $0.186 \pm 0.017$  mg/kg respectively, whereas samples of white rice from Los Ríos showed a total arsenic level of  $0.258 \pm 0.037$  mg/kg. This last arsenic concentration exceeds recommended maximum permissible limit by the FAO/WHO. Obtained data have available to estimate the Ecuadorian dietary exposure revealing serious health risk for population.

**Keywords:** Andean countries; arsenic; daily intake; healthy risk; rice; South America

Rice is the most important component of the diet by over half of the world population. In Ecuador, rice is the largest crop, and the majority of production is consumed locally being Ecuadorians the first consumers of rice in the Andean countries (INEC 2009). Unfortunately, rice could contain high levels of arsenic (As), becoming contamination with this toxic element a subject of great concern for authorities because the potential risk for consumers (HOJSÁK *et al.* 2015). Natu-

ral and anthropogenic sources contribute to arsenic contamination in water and rice in Guayas and Los Ríos, principal Ecuadorian rice production regions (POZO *et al.* 2011; BUNDSCHUH *et al.* 2012a; LÓPEZ *et al.* 2012; INEC 2017). The relationship between As and cancer had been made in Andean countries and recently a remarkable geographical pattern of cancer also had been established in Ecuador (MONTERO-OLEAS *et al.* 2017).

Although recently new data of arsenic in rice agrosystems in Ecuador have been reported (OTERO *et al.* 2016; NUNES & OTERO 2017), little is known about arsenic levels in Ecuadorian rice samples and the contribution of this staple food to the daily intake in this country. Some studies about arsenic contamination in Ecuador only have reported environmental data (CUMBAL *et al.* 2010; POZO *et al.* 2011; KNEE & ENCALADA 2014), and although may not accurately reflect an exposure level, they were useful for assessing disease risk in populations (MCCLINTOCK *et al.* 2012).

FAO/WHO recommended maximum permissible limit (ML) of 0.2 mg/kg (i-As) for polished rice (white rice) and 0.3 mg/kg for brown rice (i-As or t-As) (JECFA 2012, 2014). On the other hand, European Union established a recommended limit of 0.25 mg/kg for parboiled and husked rice (EU 2015). Although this ML is established for i-As, JECFA permits that competent authorities or importers use their own screening when employing the maximum permissible limit for inorganic arsenic in rice by determining total arsenic. When the measured t-As content is lower than proposed level for i-As, no more analysing is necessary and the sample is in conformity with established limits. If the t-As content is above the ML for i-As, more analysis shall be carried out to conclude if the i-As content exceed the maximum permissible level.

The objectives of this study were to determine the total arsenic (t-As) content in 40 different samples of rice (*Oriza sativa* L.) from provinces of Guayas and Los Ríos, main rice wetlands in Ecuador, and to estimate the dietary intake of arsenic for Ecuadorian population.

## MATERIAL AND METHODS

**Samples and reference material.** In Ecuador, main rice wetlands are placed in the low basin of the Guayas River and Los Ríos province, upstream of Guayas. In this study, forty rice samples from these two regions purchased in Ecuadorian markets were analysed. Considering that grain rice-processing and preparation methods affect the As content (BUNDSCHUH *et al.* 2012b; JAAFAR *et al.* 2012) different types of rice (brown, white and parboiled) were purchased. Of the total samples, 10 white (W), 10 brown (B) and 10 parboiled white (PW) rice were produced in the province of Guayas, whereas 10 white rice samples were produced in the province of Los Ríos. Figure 1

presents a map of Ecuador showing the location of the provinces where the rice samples were produced. In order to assess the quality of results, a reference material NIST-SRM-1568b Rice Flour (National Institute of Standards and Technology, USA) with a certified value for total arsenic level was used.

**Reagents and standards.** Ultrapure water (18.2 MΩ/cm quality) was obtained using Barnstead Easy pure II equipment (Thermo Scientific, USA) and used for all solutions and dilutions. All reagents used were of analytical grade. Working solutions were prepared by adequate dilution of arsenic standard solutions (1000 mg/l) obtained from Merck (Germany). All material was cleaned by soaking 48 h in 7.2 mol/l nitric acid solution and rinsed three times with ultrapure water prior use.

