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EDIU CARLOS DA SILVA JUNIOR

SELENIUM, BARIUM, ARSENIC AND IODINE IN BRAZIL NUT AGROECOSYSTEMS: SOIL-NUT-HUMAN RELATIONSHIP

LAVRAS – MG
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“Parece-me provável que Deus, no começo, formou a matéria em partículas sólidas, compactas, duras, impenetráveis e móveis, de tais dimensões e configurações, e com outras propriedades tais, e em tais proporções com o espaço, que sejam as mais compatíveis com a finalidade para que Ele as formou; e que essas partículas primitivas, sendo sólidas, são incomparavelmente mais duras do que quaisquer corpos porosos compostos por elas; realmente tão duras que nunca se desgastam nem se fragmentam, e não existe nenhuma força comum que seja capaz de dividir o que o próprio Deus unificou na criação original.”

“A maravilhosa disposição e harmonia do universo só pode ter tido origem segundo o plano de um Ser que tudo sabe e tudo pode. Isso fica sendo a minha última e mais elevada descoberta.”

Isaac Newton, 1642-1727

RESUMO

A castanha-da-amazônia (*Bertholletia excelsa*) é considerada um dos produtos não madeireiros mais importantes da região Amazônica, visto que suas amêndoas constituem um alimento apreciado pelas comunidades locais e internacionalmente. A amêndoa da castanha-da-amazônia é reconhecida no mundo inteiro como o alimento mais rico em selênio (Se), porém há também estudos reportando elevados níveis de Bário (Ba). No mundo inteiro, há situações tanto de deficiência, como de toxidez de Se em solos, com reflexo nos teores presente nos alimentos cultivados nestes solos. O mesmo ocorre para o arsênio (As), Ba e iodo (I) sendo que o primeiro se torna importante devido à região situar-se em uma bacia cujos solos sofrem influência de sedimentos (vulcânicos) dos Andes. O interesse com o Ba se deve a evidências da presença do mineral holandita ($Ba_2Mn_8O_{16}$) em quantidades razoáveis em solos Amazônicos, que hipoteticamente é a causa para elevadas concentrações deste elemento na castanha-da-amazônia. Já o I é um elemento pouco pesquisado no país e região Amazônica, portanto novas informações deste em solos servem como base para pesquisas futuras com biofortificação. Assim, o presente estudo busca conhecer melhor e caracterizar geograficamente os teores e a disponibilidade de Se, Ba, As e I presentes em solos da região Amazônica, verificando a acumulação na castanha-da-amazônia, bem como sua distribuição espacial nas sementes. Finalmente a fração bioacessível destes elementos é quantificada em amêndoas provenientes de diversos sítios. Amostras de solo e amêndoas foram previamente coletadas em seu ambiente natural de produção em floresta nativa e em cultivos estabelecidos em diferentes regiões da bacia Amazônica durante a época de colheita da castanha na região, entre 2014 e 2017, abrangendo sítios nos estados do Acre, Rondônia, Mato Grosso, Amazonas, Roraima, Amapá e Pará. Foram realizadas análises de teores totais de Se, Ba, As, I e outros elementos traços relevantes no solo e em castanhas, das frações disponíveis e solúveis de Se e As no solo, bem como análises de bioacessibilidade *in vitro* de Se e Ba na castanha-da-amazônia com simulação do trato gastrointestinal mediante técnica padronizada. Para complementar, foi feito um estudo de mapeamento em 2D utilizando duas técnicas complementares de µ-XRF (equipamento de bancada e luz síncrotron) para verificação da distribuição espacial de Se, Ba, Br, S e P em amêndoas de castanha-da-amazônia. Os principais resultados mostraram que o pH do solo e a CTC são, indiretamente, os principais atributos químicos que influenciam as frações de As nos solos estudados. As concentrações de Se total no solo variam de 0,05 a 2,48 mg kg^{-1}, estando, portanto, dentro da faixa segura de acordo com a legislação Brasileira. Para Ba, as concentrações no solo variaram de 8 a 765 mg kg^{-1} e, portanto, atingem níveis superiores ao valor de prevenção (150 mg kg^{-1}), com teores anormais na Amazônia Ocidental. O I-TMAH médio na camada superficial dos locais estudados é de 5,43 mg kg^{-1}, o que está acima da média mundial de 2,8 mg kg^{-1}. A técnica µ-XRF, em ambas as abordagens, com equipamento de bancada e síncrotron, revelou resultados concordantes, mostrando que o Se está acumulado principalmente no tecido do parênquima externo das sementes de castanha-da-amazônia formando um "anel" ao redor da semente. O acúmulo de Ba tende a ter comportamento semelhante, mas este concentra-se em um anel localizado mais externamente na semente, ao redor da epiderme. Os resultados gerais mostraram que a bioacessibilidade do Se na fase gástrica simulada (GP) é menor do que na fase do intestino delgado (SI-T1 e SI-T2), enquanto para Ba ocorre o contrário. Os conteúdos bioacessíveis individuais de Se e Ba em castanhas-da-amazônia variam amplamente entre e dentro dos locais estudados, mas a recomendação de ingestão de 1-3 castanhas por dia pode ser considerada segura, considerando os limites de absorção atuais estimados para Se e Ba.

Palavras-chave: Castanha-da-amazônia. Nutrição. XRF. Bioacessibilidade. Geoquímica. Amazônia.

