



Elemental composition of Brazilian rice grains from different cultivars and origins

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Abstract

Neutron activation analysis (NAA) was used for determining chemical elements in rice grains of four different cultivars (IRGA424, IRGA424 RI, Guri Inta, Puitá Inta) produced in four geographic regions of Rio Grande do Sul state, Brazil. Considering toxic elements, As varied from 0.027 to 0.646 mg/kg, while Cd was always lower than the detection limit of 0.25 mg/kg. Applying principal component and cluster analysis, As, Br, Co, Cs, Na, Rb and Zn presented potential to be used to distinguish rice grains from different regions, while no discrimination was obtained amongst cultivars.

Keywords Toxic elements in rice · Chemical elements · Neutron activation analysis · Arsenic · Cadmium · *Oryza sativa*

Introduction

Considered a global staple food, rice is part of the culture, diet and economy of people since antiquity [1]. Rich in vitamins and minerals, it is an excellent source of complex carbohydrates and the essential amino acid lysine. The cereal is often a baby's first solid food in some countries like North America and United Kingdom. More than 700 million tons of rice are produced worldwide annually [2]. Asia alone holds 90% of total production and consumption of rice, while Brazil is the largest producer and consumer outside Asia, occupying the ninth world position [2]. The

flooded fields are mainly concentrated in the Rio Grande do Sul state, the largest Brazilian producer with 70% of total rice yield. On the other hand, upland fields, corresponding to only 10% of total Brazilian production, are mostly in the state of Mato Grosso [3]. In recent years, Brazil has been increasing its share of exports, sending 700 tons of rice grains to destinations such as Peru, Senegal, Sierra Leone, Cuba, Bolivia, Panama, and Nicaragua [4].

Rice can contain toxic elements at undesirable levels from agrochemical products used for disease and pest control, contaminated soil and irrigation water [5]. The rice plant is efficient at accumulating As [5, 6], with larger amounts in the aerial part and in the grains [7, 8]. This element naturally exists in different chemical forms, including organic and inorganic species. The inorganic species arsenite As(III) and arsenate As(V) are soluble in water and readily absorbed by the gastrointestinal tract and metabolized [5, 9]. Exposure to As has been associated to cardiovascular disorders, immune system diseases, skin lesions and increased incidence of diabetes [9]. Inorganic species are considered human carcinogens class I, related to the development of skin, lung, bladder and kidney cancers [9–12]. For reducing the exposure of the population, the Brazilian Health Regulatory Agency (ANVISA) stipulated maximum limits of the element in several foods. For rice, the limit of total As was set at 0.3 mg/kg [13].

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Several studies [14–16] aimed to evaluate if the amount of total As and inorganic arsenic (iAs) in the rice grains is influenced by the genotype and cultivar. A simple alternative method to decrease As in rice is to select cultivars that biologically restrict As accumulation in the grains [17]. These cultivars may have developed ways of blocking As uptake, translocation or accumulation. Zavala et al. [14] concluded that the uptake of As and also the amount of iAs in rice are influenced by genotype rather than by environmental conditions. When different rice cultivars were submitted to the same environmental conditions in greenhouses or in the field, significant differences were found in the amounts of inorganic and organic As. Liu et al. [15] showed that the amount of inorganic As varied from 40 to 70% in six rice cultivars under flood system. Norton et al. [16] conducted a study of thirteen rice cultivars in Bangladesh, India and China, concluding that both the site and the genotype influenced total and inorganic As in rice.

In addition to As, other toxic and essential elements should also be considered in rice, since it is a staple food for millions of people. Polished rice, for example, has low amounts of Fe and Zn and, in some regions of the world, low Se [18]. Studies show that aerobic (non-flooded) cultivation conditions lead to increased Cd compared to flooded irrigation systems. Other effects of aerobic culture include decreased Se and Fe and increased Zn [19]. Although the flooded crop favors the accumulation of As, it can be a solution in areas where the soil has a high amount of Cd [18].

This study aimed to evaluate the elemental composition of rice grains from the four most planted rice cultivars in four regions of Rio Grande do Sul state, Brazil, using neutron activation analysis (NAA), which has been used for quantification of As and other elements in rice [20–22].