**Instruments.** The arsenic concentration was determined by hydride generation-atomic absorption spectrometry (HG-AAS) coupling of a PerkinElmer AA200 atomic absorption spectrophotometer to a PerkinElmer MHS 15 hydride generator (PerkinElmer Inc., USA). The HG-AAS setup consists of a continuous flow hydride generator coupled to an As-booster discharge hollow cathode lamp (Millennium Excalibur; Ps Analytical Ltd., UK). The pre-fixed wavelength of 193.7 nm was used to measure the As concentration. The gaseous arsenic hydrides were generated with NaBH<sub>4</sub> (30 g/l), NaOH (10 g/l) and diluted hydrochloric acid (1.2 mol/l). The gaseous products were carried to the sample cell, a flame-heated quartz cell placed in the light path of the spectrometer, by argon gas at a flow rate of 100 ml/min.

**Sample preparation and determination of total arsenic.** Prior analysis, rice samples were milled with a blender (Oster, 1 000W), homogenised and passed through 2 mm sieves. Then, the crushed samples were stored in a desiccator to avoid humidity. For the determination of As, around 1 g of sample was treated with 1.0 ml of an ashing aid suspension containing 200 g/l of Mg(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O plus 20 g/l of MgO and 5 ml of HNO<sub>3</sub> (7.2 mol/l).

The mixture was evaporated to dryness and mineralised in a muffle furnace at 450°C with a temperature gradual increase. White ashes were dissolved with 5 ml of HCl diluted (6 mol/l) and 5 ml of a reducing solution containing 50 g/l of KI plus 50 g/l ascorbic acid (MATOS-REYES 2010). The resulting solution was diluted with HCl solution (6 mol/l), filtered and transferred to a 50 ml volumetric flask. The accuracy of analytical method was determined using the certified reference material NIST-SRM-1568b Rice

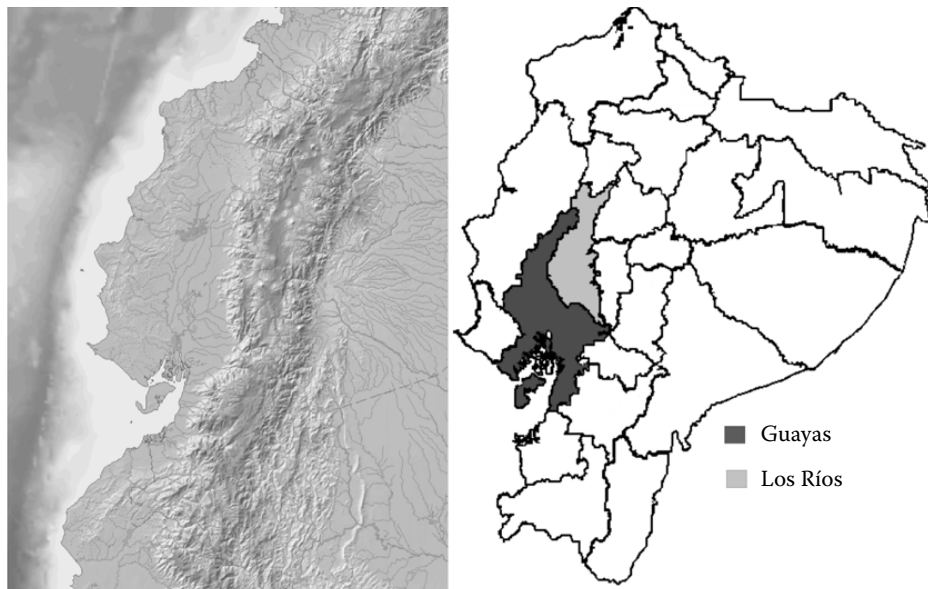


Figure 1. Map of the study areas: Guayas and Los Ríos

Flour (t-As certified value of  $0.285 \pm 0.014$  mg/kg). The recovery rates of the arsenic of the standard reference material were within the certificate values. The results of the validation showed good linearity ranges of between 1 and 5  $\mu\text{g/l}$  ( $R^2 > 0.9979$ ) with LOQ of 1.00  $\mu\text{g/l}$  and LOD of 0.20  $\mu\text{g/l}$ .