ABSTRACT

Brazil nut (*Bertholletia excelsa*) is considered one of the most important non-timber products of the Amazon region, as its nuts are a food appreciated by local communities and internationally. The nuts of the Brazil nut tree are recognized worldwide as the richest food in selenium (Se), but there are also studies reporting high levels of barium (Ba). All over the world, there are situations of both deficiency and toxicity of Se in soils, reflected in the levels present in the food grown in these soils. The same occurs for arsenic (As), Ba and iodine (I), the first of which is important because the region is located in a basin whose soils are influenced by sediments from the Andes (volcanic). The interest with Ba is due to evidence of the presence of the mineral hollandite ($Ba_2Mn_8O_{16}$) in reasonable quantities in Amazonian soils, which hypothetically is the cause for high concentrations of this element in the nuts. Iodine is a little researched element in the country and in the Amazon region, therefore, new information about it in soils serves as a basis for future research with biofortification. Consequently, the present study seeks to better understand and geographically characterize the contents of Se, Ba, As and I and their availability present in soils in the Amazon region, verifying the accumulation in Brazil nuts, as well as its spatial distribution in the seeds. Finally, the bioaccessible fraction of these elements are quantified in nuts from different sites. Samples of soil and nuts were previously collected in their natural production environment in native forest and farms established in different regions of the Amazon basin during the harvest season in the region between 2014 and 2017, covering sites in the states of Acre, Rondônia, Mato Grosso, Amazonas, Roraima, Amapá, and Pará. Analyzes of the total concentration of Se, Ba, As, I and other relevant trace elements in the soil and nuts were performed. Available and soluble fractions of Se and As in the soil were extracted and *in vitro* bioaccessibility analyzes of Se and Ba in Brazil nuts with a simulation of the gastrointestinal tract using a standardized technique. In addition, a 2D mapping study was carried out using two complementary techniques of µ-XRF (benchtop equipment and synchrotron light) to verify the spatial distribution of Se, Ba, Br, S, and P in Brazil nuts. The main results showed that soil pH and CEC are indirectly the main chemical attributes that influence As fractions in the studied soils. The total concentrations of Se in the soil vary from 0.05 to 2.48 mg kg^{-1} and, therefore, are within the safe range according to the Brazilian legislation. For Ba, the concentrations in the soil varied from 8 to 765 mg kg^{-1} and thus, reach levels above the prevention value (150 mg kg^{-1}), with abnormal levels in the Western Amazon. The average I-TMAH in the superficial layer of the studied sites is 5.43 mg kg^{-1}, which is above the world average of 2.8 mg kg^{-1}. The µ-XRF technique in both approaches, with benchtop equipment and synchrotron, agree in terms of results obtained and showed that Se accumulated mainly in the tissue of the external parenchyma of the seeds of the Brazil nut forming a "ring" around the seed. The accumulation of Ba tends to behave similarly, but it is concentrated in a ring located more externally in the seed, around the epidermis. Our findings showed that the bioaccessibility of Se in the simulated gastric phase (GP) is lower than in the small intestine phase (SI-T1 and SI-T2), whereas for Ba the opposite occurs. The individual bioaccessible contents of Se and Ba in Brazil nuts vary widely between and within the studied sites, but the recommendation to ingest 1-3 nuts per day can be considered safe, considering the currently estimated uptake limits for Se and Ba.

Keywords: Brazil nuts. Nutrition. XRF. Bioaccessibility. Geochemistry. Amazon.

DUTCH ABSTRACT

De paranoot (*Bertholletia excelsa*) wordt gezien als één van de belangrijkste niet-houtproducten (NTFP) van de Amazoneregio. De noot wordt zowel door de lokale bevolking als internationaal geapprecieerd. De noot van de paranotenboom wordt gezien als één van 's werelds rijkste voedingsbronnen van selenium (Se), maar er zijn ook studies die hoge niveaus van barium (Ba) rapporteren. Over de hele wereld zijn er situaties waar er een tekort of toxiciteit aan Se in de bodem te vinden is. Dit wordt weerspiegeld in de niveaus die aanwezig zijn in het voedsel dat geteeld wordt op deze bodem. Hetzelfde zien we ook voor arseen (As), Ba en jodium (I). Voor As is dit belangrijk aangezien deze regio gelegen is in een bekken waar de bodem wordt beïnvloed door (vulkanisch) sediment van het Andes-gebergte. Ook is er bewijs voor de aanwezigheid van redelijke hoeveelheden van het mineraal hollandiet ($Ba_2Mn_8O_{16}$) in de bodem van de Amazoneregio, er wordt verondersteld dat dit de reden is voor de hoge concentraties Ba in de noten. Jodium is een weinig onderzocht element in het land en in de Amazoneregio, daarom zou nieuwe informatie met betrekking tot de aanwezigheid in de bodem kunnen dienen als basis voor toekomstig onderzoek omtrent biofortificatie. Daarom wil deze studie proberen om de inhoud en hoeveelheid van Se, Ba, As en I in de bodem van de Amazoneregio te onderzoeken, te begrijpen en te karakteriseren, alsook de accumulatie in de paranoten te verifiëren en hun ruimtelijke verdeling in het zaad. Ten slotte wordt het biotoegankelijk deel van deze elementen gekwantificeerd in noten van verschillende sites. Er werden monsters verzameld van de bodem en noten in hun natuurlijke productieomgeving, inheemse bossen en boerderijen gelegen in verschillende regio's van het Amazonebekken tijdens het oogstseizoen tussen 2014 en 2017, op sites in de staten Acre, Rondônia, Mato Grosso, Amazonas, Roraima, Amapá en Pará. Er werden analyses van de totale concentratie van Se, Ba, As en I en andere relevante sporen in de bodem en de noten uitgevoerd. Beschikbare en oplosbare fracties van Se en As werden geëxtraheerd en er werden *in vitro* biotoegankelijkheidsanalyses van Se en Ba in paranoten gemaakt met een simulatie van het gastro-intestinaal kanaal via een gestandaardiseerde techniek. Daarnaast werd een 2D mapping studie uitgevoerd via twee complementaire technieken van µ-XRF (tafel-opstelling en synchrotron) om de ruimtelijke verdeling van Se, Ba, Br, S en P in paranoten te verifiëren. The voornaamste resultaten toonden aan dat de pH en CEC van de bodem indirect de chemische hoofdcomponenten zijn die de dynamiek van de As-delen in de onderzochte bodems beïnvloeden. De totale concentraties van Se in de bodem variëren tussen 0.05 en 2.48 mg kg^{-1} en vallen hier dus onder de veiligheidsniveau's van de Braziliaanse legislatuur. Voor Ba variëren de bodemconcentraties tussen 8 en 765 mg kg^{-1} en gaan hier dus voorbij de preventiewaarde (150 mg kg^{-1}), met abnormale niveaus in de westelijke Amazone. De gemiddelde I-TMAH in de bovenste laag van de bestudeerde sites is 5.43 mg kg^{-1}, wat boven het wereldgemiddelde van 2.8 mg kg^{-1} ligt. De µ-XRF techniek verkreeg in beide benaderingen, met tafelopstelling en synchrotron, gelijkaardige resultaten en toonde aan da Se zich vooral accumuleert in het weefsel van het externe parenchyma van de zaden van de paranoten, waarbij het een ring vormt rond het zaad. De accumulatie van Ba heeft de neiging zich op dezelfde manier te gedragen, maar is geconcentreerd in ring die zich meer op de buitenkant van het zaad bevindt, rond de epidermis. De algemene resultaten toonden aan dat de biotoegankelijkheid van Se in de gesimuleerde maagfase (GP) lager is dan in de dunne darm fase (SI-T1 en SI-T2), terwijl voor Ba het omgekeerde gebeurt. De individuele biotoegankelijke delen van Se en Ba in paranoten variëren enorm tussen en binnen de bestudeerde sites, maar de aanbeveling om 1-3 noten per dag te consumeren kan als veilig worden beschouwd, gezien de huidige geschatte opnamegrenswaarden voor Se en Ba.

