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Original Research Article

Unraveling the accumulation and localization of selenium and barium in Brazil nuts using spectroanalytical techniques

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ABSTRACT

Brazil nuts are native from the Amazon rainforest and their nuts are consumed worldwide having good sensory acceptability. However, knowledge gaps remain concerning elemental composition, localization, and interactions in Brazil nuts. This study presents a detailed assessment regarding the concentration and localization of relevant elements in Brazil nuts using different and complementary spectroanalytical techniques. Samples were collected from six sites of the Brazilian Amazon (Acre, Rondônia, Amazonas, Roraima, Pará, and Amapá) and showed results for selenium (Se) concentration ranging from 0.46 to 356 μg g^{-1} and barium (Ba) from 12.5 to 7,177 μg g^{-1}. Then, a linear regression model fitted between Se and Ba concentration in Brazil nuts provided an $R^2 = 0.30$. The spatial distribution of major and trace elements in Brazil nuts varied depending on the site of origin and concentration in the sample. The 2D maps performed via μ-XRF showed that Se accumulates mainly in the outer parenchyma tissue of Brazil nuts seeds forming a "ring" shape while Ba tends to accumulate in the epidermal tissue. The possibility of forming compounds of low solubility in Brazil nuts such as $BaSO_4$ and $BaSeO_4$ tends to increase when Ba and Se are higher respectively in the samples studied.

1. Introduction

The Brazil nut (*Bertholletia excelsa* Bonpl.), a Lecythidaceae family member, is a tree species native to South America. This species grows in upland and well-drained soils throughout the Brazilian Amazon rainforest and other countries such as Bolivia, Peru, Colombia, Venezuela, and Guianas (Mori and Prance, 1990). It is an endemic species distinguished by its economic, social, and environmental values. The commercialization of Brazil nuts is one of the main income sources for many Amazonian indigenous and riverine communities (Cardoso et al., 2017; Baldoni et al., 2020).

Brazil nut trees are huge plants that can grow up to 50 m high and can reach up to 300 cm in diameter (Salomão, 2009). The fruit measures 11−15 cm in diameter and weighs up to 2 kg, being enclosed by a woody capsule that contains 10−25 seeds (Mori and Prance, 1990). The seed of Brazil nuts is composed mainly of parenchyma tissues delimited by a ring of meristematic tissue, surrounded by an epidermal layer and a thin external lignified layer (Camargo et al., 2000). According to Corner (1976), Brazil nut seeds have an embryo of the hypocotyl type and two teguments. However, the embryo has no delimitation of differentiated cotyledons and is mostly composed of hypocotyl (Prance and Mori, 1978). Scussel et al. (2014) stated that the edible part of the nut, which

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is the storage tissue, shows several different tissue/cell layers starting from the epidermis (double/triple cells sequence of round and palisade shapes) till the endosperm tissue. Brazil nuts are rich in essential elements such as Ca, Cu, Mg, K, Mn, and P, containing also fiber, folate, and antioxidant compounds, being frequently reported to have high Se content (Silva Junior et al., 2017; Brito et al., 2019).

Selenium is a required element for all six kingdoms and three domains of life (Dolgova et al., 2018). The recommended daily intake of Se is 60 and 70 μg day^{-1} for adult women and men, respectively (Kipp et al., 2015). Due to a variety of functions, Se deficiency is associated with adverse health effects including oxidative stress, cardiovascular pathology, endocrine dysfunction, cancer, etc. Severe endemic Se deficiency is associated with the development of specific Kashin-Beck and Keshan diseases. On the other hand, at high levels, Se is toxic, and cases of toxicity and mortality have been reported following acute intoxication (Skalny et al., 2019; Hadrup and Ravn-Haren, 2020). Selenium enters the food chain via plants mostly, and plant-based food is an important source of Se to human and animal diets. Therefore, there is a significant relationship between environmental and food Se levels (Lessa et al., 2016; Silva Junior et al., 2017; Reis et al., 2017; Skalny et al., 2019).

Brazil nuts are also known for presenting high Ba concentration (Parekh et al., 2008; Gonçalves et al., 2009; Kabata-Pendias, 2011). The main reason for high Ba accumulation has been cited as the elevated Ba contents in soils from the Amazon Basin, due to the presence of the Ba-rich mineral hollandite ($Ba_2Mn_8O_{16}$) that contains around 130 g kg^{-1} of Ba, in which Ba occurs in a mobile form and is easily available for plants (Chang et al., 1995; Kabata-Pendias, 2011). Another important feature is the species' (*Bertholletia excelsa*) ability to form organic complexes that favor the mobility and redistribution of Ba to the fruits (Smith, 1971). High levels of Ba in Brazil nuts are a matter of concern, as this element can be toxic to humans. Barium at high concentration impacts the cardiovascular system in addition to causing abdominal cramps, diarrhea, vomiting, nausea, agitation, anxiety, sweating, cardiac arrhythmia, muscle weakness, and difficulties in breathing (IRIS, 2005). Concerning Ba intake, the Agency for Toxic Substances and Disease Registry (ATSDR) and the United States Environmental Protection Agency (EPA) stipulate a reference dose (i.e., the maximum acceptable oral dose) of Ba of 0.2 mg kg^{-1} day^{-1} for the adults' population (IRIS, 2005).

The importance of mineral composition assessments (major and trace elements) in food samples is due to their nutritional properties and possible beneficial health effects. Therefore, information about the elemental composition is fundamental, especially in food with great nutritional potential such as Brazil nuts. Most reported studies deal with the determination of total element contents. However, details about the localization of each element in particular tissues can provide important information on their functions, dynamics, and interaction with other elements (Welna et al., 2008).

Micro probe X-ray fluorescence (μ-XRF) techniques provide a direct and versatile means of investigating trace elements in organisms and tissues. This is useful in a wide variety of sample conditions and requires very little or no sample pre-treatment, which is important when studying delicate biological samples (Dolgova et al., 2018). This technique has been used in studies on phytoremediation, food safety, and crops' biofortification (Sarret et al., 2013; Lessa et al., 2019; Salazar et al., 2021). μ-XRF is a valuable and sensitive tool for analyzing the distribution of elements in different regions of a plant (Capobianco et al., 2018). Since X-rays can be focused on spots smaller than 1 mm, researchers have applied this technique to identify, quantify, and localize nutrients and contaminants in plant tissue (Vijayan et al., 2015).

This study presents a comprehensive characterization of Brazil nuts seeds using spectroanalytical techniques such as GF-AAS, ICP-OES for quantification and both benchtop and synchrotron-based microprobe X-ray fluorescence spectroscopy (μ-XRF) to set up 2D maps and investigate the distribution of Se, Ba and other relevant elements in Brazil nuts originated from different sites in the Amazon region. Hence, we hope to provide information on the concentration and spatial distribution of important elements in Brazil nuts seeds, which is important from both nutritional and toxicological points of view, emphasizing the accumulation of Se and Ba and the differences found depending on the concentration, geographical location and localization in seed tissues.