**Statistics.** Data analysis was performed using SPSS statistic software ver. 19.0 from SPSS Inc. (USA) and Unscrambler ver. 9.7 from Camo Software S.A. (Finland). Normality of data was confirmed by Kolmogorov-Smirnov test and Shapiro-Wilk test. Bartlett test was used to verify homoscedasticity. One-way ANOVA and *t*-test were used to compare the total arsenic concentrations among different rice groups and sampling sites. After one-way ANOVA, a Post hoc test (Tukey HSD) was used to identify groups of data that are significantly different from each other.

**Dietary exposure estimation.** It is known that the major route of exposure to arsenic is contaminated drinking water; however, research shows that dietary intake of arsenic due to rice also may be significant. To assess the underlying risk of As exposure from the consumption of rice in Ecuador, dietary exposure of As was estimated using a total diet study approach (FAO/WHO 1985). The dietary intake of As ( $\text{DI}_{\text{As}}$ ) in  $\mu\text{g/person per day}$  was calculated multiplying the rice consumption rate ( $\text{CR}_{\text{rice}}$ ) in kg/person per day by t-As concentration ( $\text{C}_{\text{tAs}}$ ) in  $\mu\text{g/kg}$ . The dietary exposure ( $\text{DE}_{\text{As}}$ ) in  $\mu\text{g/kg}$  of body weight per day was obtained as the ratio of  $\text{DI}_{\text{As}}$  and the average individual body weight of the habitant ( $\text{BW}_{\text{av b}}$ ) in kilograms.  $\text{CR}_{\text{rice}}$  and  $\text{BW}_{\text{av b}}$  data were obtained from the literature (INEC 2009; WALPOLE *et al.* 2012).

## RESULTS AND DISCUSSION

**Total arsenic in Ecuadorian rice.** Ecuadorian rice samples analysed in this study showed a wide range of total arsenic (t-As) concentration (Table 1). Mean concentration of t-As in white rice samples from Guayas was  $0.174 \pm 0.014$  mg/kg ranging from 0.157 to 0.205 mg/kg. The values for the t-As contents from Los Ríos white rice varied from 0.173 to 0.294 mg/kg, with a mean of  $0.258 \pm 0.037$  mg/kg. *T*-test was used to compare the total arsenic concentration of white rice from Guayas and from Los Ríos provinces. On the other hand, results of the *t*-test showed significantly difference between the data groups ( $P < 0.05$ ). In previous study, water, soil and rice plants in Guayas and Los Ríos provinces were studied (OTERO *et al.* 2016). This study showed a relationship between the concentrations of As in floodwater, plants and grain. Differences between provinces could not be associated to soil composition, because of their similarity. Thus, anthropogenic factors ought to be considered (POZO *et al.* 2011; BUNDSCHUH *et al.* 2012b).

With respect to brown rice, analysed samples showed t-As mean levels of  $0.232 \pm 0.021$  mg/kg ranging from 0.191 to 0.253 mg/kg. The lower As concentration in white rice compared to brown rice is probably due to the removal of the outer bran layer of rice grain during polishing. Brown rice, unlike white rice, still has the side husk and bran, plant parts where As concentration could be about ten times higher than in polished rice (white rice) (OTERO *et al.* 2016).