Trefwoorden: Paranoten. Voeding. XRF. Biotoegankelijkheid. Geochemie. Amazone.

SUMMARY

ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
AC	Acre (State)
Al	Aluminum
AM	Amazonas (State)
AN/ANO	Anori (site)
ANOVA	Analysis of Variance
AP	Amapá (State)
AR	Aruanã (site)
As	Arsenic
B	Boron
Ba	Barium
BAC	Bioaccessible Content
BaT	Total Barium
Br	Bromine
Ca	Calcium
Cd	Cadmium
CEC/T	Cation Exchange Capacity
CONAMA	Conselho Nacional do Meio Ambiente
Cr	Chromium
CRM	Certified Reference Material
Cu	Copper
DEM	Digital Elevation Model
DW	Dry weight
Eh	Redox potential
EROS	Earth Resources Observation and Science
ESe	Exchangeable Selenium
Fe	Iron
GF-AAS	Atomic Absorption Spectrometry with Graphite furnace
GLS	Generalized Least Squares
GP	Gastric phase
Hg	Mercury

HSD	Honest significant difference
I	Iodine
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
IDD	Iodine Deficiency Disorder
IRMM	Institute for Reference Materials and Measurements
K	Potassium
LOD	Limit of detection
LOQ	Limit of quantification
m	Aluminum saturation (%)
MDL	Method detection limit
Mg	Magnesium
Mn	Manganese
MT	Mato Grosso (State)
Na	Sodium
Ni	Nickel
NIST	National Institute of Standards and Technology
OM	Organic Matter
OSe	Organically bound selenium
P	Phosphorus
PA	Pará (State)
Pb	Lead
PCA	Principal Component Analysis
PC1	Principal Component 1
PC2	Principal Component 2
PFA	Perfluoroalkoxy
PV	Prevention Level
PZC	Point of Zero Charge
QRVs	Quality Reference Values
RDA	Recommended Daily Allowance
RfD	Reference dose
RO	Rondônia (State)
RR	Roraima (State)

S	Sulfur
SB	Sum of Basis
SD	Standard deviation
Se	Selenium
SEM	Standard error of the mean
SeMet	Selenomethionine
SEP	Sequential Extraction Procedure
SePP	Selenoprotein P
SSe	Soluble Selenium
SeT	Total Selenium
SGF	Simulated gastric fluid
SI	Small intestine (phase)
SIF	Simulated intestinal fluid
SI-T1	Small intestine phase after 2 h incubation
SI-T2	Small intestine phase after 4 h incubation
SM	Sena Madureira (site)
SRM	Standard reference material
SSF	Simulated saliva fluid
t	Effective cation exchange capacity
TMAH	Tetramethylammonium hydroxide
USEPA	United States Environmental Protection Agency
V	Base saturation (%)
VIF	Variance Inflation Factor
WHO	World Health Organization
XP	Xapuri (site)
Zn	Zinc

FIRST PART: GENERAL INTRODUCTION

1 INTRODUCTION

The Amazon forest is one of the most important ecosystems of the planet, comprising approximately 30% of the humid tropical region. This region is mainly covered by tropical forests and corresponds to 60% of the Brazilian territory, consisting of 4.8 million km^2. This ecosystem is very diverse in terms of biodiversity, soil types, and classes, lithology, and rainfall patterns. Oxisols rich in iron oxides dominate 75% of the region, and there is a lack of representative pedogeochemical data that characterize the region (SANCHEZ et al., 1983; LIMA et al., 2014; GARDI et al., 2015; MATSCHULLAT et al., 2020).