2. Materials and methods

2.1. Sampling sites

Fruit samples of Brazil nuts were collected in different selected sites located in six states of the Brazilian Amazon that are relevant Brazil nuts producers (Acre, Rondônia, Amazonas, Roraima, Pará, and Amapá). The sampling sites chosen for the study were either located within native areas previously studied by the Brazilian Agricultural Research Company (Embrapa) or in a cultivated farm used for commercial production of Brazilian nuts. All samples were collected during the harvest season between 2014 and 2017. Information concerning the sampling location, geographic coordinates, altitude and climatic classification is provided in Table 1.

The number of Brazil nut trees sampled on each site was: Sena Madureira-Acre: 14, Itacoatiara-Amazonas: 16, Laranjal do Jari-Amapá: 14, Caracaraí-Roraima: 14, Porto Velho-Rondônia: 7, and Santarém-Pará: 7. Brazil nuts samples collected from the site Itacoatiara-Amazonas are originated from Aruanã farm, an extensive plantation of clones from the State of Pará that were brought before the seedlings were used for grafting in the farm with the purpose of accelerating the start of the production time to ~8 years after the plants are established. Brazil nuts were sampled from below the tree canopy, collecting ten ripe fruits, which naturally fall from the trees after maturation. Brazil nuts fruits collected were opened, and a total of 50 seeds were randomly selected from each plant and put in a plastic bag. The sampling method in the farm/forest is represented schematically by Silva Junior et al. (2017).

2.2. Determination of total selenium and barium in Brazil nuts

From the 50 seeds collected from each plant, three were randomly chosen and dried in an oven (Hamco Laboratory oven, Semi-Automatic, India) at 60 °C until reaching constant weight (after ~ 72 h). Initial and final weights were recorded for all samples. After peeling, they were ground with an electric hand mill (Ika-A11 basic BS32, Germany). After grinding, 0.5 g of each sample was taken in triplicate for digestion, using the methodology described by Silva Junior et al. (2017). In short, digestion was performed using 6 mL of a mixture (2:1 v/v) composed of nitric acid ($HNO_3 \geq 65\%$) and perchloric acid ($HClO_4$, 69.7%) in a digester block (Tecnal, Bloco digestor micro TE-040/25, Brazil).

Selenium in the digested Brazil nut samples was analyzed by GF-AAS

Table 1
Information about the sampling sites of Brazil nuts samples in the Amazon region.

Municipality/ State	GPS coordinates		Altitude (m)	Climate: Köppen class
Sena Madureira/ AC	9°25′54.59″S	68°35′42.98″W	232	Am
*Itacoatiara/ AM	3°01′05.59″S	58°49′55.60″W	92	Af
Laranjal do Jari/AP	0°33′50.61″S	52°18′23.43″W	135	Am
Caracaraí/RR	1°28′10.09″N	60°44′16.96″W	107	Am
Porto Velho/ RO	8°48′30.13″S	63°50′47.17″W	103	Am
Santarém/PA	3°03′15.18″S	54°55′37.79″W	92	Am

* Brazil nut plantation in which samples were collected from clones (grafted plants) originated from the State of Pará.

(Atomic Absorption Spectrometry with Graphite furnace; AAnalyst™800 AAS, Perkin Elmer, USA). The analytical determination of Ba was performed via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Spectro - model Blue, Germany). A standard stock solution containing 1 g kg^{-1} of Se and Ba (98% purity, Fluka, Buchs, Switzerland) was used to prepare the calibration curve for both elements' determination. Data for total Se and Ba concentration in Brazil nuts samples were reported on a dry weight basis (DW) and expressed in μg g^{-1}.

For quality assurance and control in Se and Ba measurements, a standard reference material from the Institute for Reference Materials and Measurements (White Clover - BCR 402, IRMM, Geel, Belgium) and another from the National Institute of Standards and Technology (Tomato Leaves - SRM 1573a, NIST), were used respectively for Se and Ba. Additionally, three blank samples were included in each batch of digestion to calculate detection and quantification limits.

The mean recovery value obtained for the standard materials confirmed the reliability of the data. It was 100 ± 10.8% (n = 5) for Se in white clover (BCR-402) and 102 ± 7.4% (n = 7) for Ba in tomato leaves (SRM 1573a). The detection and quantification limits (LOD and LOQ) were established using eight blank extracts. The values were calculated with three and ten times the standard deviation (LOD and LOQ, respectively) of the eight individually prepared blank solutions for Brazil nut samples. The LOD and LOQ for Se were respectively 2.59 and 8.65 μg kg^{-1}. The LOD and LOQ for Ba analysis were respectively 6.61 and 22 μg kg^{-1}.

2.3. μ-XRF analyses: benchtop system

For μ-XRF 2D mapping, Brazil nuts (shelled) from each sampling site were gently cut in longitudinal and transversal sections, trying to get the slices from the middle of the nut, *ca.* 3 mm thick, using a stainless-steel blade, and then placed in a sample holder with a Kapton tape and the inner side of the seed exposed for analysis. The microanalysis was carried out using a benchtop microprobe X-ray fluorescence spectrometer (μ-XRF) system (Orbis PC EDAX, USA) operated with an Rh X-ray tube at 50 kV and 300 μA, using a 25 μm thick Ni filter and under vacuum. The spatial distribution of the elements Se, Ba, P, S, and Br in Brazil nuts seeds was performed using the benchtop μ-XRF. In order to observe the elemental distribution on a big scale (whole nut assessment), the X-ray beam spot size was delimited to 1 mm by a collimator, and the detection was carried out by a 30 mm^2 silicon drift detector with a dead time of nearly 3%. Maps were registered using a 32 × 25-pixel matrix (number of analyzed points on xy-axes) and dwell time per pixel of 5 s. The edition of the images was performed by the software Origin Pro 2016 (OriginLab Corporation, Northampton, USA).

To build up the 2D maps, the analytical signal was separated from the background using the threshold Eq. (1).

$$threshold\ (cps) = 8.45 * \sqrt{\frac{BG\ (average)(cps)}{t(s)}} \quad (1)$$

where $BG_{(average)}$(cps) corresponds to the average background calculated from 10 randomly selected points within the sample and t(s) is the dwell time per point.

2.4. μ-XRF analyses: synchrotron

2D mapping via synchrotron μ-XRF analysis was performed in the bottom extremity of the shelled Brazil nut cut in a longitudinal section with ~ 3 mm thick to observe the elemental distribution on a small scale. The spatial distribution of the elements Ca, K, P, S, Zn, Fe, Cu, Mn, Se, and Ba in the Brazil nuts seeds was performed using synchrotron-based X-ray micro-fluorescence in the XRF beamline at the Brazilian Synchrotron Light Laboratory (LNLS, Campinas, State of São Paulo, Brazil).

The cut shelled nut samples were fixed to a holder positioned at 45° from the detector and the incident beam. The sample's image plane precision was 0.5 μm with three axes (X, Y, and Z) controlled by stepper motors. The samples were excited using a 30 x 30 $μm^2$ white beam focused by a KB system. The pitch size was 30 μm in both directions and the acquisition time was 500 ms per pixel. The electron's energy in the storage ring was 3.5 GeV with a current range of 200–300 mA. The beam brightness in BL15U was 0.5 × 10^{12} photons/s/mm^2/$mrad^2$/0.1 % PC.

Fluorescence data was displayed as color maps, and the pixel brightness was displayed in RGB, with the brightest points corresponding to the fluorescence of the element at the highest concentration in the sample. The processing of the μ-XRF images was performed using the PyMca 5.2.2 software (Solé et al., 2007).