Table 1. Total arsenic concentrations found in Ecuadorian rice samples ( $n = 10$ )

Ecuadorian province	Rice type	t-As (mg/kg)
Guayas	brown rice	mean ( $\pm$ s.d.) 0.232 $\pm$ 0.021
		max. 0.253
		min. 0.191
	white rice	mean ( $\pm$ s.d.) 0.174 $\pm$ 0.014
		max. 0.205
		min. 0.157
	parboiled rice	mean ( $\pm$ s.d.) 0.186 $\pm$ 0.017
		max. 0.216
		min. 0.164
Los Ríos	white rice	mean ( $\pm$ s.d.) 0.258 $\pm$ 0.037
		max. 0.294
		min. 0.173

The obtained t-As contents for parboiled rice ranged from 0.164 to 0.216 mg/kg, with a mean of 0.186  $\pm$  0.017 mg/kg. These results showed that arsenic concentrations in parboiled rice were higher than those in non-parboiled rice (white rice). Some studies have shown that washing and soaking rice using uncontaminated water before cooking may reduce the t-As concentration of grain (SHARMA *et al.* 2014; JAAFAR *et al.* 2018), because the arsenic concentrations in food generally decreased due to solubilisation (BUNDSCHUH *et al.* 2012b). On the other hand, an increase in the concentration of As in rice cooked with As-contaminated water has been observed, depending on the type of rice, food processing, time, temperature and especially cooking medium (BUNDSCHUH *et al.* 2012b; JAAFAR *et al.* 2018). The statistical analysis showed significant differences ( $P < 0.05$ )

among the three types of rice from Guayas province. ANOVA was used to compare the total arsenic concentrations among different rice groups. Normality of data was confirmed by Kolmogorov-Smirnov test and Shapiro-Wilk test. Bartlett test was used to verify homoscedasticity. The ANOVA results suggest that concentration mean of the three different types of rice from Guayas province varied significantly. Afterward, a Tukey HSD test was applied. This *post hoc* test allowed interpreting the results. Result of Tukey HSD test suggests that white rice data set was significantly different of the other groups of rice. Figure 2 summarised in a box and whisker plot statistical data for the three types of Guayas analysed rice.

Considering the WHO-recommended maximum permissible limit or the EU-recommended maximum permissible limit for As in rice, the results obtained in Guayas rice samples did not exceed these limits of 0.2 mg/kg for polished rice, 0.25 mg/kg for parboiled rice and 0.3 mg/kg for brown rice (JECFA 2012, 2014; EU 2015).

However, Los Ríos white rice samples exceeded WHO limits; thus, the possibility of a health risk to the population with high rice consumption in Ecuador cannot be excluded and new investigation on arsenic-contaminated rice should be a priority in future works.

In previous studies conducted in different countries, data analysis of rice of various origins and types showed a very wide range of concentrations (WILLIAMS *et al.* 2005; MEHARG *et al.* 2009; BATISTA *et al.* 2011; NOOKABKAEW 2013; RAHMAN *et al.* 2014; SHARMA *et al.* 2014; LEE *et al.* 2018). In Latin America few data of As concentration in rice had been reported. In Brazil, total arsenic levels varied from 0.108 to 0.428 mg/kg, with a mean of 0.223 mg/kg (BATISTA *et al.* 2011). As concentrations in rice from Argentina

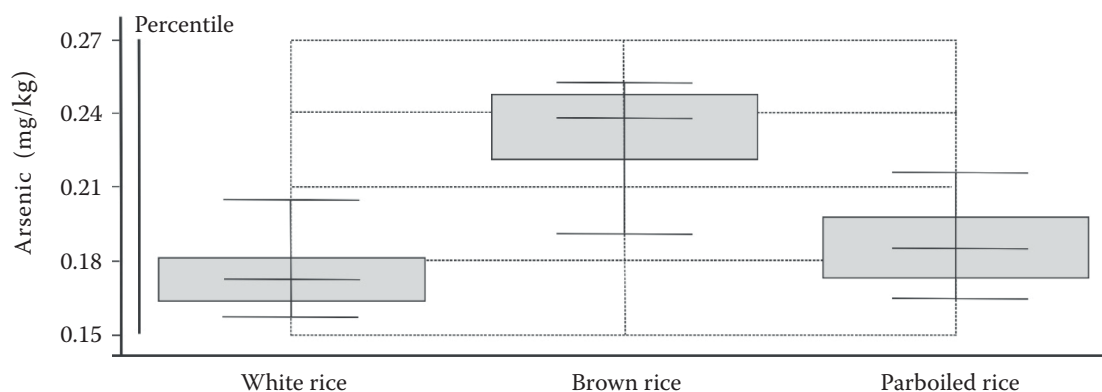


Figure 2. Total arsenic concentration in Guayas rice

boxes – 25–75% of the distribution; whiskers – min. and max.