Experimental

Samples and treatments

Samples of rice grains were collected directly from producers of certified rice, where the information was guaranteed in relation to cultivar, location and production system. Sampling was performed in the flooded system of Rio Grande do Sul state. Table 1 shows the origin and cultivar of all samples, and Fig. 1 illustrates the sampling sites. According to Rio Grande do Sul Rice Institute (IRGA), the state was divided into six rice producing regions, i.e. west frontier (WF), campaign (CAM), central depression (CD), internal coastal plain (ICP), external coastal plain (CP) and south zone (SZ). In this work, this division will be used to locate the samples.

The husk of rice grains was removed using the rice mill Yanmar ST 50. After that, samples were dried in oven at 50 °C for 12 h, and grinded in a cryogenic equipment Freezer Mill 6870. Then, they were placed in polyethylene bottles, and stored at 18 °C under controlled humidity and luminosity conditions. The evaluation of residual moisture was performed using 1 g aliquots in triplicate, after drying in oven at 80 °C for 6 h.

Naa

Duplicate 300 mg aliquots of samples and reference materials SRM 1568a Rice Flour (National Institute of Standards and Technology—NIST) and CRM-AGRO C1001a—Brown Rice (CRM-Agro Reference Materials for Agriculture, Livestock and Toxicology, CENA/USP) were inserted into polyethylene capsules from Posthumus Plastics, Beverwijk, The Netherlands. Empty capsules were included as analytical blanks. Pieces of 10 mg of a Ni–Cr alloy with known chemical composition [23] were placed between capsules to measure the neutron flux during irradiation, which was carried out for 4 h, under thermal neutron flux on the order of $1.2 \times 10^{13} \text{ cm}^{-2} \text{ s}^{-1}$, in the nuclear research reactor IEA-R1 of the Nuclear and Energetic Research Institute (IPEN), from the Brazilian Nuclear Energy Commission (CNEN) located in the city of São Paulo, São Paulo state.

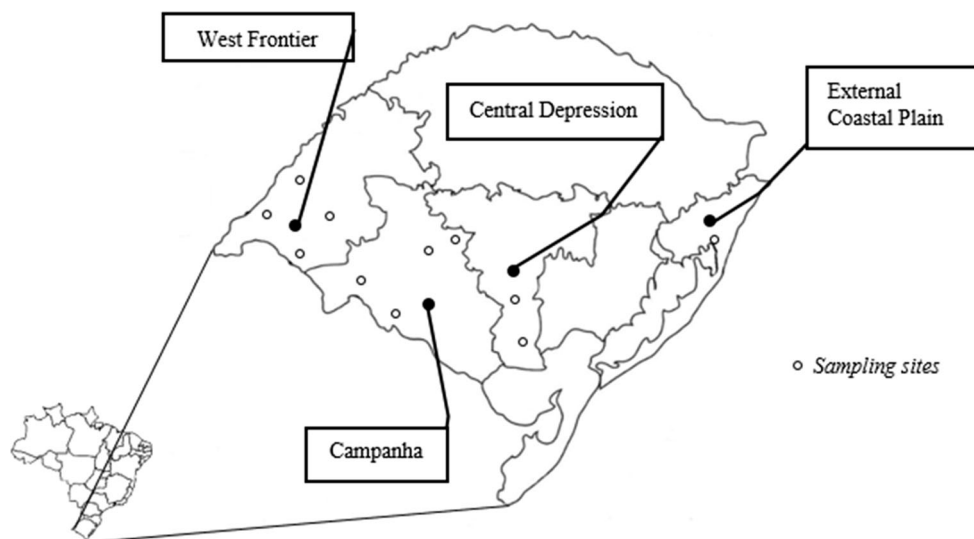
The induced activity was measured by high-resolution gamma-ray spectrometry at CENA/USP, using HPGe detectors with 50% relative efficiency at the 1332 keV photopeak of ^{60}Co . For better detection of gamma-rays from radionuclides with different half-lives, four measurements were performed with approximate decay times of 3, 7, 15 and 30 days, and respective counting times of 15 min, 30 min, 60 min and 120 min. Mass fraction of elements and the respective standard uncertainties were calculated using the software package Quantu [24] based on the k_0 method.