Besides the total quantification of Se and Ba in Brazil nuts, both elements and sulfur (S) had their concentrations estimated using PyMca fit results of each mapped region of the studied samples (bottom section of longitudinal cuts). The values were obtained using White Clover (BCR 402, IRMM, Geel, Belgium) as a standard reference material prepared in a tablet, with known density (0.33 g cm^{-3}). After the acquisition, the spectrum was used to estimate the elemental concentrations in Brazil nut samples. The calculation of the sample composition was based on the general relationship between the concentration (*Ci*) of the analyte *i* and the measured net intensity (*Ii*), which is simply expressed by Eq. (2)

$$Ci = Ki\ x\ Ii\ x\ Mis \quad (2)$$

Where *Ci* = concentration of analyte *i*, Ki = calibration constant factor of *i*, *Ii* = measured net intensity of *i* and *Mis* = correction factor for matrix effects of specimen "*s*" on *i*. The advanced fitted results are presented in Section 2 of the supplementary material, providing the calibration parameters.

2.5. Statistical analyses

A descriptive analysis of Se and Ba concentrations was performed using box plots for illustration of data distribution using the software R 3.6.2 (R Development Core Team, 2020). Regression analysis for Se x Ba levels in Brazil nuts was performed using a linear regression model. To attend the statistical assumptions previous to regression analysis (i.e., normality, linearity, and homoscedasticity), data for Se and Ba were log-transformed before fitting the equation. To complement the results, scatter plots showing correlation among Se, Ba, and S using μ-XRF intensities were performed with the PyMca image correlation tool (PyMca 5.2.2) (Solé et al., 2007), and are presented in Fig. S2.

3. Results and discussion

3.1. Selenium and Ba concentration in Brazil nuts assessed by GF-AAS/ICP-OES

Selenium concentrations in Brazil nuts ranged from 0.46 (Sena Madureira-Acre) to 356 μg g^{-1} (Santarém-Pará) with an average concentration of 52.2 μg g^{-1}. The nuts sampled from the sites Santarém-Pará and Itacoatiara-Amazonas presented the highest Se concentrations (median of 249 and 68.2 μg Se g^{-1}, respectively) and the samples from Sena Madureira-Acre and Porto Velho-Rondônia the lowest Se concentrations (median of 2.52 and 8.83 μg Se g^{-1}, respectively). Caracaraí-RR and Laranjal do Jari-AP had nuts with intermediate Se concentrations (median of 10 and 48.6 μg Se kg^{-1}, respectively) (Fig. 1).

The geographical location of each Brazil nut stand, where the samples were collected, becomes an important variation factor because it reflects the soil physicochemical attributes and environmental characteristics that affect Se and Ba bioavailability. Selenium concentrations in Brazil nut samples in the present study were higher when compared with a previous study performed by Lima et al. (2019) with Se levels ranging from 10 to 79 μg g^{-1}, and the values observed by Silva et al. (2013),

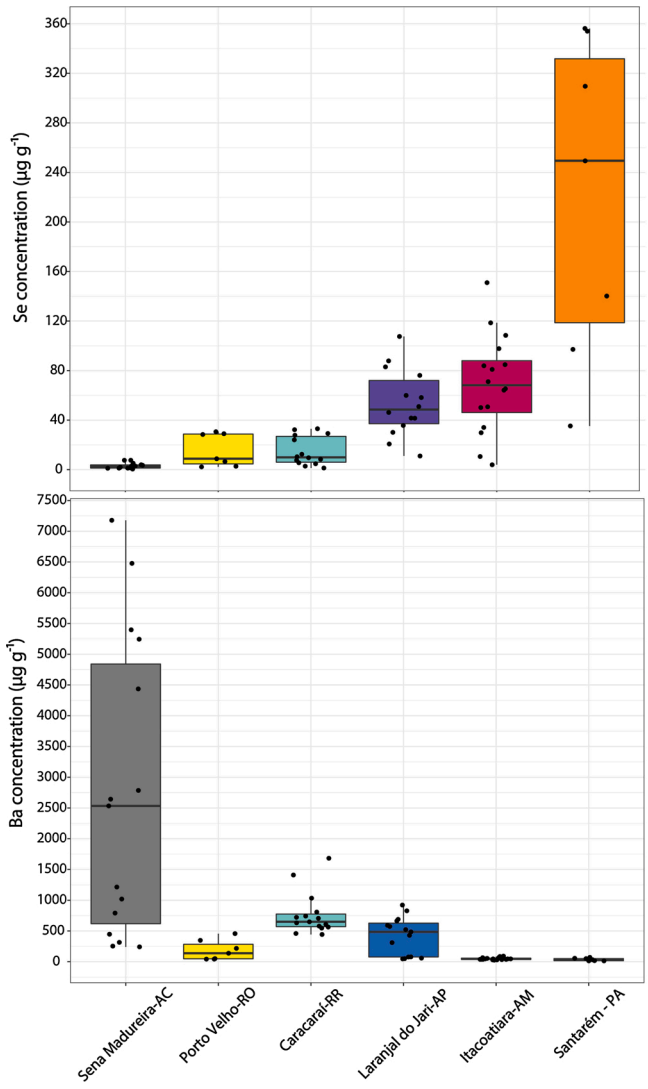

Fig. 1. Box plot for total Se and Ba concentrations in Brazil nuts samples from the respective sites: Sena Madureira-Acre (n = 14), Porto velho-Rondônia (n = 7), Caracaraí-Roraima (n = 14), Laranjal do Jari-Amapá (n = 14), Itacoatiara-Amazonas (n = 16), and Santarém-Pará (n = 7). Obs.: Data for Se in Brazil nuts from the sites Sena Madureira-Acre, Caracaraí-Roraima, Laranjal do Jari-Amapá, and Itacoatiara-Amazonas were previously reported by Silva Junior et al. (2017).

where samples acquired in local market had 54.8 (± 4.6) µg g^{-1} (n = 3) and Dumont et al. (2006) who reported values ranging from 5.1 to 49.9 µg g^{-1}. However, the wide variation trends among different samples/sites remain similar.

Barium concentration varied from 12.5 (Santarém-Pará) to 7177 µg g^{-1} (Sena Madureira-Acre). The highest median concentrations are from Sena Madureira-Acre and Caracaraí-Roraima (2533 and 650 µg g^{-1}, respectively), while the lowest median concentrations are found in the sites Itacoatiara-Amazonas (genotypes from the State of Pará) and Santarém-Pará (median of 45.3 and 26.9 µg g^{-1}, respectively) (Fig. 1).

An early study performed by Smith (1971) observed the unique capacity of Brazil nuts to accumulate Ba. The authors used Neutron Activation Analysis (NAA) for Ba determination and reported concentrations ranging from 190 to 5890 µg g^{-1} for the endosperm, being two to three times as high as for the outer tissues of the fruit, and about twenty times greater than the sapwood of the trunk. Comparatively, Ba concentrations in the present study reached values higher than those found by Parekh et al. (2008), who reported concentrations ranging from 96 to 1990 µg g^{-1} in nuts from Brazil, Bolivia, Peru, and Northern South America, and also higher than those reported by Gonçalves et al. (2009), who referred to concentrations ranging from 860 to 2084 µg g^{-1} in nuts from several Brazilian samples.