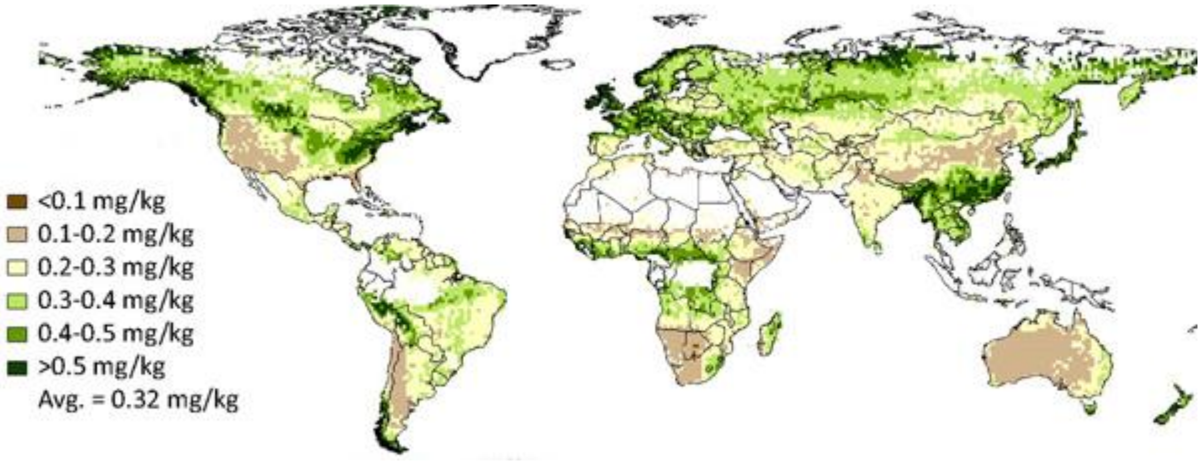
Brazil nut (*Bertholletia excelsa* Bonpl.), a member of the *Lecythidaceae* family, is a species native to South America (Figure 1). This species grows on well-drained upland soils throughout the Brazilian Amazon forest and in other countries such as Bolivia, Peru, Colombia, Venezuela, and Guyanas (SHEPARD; RAMIREZ, 2011). The species is found in areas with average annual precipitation between 1400 and 2800 mm, average annual temperature between 24 and 27 °C, and relative humidity of 79% to 86% (DINIZ; BASTOS, 1974). Brazil nuts are consumed worldwide, being known as the richest food in Se, with values of up to 512 mg Se kg^{-1} reported in the literature (CHANG et al., 1995; DUMONT et al., 2006). It is an endemic species distinguished by its economic, social, and environmental values. Brazil nut commerce is one of the main sources of income for many indigenous and riverside communities in the Amazon (CARDOSO et al., 2017; BALDONI et al., 2020).

Figure 1 - Brazil nut tree from the state of Acre.

Source: Embrapa.br. Photo: Renata Silva.

Selenium is one of the elements that play an important role in animal and human health, being essential also for other organisms including bacteria and algae (VRIENS et al., 2016; BLAZINA et al., 2017). In the environment, however, there are concerns about both toxicity and its deficiency. It has been shown that Se ingestion and its status in a given population may be closely related to concentration, distribution in the solid phase of the soil, and speciation in the local environment, which is largely determined by geochemical characteristics (NOTHSTEIN et al., 2016). The knowledge of such factors can suggest health risks and be relevant to a country or region and can help to predict which places may be characterized by deficiency or toxicity in the soil. Figure 2 shows the global distribution of Se in soil.

Figure 2 - Global distribution of selenium in the soil.



Source: Support health clinic.

On a global scale, it is estimated that 0.5 to 1 billion people are directly affected by Se deficiency. In general, areas with low natural Se content in the soil are more widespread and extensive than areas with excessive levels of Se (HAUG et al., 2007; JOHNSON et al., 2010; WINKEL et al., 2012). In Brazil, information on Se values in the soil is scarce, especially concerning the fraction of Se available for plants. Particularly in the Amazon region, analytical determinations of Se in the soil are practically non-existent, except for studies assessing Se levels in areas of agricultural production. Due to concerns about Se deficiency, there is a need to search for more information regarding the status of Se in soils on a national scale in order to understand the main factors that influence their concentrations and their availability for plants and animals (COMBS JÚNIOR, 2001).

Barium (Ba) is another important element with a few investigations in Amazonian agroecosystems, even when compared with studies on Se. It is identified as a dense alkaline earth metal, considered non-essential for organisms and harmful to humans, animals, and plants. Its exposure can cause multiple deleterious effects in animals, including damage to the cardiovascular, renal, respiratory, hematological, nervous systems and even lead to death. The toxicity of the Ba compounds is significantly related to their solubility, and the more soluble the compound is, the more toxic it will be in the environment (LAMB et al., 2013; ÇANLI, 2018).

Barium is known to be not very mobile in soils because it is easily precipitated as sulfates ($BaSO_4$), carbonates ($BaCO_3$), the two most prevalent and naturally occurring forms of Ba in the environment, and also strongly adsorbed on clays. Barium contents in the soil surface layer range from 10 to 1500 mg kg^{-1}. Barium has a lithophilic affinity and is likely to concentrate on igneous and sedimentary acid rocks. The ionic radius of Ba is quite similar to that of K, and therefore generally has similar behavior in geochemical processes (KABATA-PENDIAS, 2011).

Barium solubilization and consequently the release of Ba^{2+} ions in soil solution may occur in acidic conditions such as those found in tropical soils, in absence of oxygen, or even due to microbial action, but in contrast, it precipitates as sulfate and/or carbonate salts in neutral or basic pH conditions (BALDI et al., 1996; MENZIE et al., 2008; ABREU et al., 2012). Therefore, when available at high levels in soils, it can interfere with the availability of S, due to the formation of low solubility sulfates (COSCIONE; BERTON, 2009).