Statistical analysis

The statistical tests were performed using the Statistic Analysis System SAS. Normality and homoscedasticity of data were tested before the analysis of variance (ANOVA) was applied to verify significant differences between the composition of rice grains of diverse cultivars and origins at the 95% confidential level ($p < 0.05$). Factorial ANOVA was used for identifying possible significant interactions between the two independent variables (cultivar and origin) on the elemental composition of rice [25, 26]. In the presence of significant interaction, the evaluation of results requires a comparison of the levels of a variable inside the fixed levels of another variable [27]. The chemical

Table 1 Number of rice grain samples taken in Rio Grande do Sul state, Brazil

Region	Cultivar				Total
	Guri Inta	Puitá Inta	IRGA424	IRGA424 RI	
Campanha (CAM)	2	2	1	1	6
Central depression (CD)	1	1	–	1	3
West frontier (WF)	4	4	5	3	16
External coastal plain (CP)	1	1	–	1	3
Total	8	8	6	6	28

Fig. 1 Location of sampling in Rio Grande do Sul state, Brazil

elements with significant differences identified by ANOVA were evaluated by Duncan's new multiple range test [26, 28]. Principal component analysis (PCA) was used to select a subset of elements (components) that had the largest contribution to the variation between rice grains from different cultivars and origin, using Ward's method with Euclidian distances [29, 30]. Finally, cluster analysis was applied using the main components of the PCA. Cluster analysis is an exploratory data analysis that can order samples in a dendrogram, where samples with the highest similarities are grouped together, while samples with small similarities are widely separated [29]. PCA and cluster analysis were performed using Statistica software.

Results and discussion

Quality control

To evaluate the accuracy obtained for two CRMs used in the quality control (SRM NIST 1568a Rice Flour and CRM-AGRO C1001-a Brown Rice), En scores were calculated comparing the results to the certified values, taking into account the respective uncertainties [31]. Values between -1 and 1 are considered admissible. The mean

scores ($n = 5$) were within the limit of ± 1.0 for all the elements, with exception of Na in NIST 1568a, as shown in Fig. 2.

The mean mass fraction and standard deviation ($n = 5$) of Na in the SRM 1568a were 13.41 ± 0.38 mg/kg, with a RSD of 3%, while Masson et al. [32], using ICP-MS, reported a mean mass fraction and standard deviation ($n = 5$) of 8.5 ± 5.6 mg/kg, against a certified value of 6.6 ± 0.8 mg/kg. From calculation using the mass fractions of Al and Mg in the SRM 1568a, the possible interference reactions in NAA, $^{27}\text{Al}(n,\alpha)^{24}\text{Na}$ and $^{24}\text{Mg}(n,p)^{24}\text{Na}$, were not responsible for the higher results of Na obtained here. As blank was checked and appropriately discounted, no other analytical problem was identified for the disagreement of Na results in the SRM. Therefore, the element was also considered in the subsequent statistical analysis and discussion, taking into account that possible contamination of certified reference materials with Na from the glass bottles was already suggested [33] and that the results were in good agreement with the reference value of Na in the other reference material (CRM-AGRO C1001-a), which was obtained from independent measurements in twelve laboratories using diverse analytical techniques.

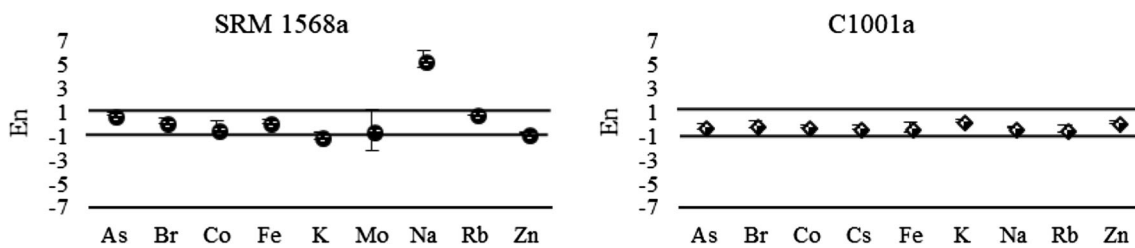


Fig. 2 Mean en-score and standard deviation ($n = 5$, mg/kg) obtained for CRM's NIST SRM 1568a and CRM-AGRO C1001a

Chemical elements in rice

NAA allowed the evaluation of As, Br, Cd, Co, Cs, Fe, K, Mo, Na, Rb and Zn in the 28 samples of rice grains of four cultivars and four regions of Rio Grande do Sul state, Brazil. The mass fractions and analytical uncertainty ($n = 2$) obtained for total As can be verified in Fig. 3. Of the seven samples with levels above the maximum limit of total As in rice (0.3 mg/kg) established by ANVISA [13], four were Guri Inta, and four were from the region of Campanha (CAM). The mean mass fraction ($n = 28$) of total As was 0.243 ± 0.183 mg/kg (from 0.027 to 0.646 mg/kg), with a RSD of 76%. Former studies reported similar ranges of total As in Brazilian brown rice (0.189–0.393 mg/kg [34] and 0.101–0.190 mg/kg [35]).