It is important to mention the comparison between samples from the sites Itacoatiara-Amazonas and Santarém-Pará, which presented similar behavior in terms of accumulation of Se and Ba in Brazil nuts (i.e., highest Se concentration and lowest Ba concentration). This particular case may be explained because the clones grown in the site Itacoatiara-Amazonas (Aruanã farm) originated from the state of Pará. Therefore, the genotype of these two groups (populations) of plants share closer characteristics than those from the other studied sites. Apart from that, they are cultivated in different soils and therefore will have different environmental and physicochemical soil properties influencing the plant's uptake for Se and Ba. However, we recognize that the genotype may represent a great influence on the amount of Se and Ba accumulated by the plants.

A 99-fold difference was observed between the median Se concentration from the site Sena Madureira-Acre (lowest Se concentration reported) and Santarém-Pará (highest Se concentration reported). For Ba, a 94-fold difference between the median concentration from the site Santarém-Pará (lowest Ba concentration reported) and Sena Madureira-Acre (highest Ba concentration reported) was observed. Remarkably, the sites with highest concentrations for both Se (Santarém-Pará, in the northern portion of the Brazilian Amazon) and Ba (Sena Madureira-Acre, in the southern part of the Brazilian Amazon) present wider variation in concentrations within the site than the other sites with lower concentrations reported (Fig. 1). Geographically, taking the Amazon region as a whole (the whole rainforest covering South America), the concentrations reported in the present study agree with the ones described by Parekh et al. (2008), who found higher Ba concentrations for samples collected in the south of Amazon (Bolivia) and greatest Se concentrations measured in samples from what they described as Northern South America (presumably Venezuela or Colombia).

A linear regression model with the data log-transformed was fit for the studied variables (Se x Ba concentration in Brazil nuts). The equation demonstrated low but noticeable (R^2 = 0.30) trending between the levels of Se and Ba in Brazil nuts (Fig. 2). The scatter plot shows that when Se concentration in Brazil nuts is high, the levels of Ba decreases, being this behavior confirmed in both graphics (Figs. 1 and 2). Supporting the present results, Armelin et al. (2019) observed an inverse correlation between the concentrations of Se and Ba in Brazil nuts. Even

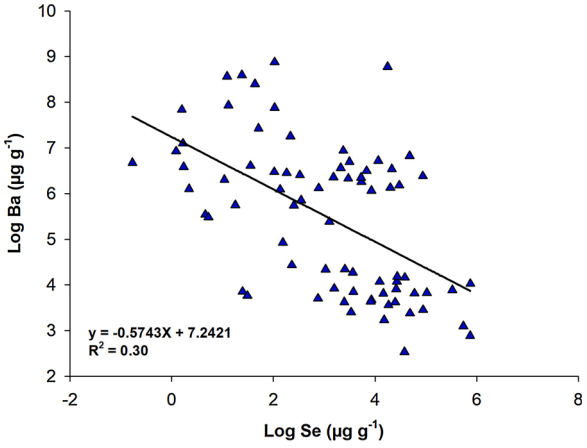

Fig. 2. Linear regression model for total Se x Ba concentration in Brazil nuts from the six sites studied. Data were log-transformed before applying the linear regression model to meet parametric statistical assumptions. The information expressed in the graphic represents the equation with its regression coefficient (R^2).

though the data in the present study is not large enough to better visualize the relationship between Se and Ba concentrations in Brazil nuts this topic opens a new field of discussion for the possible negative effect caused by a preferential uptake, translocation, and accumulation of Se over Ba or vice-versa by the Brazil nuts tree from the roots to the edible seeds. This preferential uptake is assumed to occur depending on the availability of both elements in soil and the plant's ability and affinity to absorb the elements.

3.2. XRF 2D mapping with benchtop system

The investigation of longitudinal and transversal sections of shelled Brazil nuts seeds by μ-XRF showed wide variation in the spatial distribution of the elements in the tissues, following sometimes irregular distribution with some hot spots scattered throughout the seed. The average spectrum of the samples analyzed via μ-XRF benchtop system for the sites Sena Madureira-Acre, Itacoatiara-Amazonas, Laranjal do Jari-Amapá, and Caracaraí-Roraima is shown in Fig. S1 of the supplementary material.

Selenium and Ba distribution in Brazil nuts seeds presented different behavior according to the origin (site studied), reflecting the concentration of the elements in the seed. According to the concentration range in which the sample location site could fit, it was possible to identify four different scenarios in terms of variations in elemental distribution as follow:

1) For the site Sena Madureira-Acre (with low Se, < 10 μg g^{-1}; and high Ba, > 1000 μg g^{-1}), Se is distributed randomly with a few scattered hot spots filling the seed and presenting similar distribution in the transversal section. On the other hand, Ba, for both longitudinal and transversal sections, shows a notable higher intensity at the seed's border, where the epidermal layer is located. Barium surrounds the external layer of the seed, and the presence of the element internally is much less visible than in the epidermal tissue, remarkably in the longitudinal section (Fig. 3). Therefore, in theory, if during the peeling of Brazil nuts, the epidermal tissue is removed partly or completely, Ba concentration would be lower in the remaining edible nut.
2) For the site Laranjal do Jari-Amapá (with high Se, > 50 μg g^{-1}; and medium Ba, 100−1000 μg g^{-1}), Se tends to be more concentrated in the bottom of the seed, which happens to be the seed's root apex (radicle), perfectly visible in the longitudinal section. Curiously, the root pole (seed base), from where the primary root originates when the seed germinates, could be identified because it is larger than the stem pole, where originates the aerial part of the plant (Simone and Gurgel, 2006). For the transversal section, it is possible to observe a "ring shape" where Se intensity is higher. Such results indicate that Se is possibly being concentrated in the ring formed by the outer parenchyma layer, in the frontier with the seed's procambium ring. For Ba, the behavior is similar to that reported for the sample from Sena Madureira-Acre, in which Ba contours the external epidermal layer of the seed in the longitudinal section, but for the image of transversal section, this ring formed is a lot more visually expressive than the image in transversal section of Sena Madureira's sample. When comparing the hot spots formed by Se and Ba in this sample, the difference is that Ba "ring" is more externally located, adjacent to Se, and comes right above the seed's epidermal layer (Fig. 3).
3) For the site Itacoatiara-Amazonas (with high Se, > 50 μg g^{-1}; and low Ba, < 50 μg g^{-1}), Se presents a hot spot in the seed's root apex (radicle), which follows the same pattern as the sample from Laranjal do Jari-Amapá in the longitudinal cut. The Se ring shape is not regular in the transversal section because of the unusual anatomy of the seed sampled. Still, it forms a layer in the parenchyma region that is even thicker than the observed in other samples. Barium was not