It is known that in the southeast Amazon, manganese deposits accumulated during the Proterozoic period, in which sedimentary, hydrothermal, and lateritic environments produced a wide and complex variety of Mn-minerals (coronadite, hollandite, and romanechite). Among these rocks containing Mn and duricrusts are those with a higher concentration of Ba (up to 205,541 mg kg^{-1}). Therefore, the weathering of these rocks is a possible cause of soils with high Ba content occurring in the region (ALBUQUERQUE; HORBE; BOTELHO, 2017). Evidence has also been published on the abnormally high concentrations of Ba in Brazil nuts (up to 5890 mg kg^{-1}), which is in agreement with the above mentioned about the cause for high concentrations of this element in soils of the region (SMITH, 1971). Figure 3 is a 5 mm scale picture of a gray, feathery aggregate of hollandite.

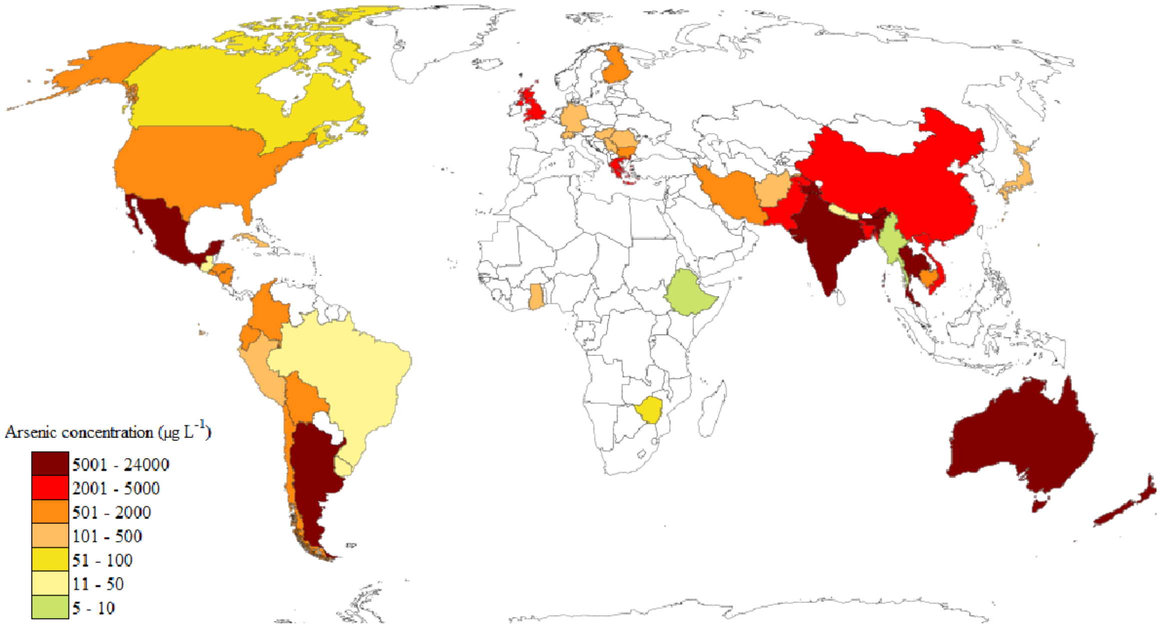
Figure 3 - Hollandite ($Ba(Mn^{4+}{}_6Mn^{3+}{}_2)O_{16}$), found in Sieben Brüder Mine, Langenberg, Schwarzenberg District, Erzgebirge, Saxony, Germany.

Source: webmineral.com, coyright: ©Thomas Witzke/Abraxas-Verlag.

It is known from the literature that there is great variability for both Se and Ba content in Brazil nuts, depending on the location and soil in which they were grown, probably also reflecting the quantities of these elements in regional soils and also the capacity of each genotype and plant population to accumulate them in the edible nuts. The variations in Se concentrations in Brazil nuts were reported in studies previously carried out by Silva Junior et al. (2017) (<0.5 to 147 mg kg^{-1}), Chang et al. (1995) (0.03 to 512 mg kg^{-1}, fresh weight), Dumont et al. (2006) (nuts without shell containing on average 5.1 mg kg^{-1} and nuts with shell containing on average 50 mg kg^{-1}), Pacheco and Scussel (2007) (8.5 to 69.7 mg kg^{-1}), Parekh et al. (2008) (1.6 to 20.2 mg kg^{-1}), Secor and Lisk (1989) (0.2 to 253 mg kg^{-1}), Brito et al. (2019) (1.2 to 151 mg kg^{-1}) and Lima et al. (2019) (10 to 79 mg kg^{-1}). For Ba concentration in Brazil nuts, the variations were reported by Smith (1971) (190-5890 mg kg^{-1}), Parekh et al. (2008) (96 to 1990 mg kg^{-1}), Gonçalves et al. (2009) (86 to 2084 mg kg^{-1}), Welna et al. (2008) (66.2 ± 12.3 mg kg^{-1} on average) and Brito et al. (2019) (2.4 to 1054 mg kg^{-1}).