A large range of total As mass fraction within the same cultivar suggests that the presence of this toxic element is more related to the origin of the samples than to the cultivars. In fact, there was no statistical difference between the cultivars for As by ANOVA ($p > 0.05$). Evaluating the

presence of As by region (CAM = campanha, CD = central depression, CP = external coastal plain, WF = west frontier), there was statistical difference between the mean As mass fractions of rice from CAM and WF ($p < 0.05$) by Duncan's test (Fig. 4), with CAM showing the highest mass fractions.

Cd content in all samples was below the detection limit of 0.25 mg/kg, which was lower than the limit of 0.4 mg/kg established by ANVISA. Other authors [20] also found Cd below the detection limit in polished and parboiled rice from Sri Lanka analyzed by NAA. Mataveli et al. [35], using ICP-MS, found one sample of Brazilian polished rice with 0.042 ± 0.008 mg/kg of Cd, out of a group of 37 samples that were below the detection limit.

The mean mass fractions of ten chemical elements in rice grains from four cultivars can be seen in Table 2. Factorial ANOVA was applied to verify the interaction between cultivar and origin in relation to chemical elements, observing significant interaction only for Na ($p < 0.001$), meaning that the variables cultivar and origin

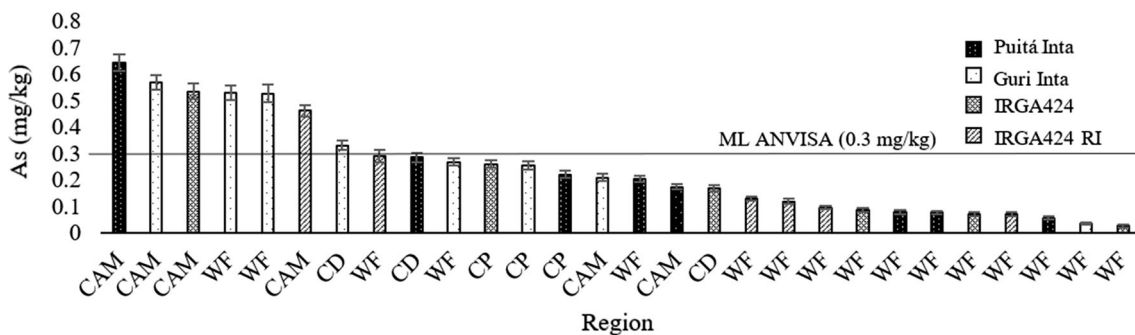


Fig. 3 As mass fractions and respective standard uncertainties ($n = 2$, mg/kg) in brown rice of different cultivars and regions from Rio Grande do Sul state, Brazil

Fig. 4 Total As and standard deviation (mg/kg) in brown rice from different regions of Rio Grande do Sul state, Brazil. Means followed by letters in common do not differ statistically ($p < 0.05$)

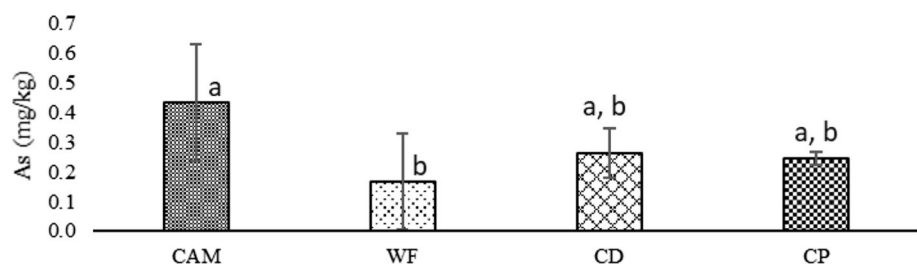


Table 2 Mean mass fractions and standard deviations (mg/kg, dry matter) of chemical elements in Brazilian brown rice from different cultivars