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of 0.30 mg/kg (FARÍAS *et al.* 2015) and 0.056 mg/kg in raw rice (JAAFAR *et al.* 2018) have been reported. Figure 3 shows the concentrations of total arsenic in rice from different countries obtained from the literature, which provides useful information about the range of arsenic concentration in rice worldwide.

**Estimated dietary intake of total arsenic.** In this study, dietary intake of As from rice consumption in Ecuador was calculated using data analysis and other data reported in literature. In Ecuador, the consumption rate ( $CR_{\text{rice}}$ ) is 0.326 kg/person per day (INEC 2009) and the average individual body weight of the habitant ( $BW_{\text{avb}}$ ) is 67.9 kg for Latin American and Caribbean (WALPOLE *et al.* 2012). Table 2 summarised results obtained by calculating dietary intake and dietary exposure following the approach previously mentioned

in material and method section. The JECFA established the provisional tolerable daily intake (PTDI) of 2.1 µg/kg of body weight per day. In the present work, total arsenic data obtained did not exceed these recommended values. However, in the opinion of Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) this PTDI is no longer appropriate as data had shown that arsenic causes cancer. CONTAM Panel identified a range of benchmark dose lower confidence limit for 0.1% (BMDL01) values between 0.3 and 8 µg/kg of body weight per day for cancers of the lung, skin and bladder, as well as skin lesions (EU Commission Regulation 2015). Consequently, the estimated dietary exposures to arsenic for average and high level consumers in Ecuador are within the range of the BMDL01 values identified, and therefore the possibility of a risk to some consumers