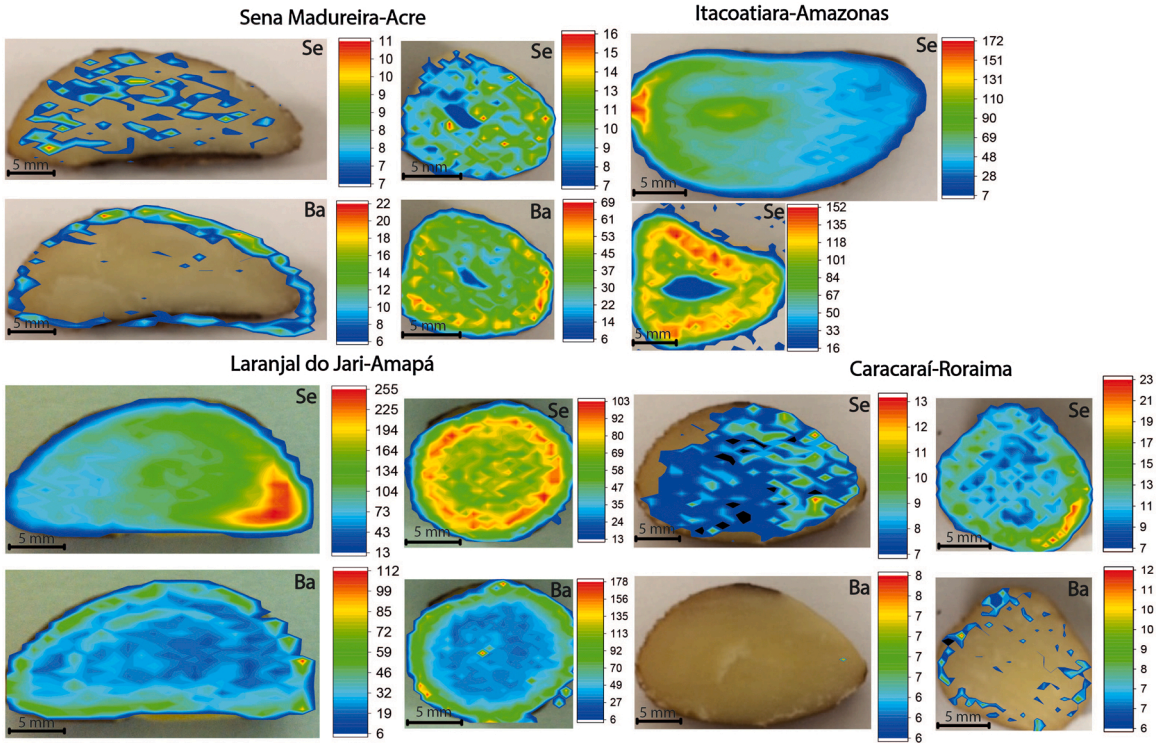

Fig. 3. Selenium and Ba distribution in Brazil nuts seed samples from the sites Sena Madureira-Acre, Itacoatiara-Amazonas, Laranjal do Jari-Amapá and Caracaraí-Roraima, represented in longitudinal and transversal sections. Selenium and Ba average concentrations are respectively for each site: Sena Madureira-Acre: 3.09 and 2732 μg g^{-1}; Itacoatiara-Amazonas: 69.1 and 48.4 μg g^{-1}; Laranjal do Jari-Amapá: 53.6 and 422 μg g^{-1}; and Caracaraí-Roraima: 14.9 and 772 μg g^{-1}. The temperature bars indicate Se-Kα and Ba-L Net counts obtained by the μ-XRF Orbis PC EDAX. Obs.: Barium was not detected by the XRF technique in the sample from Itacoatiara-Amazonas.

detected by the μ-XRF scanning in the sample from the site Itacoatiara-Amazonas because it was below the limit of detection of the technique (Fig. 3).

4) For the site Caracaraí-Roraima (with medium Se, 10−50 μg g^{-1}; and medium Ba (100−1000 μg g^{-1}), interestingly Se intensity and distribution reflected its intermediary concentration reported for both longitudinal and transversal sections. Barium intensity and distribution, on the other hand, were barely noticed by a tiny spot in the longitudinal section and a few hot spots surrounding the external layers in the transversal section.

The results demonstrated that Se forms a "ring shape" in the samples containing higher total concentrations and also presents a higher intensity in the inferior apex of the seed (root pole). But this "hot spot" with higher Se intensity in the bottom is not visible for the samples with low and medium Se content for example (Sena Madureira-Acre and Caracaraí-Roraima with average Se = 3.08 and 14.9 μg g^{-1}, respectively), and therefore Se tends to be more homogeneously distributed in the whole seed. Barium distribution in the longitudinal sections of samples from Sena Madureira and Laranjal do Jari (with Ba = 2732 and 422 μg g^{-1}, respectively) was more concentrated in the seed's peripheral region, forming an almost continuous line in the border of the seed along with the epidermal tissue. In the transversal sections, the distribution pattern is similar and supports what was observed in the longitudinal cuts, except for the sample from Caracaraí-Roraima, which showed fewer hot spots (Fig. 3).

Therefore, since Se is more internally located than Ba, we hypothesize that this element is present in the outer layer of cells of the undifferentiated parenchyma of the hypocotyl, which is more active, with the potential to restart meristematic activities. In contrast, Ba is more externally located, mainly present in the epidermal layer that constitutes the endosperm (Camargo et al., 2000). Similarly to what was observed in our study, μ-XRF analyses in a cross-cut shelled Brazil nut performed by Lima et al. (2019) showed a "ring-shaped" hot spot of Se concentration 1–2 mm from the seed's exterior, in agreement with the distribution found in the longitudinally cut seed as well. As suggested by these authors, Se might be accumulating in such a way that it can be readily distributed to the growing meristems during seed germination and serve to protect these tissues from biotic stresses. The high Se concentrations found in the nuts from the present study are reasonably comparable with other researches, with hyperaccumulating plants demonstrating Se as protection from herbivores, pathogens, or even toxic substances (Mehdawi and Pilon-Smits, 2012; Huang et al., 2017).

The maps with spatial distribution of S and P are presented in Fig. 4. Observing the samples from Laranjal do Jari-Amapá in longitudinal and transversal sections and Itacoatiara-Amazonas in transversal sections, it is possible to identify that both S and P present higher intensities in the external layers of the endosperm, which can be assumed to be the region of the seed with higher meristematic activity. In the samples from Caracaraí-Roraima only P in the longitudinal cut was detected, and yet in a small spot in the center of the seed. The other maps were below the detection limit of the technique for both S and P. For the samples from Sena Madureira-Acre, P and S in transversal sections presented intensities below the calculated threshold (Eq. 1), and for the longitudinal sections, just a few visible spots were above the threshold for P and S.

Despite not being quantified by the conventional approaches (GF-AAS/ICP-OES), bromine (Br) was mapped with surprisingly high intensity in Brazil nuts from all four studied sites, as can be observed by the prominent peaks of Br visible in the mean spectrum of the μ-XRF analyses (Fig. S1) and the estimated concentration in the μ-XRF mapped area for the sample RR7 (Caracaraí-Roraima) was 307 μg g^{-1} (Table S1). It was not possible to identify a clear pattern of spatial distribution in the samples studied. The maps show a different distribution behavior for each of the samples assessed. In general, what could be observed was that intensities were higher more internally in the endosperm than externally in the epidermal tissue, and there was a remarkable hotspot in the core of the sample from Caracaraí-Roraima, in the longitudinal section (Fig. 5).