High levels of arsenic (As) have also been observed in groundwater around the world (Figure 4) and also in soils including sites located in Brazil, mainly due to mining activities (BUNDSCHUH et al., 2012). Hypothetically, we could expect high concentrations of As in soils close to the Amazon river basin because of the sediments that reach the Amazon River (and its tributaries) coming from the Andes, which are also expected to be naturally enriched in volatile trace elements (for example, Se and Hg) (FADINI; JARDIM, 2001; SILVA JUNIOR et al., 2017). However, there is a lack of information on the natural levels of As in the soils of the Amazon basin. Most recent studies have focused only on specific states in the Eastern Amazon (FERNANDES et al., 2018). Therefore, it is necessary to conduct a more comprehensive study on the occurrence/distribution of As in different agroecosystems in the Amazon, in order to provide information about the variability and levels of this element, which will aid in avoiding health problems in the local population due to the use of soil and water resources for subsistence, construction, and agriculture in this region.

Figure 4 - Arsenic concentration in groundwater around the world (elaborated with data from Rasheed et al., 2016).



Source: Saldaña-Robles et al. (2018).

Iodine (I) has been known to be an essential element for humans since the middle of the 19th century, it being a constituent of the thyroid hormones triiodothyronine (T4) and thyroxine (T3), which are crucial for proper growth, metabolism, and development of the human body. In particular, a great body of literature has been published on the link between thyroid hormones and brain development and functioning. Although required in small amounts, iodine deficiency is widespread around the globe. It is considered one of the most common micronutrient deficiencies, although easy to prevent. At a global scale, approximately 2 billion people suffer from iodine deficiency (ID) of which approximately 50 million presents with clinical manifestations (BIBAN; LICHIARDOPOL, 2017; BOWLEY et al., 2019; PINTO et al., 2020).

The I geochemical cycle is of great importance in the environmental controls on IDD (Iodine Deficiency Disorders), and this is because the concentrations in the soil reflect the concentration in the food grown locally. The behavior and distribution of iodine in the environment are of significant interest in a range of scientific disciplines. The information about the distribution of I in Amazonian agroecosystems would be relevant because of the lack of research on this topic, which is essential to support research on the biofortification to solve eventual problems related to insufficient supply of iodine (I) for the population (FUGE; JOHNSON, 2015; SMOLE et al., 2019).

The terms “soluble” and “available” are introduced here and represent important steps in the soil-solution interface before the trace elements can reach plants, microrganisms, and animals. The distinction is made because they represent two different pools in terms of elemental occurrence in the soil and play different roles in the element cycle. These terms classify trace elements in soil into different functional fractions with different metal-binding strengths (WAN et al., 2017). Soil chemical extractions for soluble and available trace elements can help understand the element's dynamic chemical behavior and its possible entrance into the food chain. Many extractants can be used for this purpose, for example, the soluble fraction can be extracted using water (water-soluble) (WENZEL et al., 2001). The available fraction is not well defined in the literature in terms of method of extraction, but would be better classified as the element retained in the solid phase interacting with the soil solution and occupying exchangeable sites as diffuse ion or as outer-sphere complexes (HERREWEGHE et al., 2003). Using As as an example, the available fraction can be extracted by using Na_2HPO_4 (KIM et al., 2003), $MgCl_2$ (JIANG et al., 2014), CH_3COOH (WAN et al., 2017), $(NH_4)_2SO_4$ (WENZEL et al., 2001) and KH_2PO_4 (THINH et al., 2018) among others.

Beforehand, to better explain the whole process, the uptake of trace elements by plants is a key stage in the soil-plant-animal/human pathway and is second only to intake via drinking water, concerning the link between geochemistry and human health apart from where there is excessive ingestion of soil. Plants readily take up ionic or soluble complexed forms of trace elements present in the soil solution. The factors affecting the amounts of elements absorbed through the roots are those controlling: (1) the concentration and speciation of the element in the soil solution, (2) movement of the element from the bulk soil to the root surface, (3) transport of the element from the root surface into the root, and (4) its translocation from the root to the shoot. The absorption of mobile ions present in the soil solution is mainly determined by the total quantity of this ion in the soil. However, in the case of strongly adsorbed ions, absorption into the root is more dependent on the amount of root produced and its ability to explore a large volume of soil (ALLOWAY, 2013).

Particular information on soluble and available concentrations of Se and As in soils is missing for Amazonian environment, having in mind that the total concentration in soil is an inaccurate predictor of the availability of these trace elements. Therefore, to obtain valuable information about the availability of Se and As, a chemical extraction procedure must be applied to identify the fraction in the soil which is available to organisms and plants. This is highly relevant, as this information can support research and institutions that are involved in decision-making, to provide better planning for sustainable land and natural resources used by the local population, also guaranteeing health and food security.

Moreover, since there is a great diversity of Brazil nuts originating from different sites in the Amazon region (Acre, Rondônia, Amazonas, Roraima, Pará, Mato Grosso, and Amapá), it can also be hypothesized that their population genetic diversity has great influence in the overall composition of the nuts, and therefore, on the bioaccessibility and bioavailability of Se and Ba to humans consuming the nuts. This is because the potential for tolerance, translocation, and accumulation of a certain element is driven by the plant genotype. Therefore, it should be known which fraction of these elements can be released from the food matrix in the intestinal tract (bioaccessibility) and becomes available for uptake by the body (bioavailability), as higher total concentrations do not necessarily reflect in higher toxicity. Despite being carried out in the laboratory, standardized *in vitro* bioaccessibility protocols constitute an excellent simulation of gastrointestinal digestion processes. *In vitro* bioaccessibility assessments of Se and Ba in Brazil nuts can contribute to updating nutritional recommendations on the human consumption of Brazil nuts and its derivates.

Table 1 provides a summary of background values for As, Ba, I, and Se in worldwide and regional soils besides information on deficient, adequate, and toxic daily intake levels for these elements in humans.

Table 1 - Background values in soil (total concentrations), deficient, adequate, and toxic intake levels for As, Ba, I, and Se for humans.