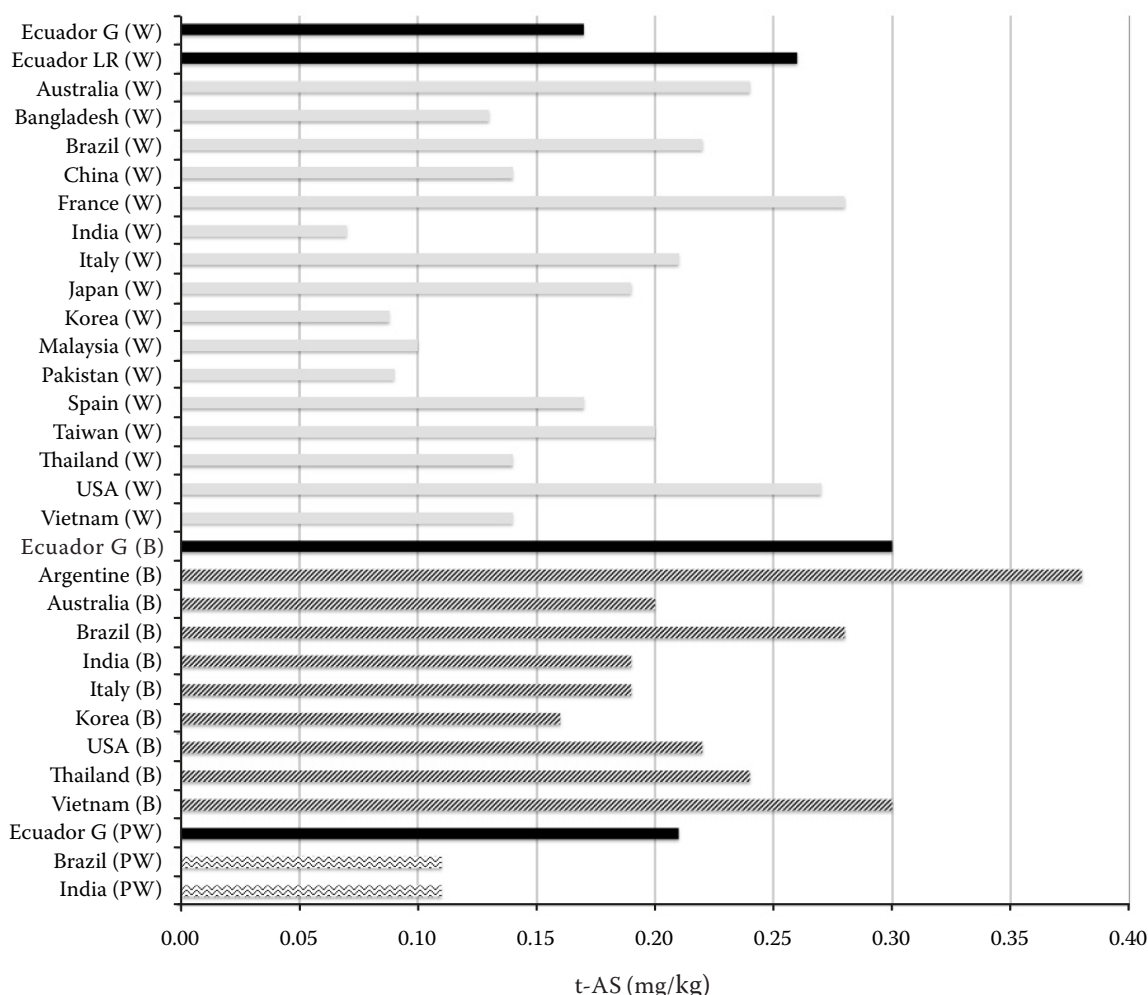


Figure 3. Concentration of total arsenic in market rice from different countries

W – white rice; B – brown rice; PW – parboiled white rice (PW) are in cross-hatched; LR – rice from Los Ríos province (Ecuador); G – rice from Guayas province (Ecuador) (WILLIAMS *et al.* 2005; MEHARG *et al.* 2009; BATISTA *et al.* 2011; NOOKABKAEW *et al.* 2013; RAHMAN *et al.* 2014; FARÍAS *et al.* 2015; LEE *et al.* 2018)

Table 2. Estimated dietary intake of total arsenic ( $DI_{As}$ ) and the dietary exposure ( $DE_{As}$ ) from Ecuadorian rice

Ecuadorian province	Type of rice		t-As concentration ( $C_{tAs}$ ) ( $\mu\text{g/kg}$ )	Dietary intake of As ( $DI_{As}$ ) ( $\mu\text{g/person/day}$ )	Dietary exposure ( $DE_{As}$ ) ( $\mu\text{g/kg}$ of body weight/day)
Guayas	brown rice	mean	232	75.6	1.10
		max.	253	82.5	1.21
		min.	191	62.3	0.92
	white rice	mean	174	56.7	0.84
		max.	205	66.8	0.98
		min.	157	51.2	0.75
	parboiled rice	mean	186	60.6	0.89
		max.	216	70.4	1.04
		min.	164	53.5	0.79
Los Ríos	white rice	mean	258	84.1	1.24
		max.	294	95.8	1.41
		min.	173	56.4	0.83

cannot be excluded. NUNES & OTERO (2017) calculated that the estimated daily intake of arsenic for infants living in urban areas of Ecuador is around four times higher than that of European infants, being equal for those livings in rural areas of Ecuador.

## CONCLUSIONS

Forty samples from the main rice producing regions purchased in Ecuadorian markets were analysed. Results obtained in the Guayas rice did not exceed the FAO/WHO or EU recommended maximum permissible limit. However, Los Ríos white rice samples exceeded this value and new investigation on rice from this region should be a priority in future researches. Estimated diary intake was below t tolerable daily intake established by JECFA. However those estimated values were within the range of the benchmark dose lower confidence limit for 0.1% (BMDL01) identified by CONTAM panel, and therefore the possibility of a risk to some consumers cannot be excluded.

The results of this work provide the important data about As content in Ecuadorian rice and contribute to the lack of knowledge about this contaminant in rice from Andean countries.

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