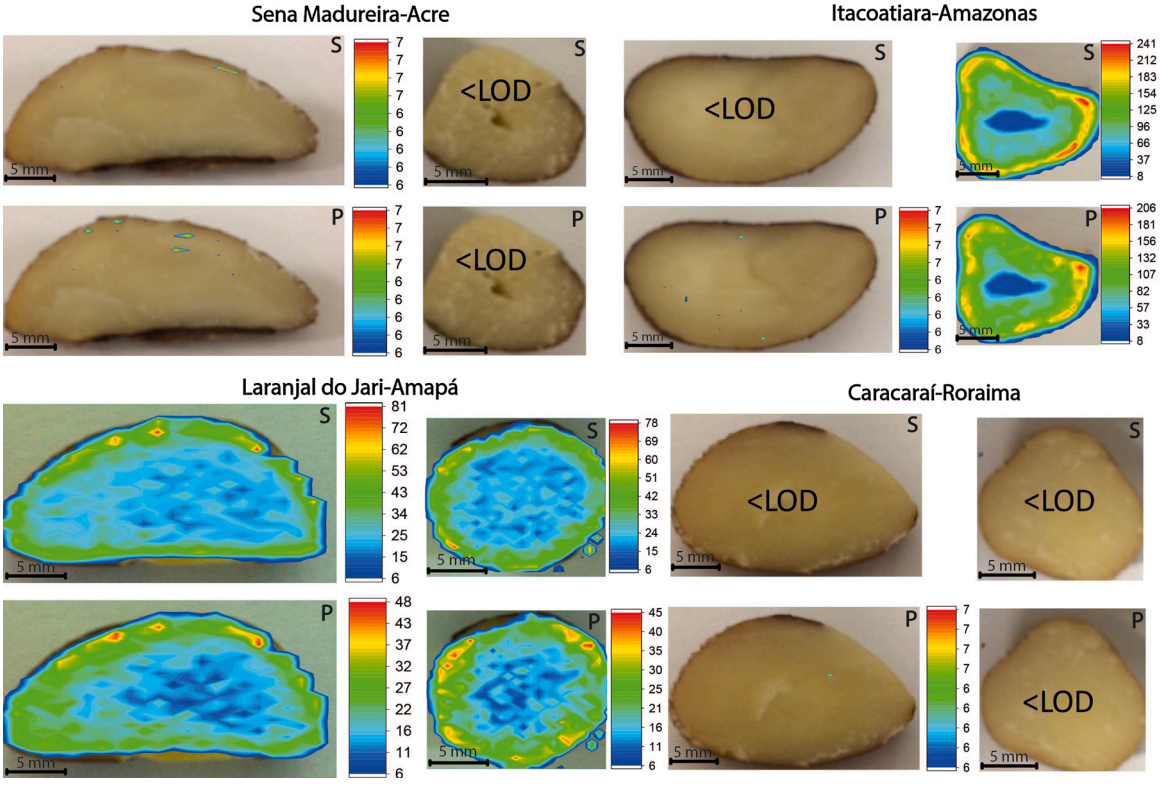

Fig. 4. Sulfur and P distribution in Brazil nuts samples from Sena Madureira-Acre, Itacoatiara-Amazonas, Laranjal do Jari-Amapá and Caracaraí-Roraima in transversal and longitudinal sections. The temperature bars indicate net counts of each element obtained by the μ-XRF Orbis PC EDAX.

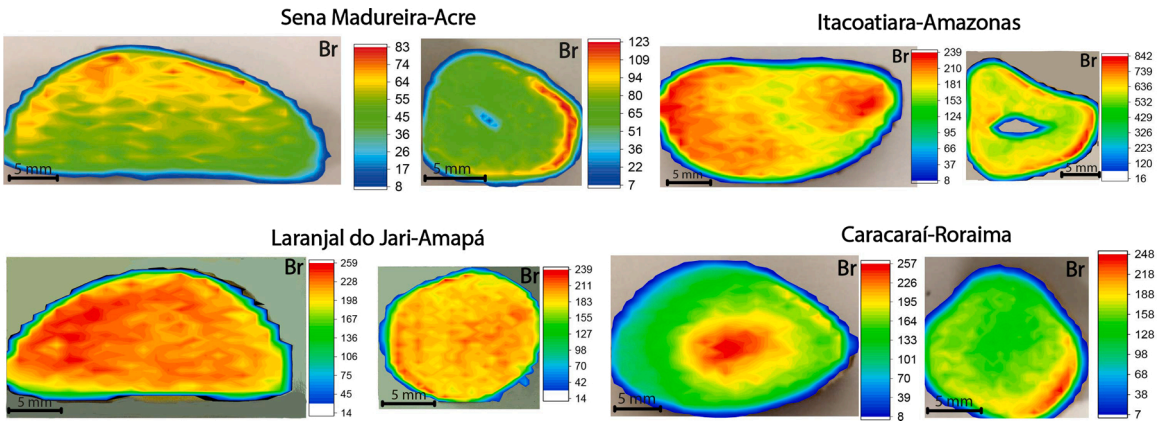

Fig. 5. Bromine (Br) distribution in Brazil nuts seed samples from the sites Sena Madureira-Acre, Itacoatiara-Amazonas, Laranjal do Jari-Amapá and Caracaraí-Roraima, represented in longitudinal and transversal sections. The temperature bars indicate Br-Kα Net counts obtained by the μ-XRF Orbis PC EDAX.

To note, bromine is considered non essential to human health, but in suitable doses, it has been recommended as an antiepileptic agent, yet it can also be combined with hemoglobin causing hematologic diseases at high doses. The intake of high concentrations can reduce iodide accumulation - not only in the thyroid but also in mammary glands - and increase iodide elimination through the kidneys (Nascimento et al., 2017). A high Br concentration was reported in Brazil nuts by Furr et al. (1979) with 87 μg g^{-1} dry weight, besides high levels of Se (11 μg g^{-1}) and Ba (1764 μg g^{-1}). There is no recent literature reporting Br in Brazil nuts specifically. Therefore, further studies are required to better understand its accumulation and concentration range.

3.3. X-ray synchrotron microprobe analysis

The RGB images obtained by synchrotron XRF served as complementary information for the images obtained in the benchtop system. These RGB maps are on a smaller scale to obtain detailed information from the bottom extremity of the Brazil nuts seeds. This seed section contains "hot spots" with higher Se intensities as observed by the previous approach and where we assumed that the presence of Se, Ba, or S