Element	Soil background (mg kg^{-1})		Deficient intake (µg day^{-1})	Adequate intake (µg day^{-1})	Toxic level (µg day^{-1})
As	6.83 (Kabata-Pendias, 2011)	1.10 ± 1.34 in Eastern Amazon-Brazil (Fernandes et al., 2018)	-	-	>2 µg/kg body weight (EFSA, 2009)
Ba	362-580 (Kabata-Pendias, 2011)	13.44 ± 18.94 in Eastern Amazon-Brazil (Fernandes et al., 2018)	-	-	>200 µg/kg body weight (USEPA, 2005)
I	2.8 (Kabata-Pendias, 2011)	1.1 and 2.3 in San Juan and La Pampa-Argentina (Watts et al., 2010)	<100 (Abel et al., 2018)	150 (Institute of medicine, 2000)	>1100 (Leung et al., 2015)
Se	0.44 (Kabata-Pendias, 2011)	0.19 in São Paulo-Brazil (Gabos et al., 2014)	<30 (Fairweather-Tait et al., 2011)	60 and 70 for women and men (Kipp et al., 2015)	>900 (Fairweather-Tait et al., 2011)

2 LITERATURE REVIEW

2.1 Brazil nuts: species characteristics and market

The Brazil nut tree (*Bertholletia excelsa* Bonpl., *Lechytidaceae*) is one of the symbol trees of the Amazon, due to its social, ecological, and economic importance for the region and local communities. Thousands of families of extractivists and rural producers use Brazil nut as an important source of income (AMARAL et al., 2013; SOUZA et al., 2008). It is popularly known as Brazil nut or Pará nut and, more recently, Amazon nut (WADT; KAINER, 2012).

Brazil nuts are native to the Amazon, occurring throughout the region, including the states of Rondônia, Acre, Amazonas, Pará, Maranhão, Amapá, Roraima, Mato Grosso, and northern Goiás, as well as in Venezuela, Colombia, Peru, Bolivia, and Guyana (MULLER et al., 1980). The Brazil nut tree is a large plant that can reach up to 50 meters in height, and its trunk can measure on average up to 2 meters in diameter, being able to live more than 300 years. Its fruits (Figure 5) can weigh up to 2 kg, containing 10-25 nuts inside, and having a high content of vitamins and proteins. The fruits are highly appreciated abroad, where they are known as “Brazilian nuts”, and the main consumers of Brazil nuts are located in the USA and Europe. More than 90% of the product produced in Brazil is exported to the United States, France, England, Germany, and Italy. Therefore, all this marketing practice within our country becomes a great source of income for the people who live and depend on this plant in the Amazon (AMARAL et al., 2013; SOUZA et al., 2008).

The opening of ports in the Amazon for foreign trade, in 1866, allowed the expansion of the production and the commercialization of Brazil nuts outside the country. From that time, the collection of Brazil nuts became an important economic activity in the Amazon, especially in the regions of Manaus and Belém. Like rubber, Brazil nuts were and have been one of the most important products in the Amazon in social and economic terms. Even today, practically all Brazil nut production comes from native forest extraction (WADT; KAINER, 2012).

The striving of the population towards healthy nutrition, along with doctors' recommendations to include nutritious foods in the diet have been important factors in driving the growth of the Brazil nuts market in Brazil. Bolivia, Brazil, Peru and Gambia are the main producers of Brazil nuts as well as the key suppliers for exports. A remarkable number of products is supplied from other countries that are not growing Brazil nuts, in this case, re-exports of products in processed or unprocessed form take place. Brazil nuts, being an exotic product, have great prospects in the global market. Despite the temporary stagnation of the

Brazil nut market, it is possible to note many positive aspects, contributing to its development. Among those are the increases in the share of shelling in the Brazil nut producing countries, diversification both in producing countries and importing countries, popularization of healthy nutrition among the population, and urbanization. Some of the constraints are the global crisis, political and economic phenomena in the world, hindered investments in the industry, and the purchasing power of the population. Countries in West Africa have become more noticeable due to Brazil nut production, although they still represent a small percentage of the global market (INDEXBOX, 2016).

Concerning the market and commercialization of multiple products of the species, Brazil stands out as the largest producer and exporter. In 2015, Brazil exported the equivalent of US$ 41.56 million in Brazil nuts. However, in 2017, export revenue was only US$ 11.96 million, the lowest value since 2009 (OLIVEIRA et al., 2020). Brazilian firms used to dominate the Brazil nut market to such an extent that the product still carries the country's name. In a surprising twist, 77% of all Brazil nuts are now processed and exported by Bolivia, a country with far fewer resources than its neighbor. The reason by which Bolivian producers prevailed was because they joined forces to revamp their manufacturing practices and meet EU sanitary standards despite continued mutual mistrust. In contrast, Brazilian producers have been unable to work cooperatively and lost access to the European market entirely (COSLOVSKY, 2014).

There is considerable national and international interest in promoting non-timber forest products (NTFPs) as a strategy for conserving tropical forests and maintaining the ways of life and particular cultures linked to extraction. Brazil nuts are a classic NTFP and only in the Brazilian Amazon (TOMASI, 2016). The extensive use of Brazil nuts in the cosmetic industry allows the generation of income for approximately 15,000 families living on the extraction of the product, ensuring the conservation of the species (SANTOS; SOUZA, 2012). As an additional form, processing and marketing of primary production directly by extractivists is an important step in the production chain (MACIEL; REYDON, 2008).

It is also being observed that Brazil nuts sold at local markets are commonly originated in another state. Moreover, details about their origin are not usually available from the vendors. Such information is relevant, as there are significant differences in Se concentration among samples with different origins (CARDOSO et al., 2017).