Cultivar	As	Br	Co	Cs	Fe	K	Mo	Na	Rb	Zn
Guri Intá (n = 8)	0.34 ± 0.19a	1.07 ± 0.19a	0.16 ± 0.74a	0.07 ± 0.16a	11.9 ± 2.1a	2400 ± 120a	0.74 ± 0.25a	4.1 ± 1.8	14.8 ± 6.9a	24.4 ± 2.3a
Puitá Intá (n = 8)	0.22 ± 0.19a	1.49 ± 1.20a	0.17 ± 0.11a	0.21 ± 0.11a	13.3 ± 3.1a	2360 ± 210a	0.68 ± 0.22a	4.9 ± 2.2	19.1 ± 9.3a	26.2 ± 2.5a
IRGA424 (n = 6)	0.20 ± 0.15a	1.73 ± 1.06a	0.40 ± 0.16a	0.26 ± 0.16a	11.1 ± 1.7a	2370 ± 210a	0.65 ± 0.24a	4.0 ± 0.2	25.0 ± 5.5a	23.4 ± 2.0a
IRGA424 RI (n = 6)	0.19 ± 0.19a	1.23 ± 0.84a	0.15 ± 0.11a	0.26 ± 0.15a	10.4 ± 1.2a	2410 ± 350a	0.73 ± 0.28a	5.2 ± 3.1	22.3 ± 10a	25.0 ± 1.9a
Average	0.24 ± 0.18	1.37 ± 0.96	0.19 ± 0.16	0.17 ± 0.16	11.7 ± 2.2	2380 ± 220	0.74 ± 0.28	4.5 ± 2.0	19.8 ± 8.6	24.8 ± 2.4

Means with letters in common in the column do not differ statistically by Duncan's Test ($p > 0.05$)

*Significant interaction between cultivar and origin by Factorial analysis

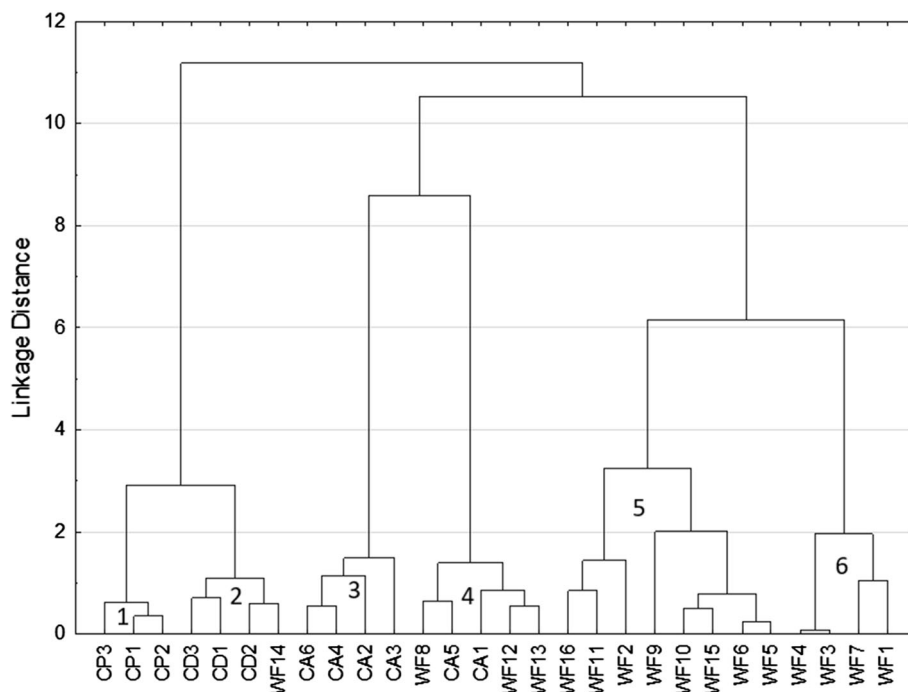
Table 3 Mean mass fractions and standard deviations (mg/kg, dry matter) of chemical elements in brown rice from different regions of Rio Grande do Sul state, Brazil