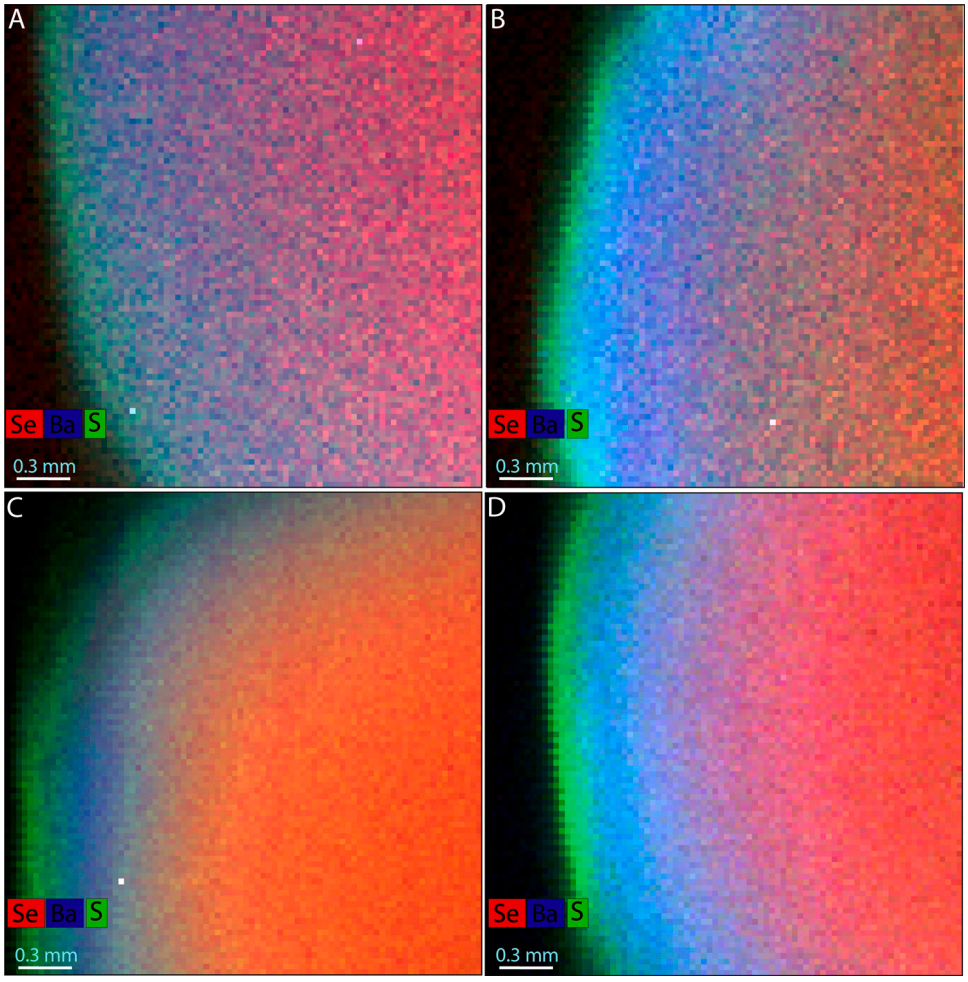

Fig. 6. XRF-Synchrotron analyses. RGB images for selenium, barium and sulfur in the basal region of the Brazil nut seed (longitudinal section). The legends for the sites, Se, Ba and S concentrations are respectively: **A** = Sena-Madureira-Acre (AC9, Se = 1.26, Ba = 2914, S = 533 μg g^{-1}); **B** = Porto velho-Rondônia (RO14, Se = 6, Ba = 511, S = 1268 μg g^{-1}); **C** = Laranjal do Jari-Amapá (AP13, Se = 314, Ba = 646, S = 1700 μg g^{-1}); **D** = Santarém-Pará (PA1702, Se = 67.5, Ba = 24.9, S = 1293 μg g^{-1}).

would play key roles in the meristematic seed tissues such as in the differentiation of the plant when it germinates and also in the solubility of Se and Ba themselves, which makes them more or less bioaccessible for humans.

Brazil nut samples were grouped according to their respective Se concentrations and depending on the sub-region in which they were collected, as follow: 1) For samples with lower Se concentrations (Porto Velho-Rondônia and Sena Madureira-Acre, located in the southern Amazon), there is a more diffuse transition between the color layers corresponding to the intensities of S, Ba, and Se. This distinction starts in the perisperm layer where S is more concentrated, going through the procambium and epidermal layers (higher Ba intensity) and finally the outer parenchyma (Se) in the bottom of the seed. The predominance of blue color in the area mapped of the endosperm for samples from the sites Sena-Madureira-Acre and Porto Velho-Rondônia indicates the high concentration of Ba for those samples, and therefore a certain predominance of this element over Se and S (Fig. 6A and B); and, 2) For samples with higher Se concentration (Santarém-Pará and Laranjal do Jari-Amapá, located in the northern Amazon), the transition between the layers is more apparent, following the division from the outer layer (periderm tissue) with predominant S, procambium and epidermal tissue with Ba, and beginning of the parenchyma with the predominance of Se. Remarkably, these sample sections showed the edge (beginning) of the ring formed by the outer parenchyma tissue with the clear predominance of Se, which indicates the high Se concentration in this region of the seed (Fig. 6C and D). The predominance of Se in tissues with higher meristematic activity may reveal its importance in protecting the seed against biotic and abiotic stress or even playing key roles during the germination process. These findings agree with the results observed by the whole seed's images (benchtop equipment) (Fig. 3).

The relationship between Se, Ba and S in the mapped region of the Brazil nuts seeds was of interest because of the possibility of an elemental association forming compounds of low solubility such as barium selenate ($BaSeO_4$) and barium sulfate ($BaSO_4$) as the colors are visibly mixed and the transition is diffuse from one tissue layer to the other depending on the concentration of the mentioned elements (Fig. 6). The mass ratios for Ba:S present in $BaSO_4$ is 4.28 and for Ba:Se present in $BaSeO_4$ is 1.74. Comparing these ratios with the values observed for the samples presented in Fig. 6, the one with the closer ratio to form $BaSO_4$ is the sample from Sena Madureira-Acre (Ba:S mass ratio = 5.47) whereas $BaSeO_4$ could be formed in the sample from Laranjal do Jari-Amapá (Ba:Se mass ratio = 2.05) (Table S2). Remarkably, according to the mass ratios, the samples with higher possibility of forming $BaSO_4$ and $BaSeO_4$ are respectively the ones with highest Ba and Se concentration. Based on the concentration of these elements present in the seeds (Se, Ba, and S), for most Brazil nuts samples there is a chance that it would be partially forming either barium sulfate or barium selenate, but the existence of such relationships would only be confirmed through chemical speciation analyses focused in the different tissue layers of the seeds.

For Brazil nuts seeds, it is still not possible to confirm if Se, which is believed to be accumulated in the parenchyma tissue cells (most of the

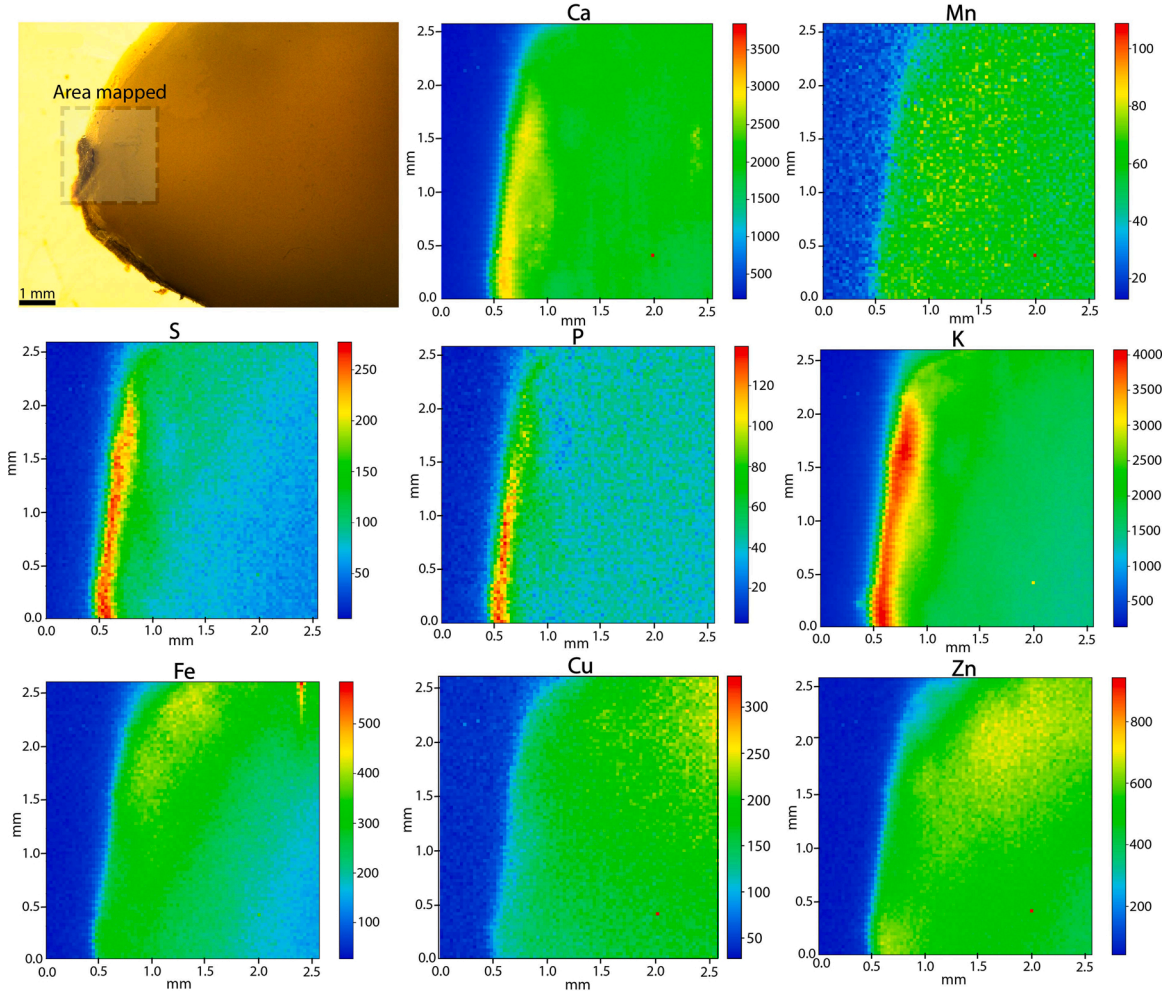

Fig. 7. Images of the mapped region of a Brazil nut sample from Caracaraí-Roraima (RR7) in a small scale (root pole in the bottom extremity of the seed), in longitudinal section with the spatial distribution of chemical elements. The temperature bars indicate net counts of each element obtained by Synchrotron μ-XRF.

endosperm), will be more associated with the proteins themselves or dissolved with the lipids fraction contained in this tissue. For seeds of staple food such as rice and wheat, there is evidence that Se distribution in tissues is strongly associated with the protein distribution, especially a protein matrix present in the endosperm (Lessa et al., 2019; Reis et al., 2020).

Brazil nuts seeds' germination process is particularly very slow and takes up to 6 months in total if no special treatment is used. Such delay is due to endogenous seed dormancy, which may be attributed to Se and/or Ba's high levels in the Brazil nuts seed embryo. To prove if this dormancy mechanism exists would be needed to explore this research gap and determine whether the dormancy is due to a chemical inhibitor, an immature embryo, or both (Kainer et al., 1999; Santos et al., 2013).

The XRF images shown in Fig. 7 represent data from a particular sample from the site Caracaraí-Roraima (RR7). The major elements S, P, and K (except for Ca) are more concentrated at the edge of the seed. The microscope picture with the area mapped shows a slice of the perisperm tissue mostly composed of lignin and dead cells, where those elements coincide with their hotspots. On the other hand, the micronutrients (Mn, Fe, Cu, and Zn) are more homogeneously distributed throughout the bottom of Brazil nut seed mapped. The estimated concentrations via XRF technique are: P = 1801 μg g^{-1}, S = 1270 μg g^{-1}, K = 3404 μg g^{-1}, Ca = 2021 μg g^{-1}, Mn = 4.42 μg g^{-1}, Fe = 23.7 μg g^{-1}, Cu = 18.7 μg g^{-1}, and Zn = 72.8 μg g^{-1}. Detailed information of the sample RR7 elemental mapping is presented in Table S1.

Quantification of major and microelements in Brazil nuts was performed previously by Brito et al. (2019), who reported ranges for K (337–2981 μg g^{-1}), Ca (142–3436 μg g^{-1}), P (64.9–6708 μg g^{-1}), Fe (5.7–36.8 μg g^{-1}), Mn (0.1–11.4 μg g^{-1}), and Zn (3.1–48.9 μg g^{-1}). Lima et al. (2019) also measured S (1.6–4.8 g kg^{-1}) and Cu (11.2–56 μg g^{-1}). Comparatively, in the present study, only K and Zn levels were higher than those previously mentioned, with levels for other elements falling within the ranges reported. The overall presence of high concentrations of P and S in Brazil nuts can be associated with the relatively high concentration of proteins (Naozuka et al., 2011). Also, the concentration of these elements may vary according to climate and soil characteristics, which influence nutrient uptake levels by the plant (Cardoso et al., 2017).

4. Conclusions

Selenium and Ba total concentrations in Brazil nuts can be considerably high depending on the origin site of the sample. Also, their spatial distribution in Brazil nuts seeds varies depending on the site where the samples are obtained.

Selenium tends to accumulate in the outer parenchyma tissue of Brazil nuts seeds, forming a "ring shape" surrounding the seed. Another accumulation pattern observed was that Se intensity for the high-Se concentration samples presented a hot spot in the bottom extremity. Barium accumulation tends to have similar behavior, but this element concentrates in a "ring" more externally located in the seed, around the epidermal tissue.

The possibility of forming low solubility compounds such as $BaSO_4$ and $BaSeO_4$ is higher when Ba and Se concentrations are higher respectively. But to confirm this assumption, it would be necessary to perform future speciation analyses and find out how these elements are chemically present in Brazil nuts seed tissues.

The μ-XRF technique in both benchtop and synchrotron approaches agree in terms of results obtained and showed to be successful for mapping the spatial distribution of Se, Ba, and other relevant elements in Brazil nuts from different Amazon agroecosystems. Nevertheless, further studies are necessary to elucidate the mechanisms driving the accumulation of these elements in Brazil nuts seeds.

Author statement

ECSJ did the conceptualization, investigation, formal analysis, data curation, and writing the original draft; **NMD** helped in the methodology, writing - review & editing; **JHLL** was in charge of the investigation and methodology; **PGR** helped in the investigation, writing - review & editing; **LHOW** provided resources, writing - review & editing; **KES, RMBL, KDB, MCG** and **RCOJ** provided resources, writing - review & editing; **HWPC, ARR and GL** supervised, helped in review & editing; **LRGG** is the senior author and collaborated in the supervision, funding acquisition, conceptualization, writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2021.104329.

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