Region	As	Br	Co	Cs	Fe	K	Mo	Na*	Rb	Zn
CAM (n = 6)	0.43 ± 0.20a	1.60 ± 0.94ab	0.08 ± 0.09b	0.08 ± 0.07b	10.9 ± 0.9a	2450 ± 150a	0.93 ± 0.40ab	3.8 ± 0.6	25.4 ± 5.6a	24.7 ± 2.5a
WF (n = 16)	0.17 ± 0.16b	1.68 ± 0.16a	0.29 ± 0.14a	0.24 ± 0.12a	12.1 ± 2.7a	2300 ± 210a	0.62 ± 0.17a	3.8 ± 0.3	21.5 ± 7.8a	24.0 ± 2.1a
CD (n = 3)	0.26 ± 0.08ab	0.37 ± 0.07ab	0.07 ± 0.04b	0.04 ± 0.02b	11.7 ± 0.9a	2600 ± 210a	1.10 ± 0.09b	4.3 ± 1.1	11.1 ± 5.7b	26.8 ± 0.9a
CP (n = 3)	0.25 ± 0.02ab	0.24 ± 0.02b	0.02 ± 0.01b	0.15 ± 0.09ab	11.4 ± 1.9a	2520 ± 50a	0.68 ± 0.09ab	10.0 ± 1.6	8.5 ± 2.8b	27.5 ± 1.8a
Average	0.24 ± 0.18	1.37 ± 0.96	0.19 ± 0.16	0.17 ± 0.13	11.7 ± 2.2	2380 ± 220	0.74 ± 0.28	4.5 ± 2.0	19.8 ± 8.6	24.8 ± 2.4

Means with letters in common in the column do not differ statistically by Duncan's Test ($p > 0.05$)

*Significant interaction between cultivar and origin by Factorial analysis

Fig. 5 Dendrogram comparing rice grains of different regions from Rio Grande do Sul state, Brazil, considering the variables As, Br, Co, Na, Rb and Zn. CA Campanha, CP external coastal plain, CD central depression, WF west frontier



for this element are not independent. For this element, the behavior of the cultivars was investigated for each region of origin, finding significant differences for all the cases ($p < 0.01$). There was no statistical difference ($p > 0.05$) among the rice grains from the four cultivars for all other elements by ANOVA, suggesting that the elemental composition of rice grains is more influenced by origin than by cultivar.

Table 3 shows the mass fractions of the elements in rice grains from different regions of Rio Grande do Sul state. There was statistical difference among the rice grains from different regions for As ($p < 0.03$), Br ($p < 0.05$), Co ($p < 0.009$), Cs ($p < 0.002$), Mo ($p < 0.05$), Na ($p < 0.001$) and Rb ($p < 0.004$). Since there was a significant interaction for Na by the factorial analysis between cultivar and region, comparisons were made between the regions for each cultivar, presenting highly significant differences in all cases ($p < 0.01$). It is interesting to notice that the mean mass fraction of Na in the rice grains from CP is much higher ($p < 0.05$) than those from other regions of Rio Grande do Sul. In fact, CP is the only region near the ocean, which could be a reason for this high Na content in the grains.

PCA was applied for investigating possible grouping between the samples and cluster analysis was carried out with the main components. The elements As, Br, Co, Cs, Na, Rb and Zn were used to compare rice grains from the four regions (Table 3). Six clusters were observed among the samples, as identified in Fig. 5 (three main components and 85% of the information, applying Ward's method and

Euclidian distances). Cluster 1 formed by the samples from CP, cluster 2 by the samples from CD and one sample of WF, cluster 3 grouped four samples from CAM, and cluster 5 and 6 grouped twelve samples from WF. The most dispersed sample group was cluster 4, with three samples from WF and two samples from CAM, which can be explained, in part, by the fact that these regions are geographically close.

Conclusions

Neutron activation analysis allowed the determination of As, Br, Co, Cs, Fe, K, Mo, Na, Rb and Zn in the samples of rice grains of four different cultivars (IRGA424, IRGA424 RI, Guri Inta, Puitá Inta) produced in four distinct geographic regions of Rio Grande do Sul state, Brazil. The total As mass fraction was above the maximum limit of 0.3 mg/kg established by Brazilian legislation for 7 out of 28 samples, while Cd was lower than the detection limit of 0.25 mg/kg, demonstrating that all samples were below the Brazilian legislation limit of 0.4 mg/kg.

Significant differences were observed for the composition of rice grains of different regions of Rio Grande do Sul state. From results of PCA and cluster analysis, As, Br, Co, Na, Rb and Zn showed potential for grouping samples of rice grains according to the region of origin.

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