Figure 5 – Brazil nuts fruits and their unshelled and shelled seeds.

Source: Amazônia.org.br.

2.2 Selenium, barium, arsenic, and iodine in human health

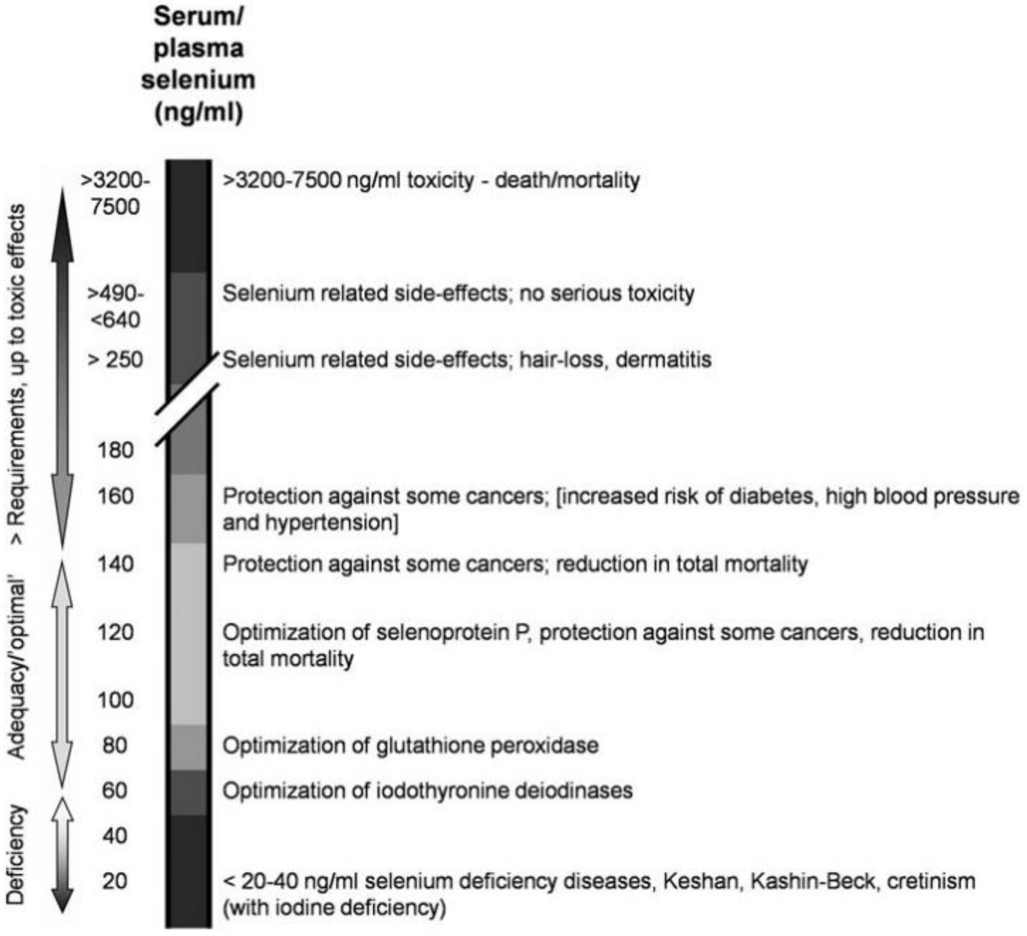
Selenium is an essential nutrient that plays a key role in human biology. This has become increasingly obvious as new research shows its unquestionable role in important areas of human health. As a constituent of 25 selenoproteins, it plays fundamental roles in the immune system, in reducing negative health impacts of viral infections, being essential for fertility and reproduction, acting in the metabolism of thyroid hormone, and protecting against cardiovascular diseases, in addition to alleviating oxidative stresses or inflammatory conditions in the human organism (RAYMAN, 2012).

Selenium plays an antioxidant role because it is the main constituent of antioxidant enzymes that are expressed in various tissues, including the brain. Thus, this mineral has an important role in offering protection against cell damage induced by ROS (reactive oxygen species) and the proposed mechanisms mainly invoke the functions of glutathione peroxidase and selenoprotein P (CHEN; BERRY, 2003). Dietary Se acts principally through selenoproteins, most of which are oxidoreductases involved in diverse cellular functions. It is essential to predict the concentration of Se present in ingested foods that can be incorporated into these proteins to optimize their activity, thereby reflecting on the level of Se in the blood plasma (HATFIELD et al., 2014).

The Nutrition Societies of Germany, Austria, and Switzerland revised the reference values for Se ingestion using the saturation of selenoprotein P (SePP) in plasma as a criterion for deriving the values instead of using the measurement of GPx activity in the plasma, which is used to derive most reference values. The resulting estimated recommended values for dietary intake were 70 µg/day for adult men and 60 µg/day for adult women. Despite being considered an essential element, Se can also be toxic to humans and animals, at higher levels of consumption (KIPP et al., 2015).

The beneficial effects of Se thus occur in a very narrow dose range, with current estimates suggesting that intakes below 30 µg/day are inadequate and those exceeding 900 µg/day are potentially harmful. Therefore, below this range, Se cannot perform all essential functions in the body and above it, it becomes toxic. Also, the health effects are related to the level of exposure, current Se status, and the species of Se in the food. The existing possible relationships between blood Se concentration and its function or health effects are summarized in Figure 6 (MAIHARA et al., 2004; KHANAL; KNIGHT, 2010; FAIRWEATHER-TAIT et al., 2011).

Figure 6 - Range of blood selenium concentrations with possible related health effects from deficiency to toxicity. Various parameters associated with selenium function or health have been assessed in relation to a range of selenium intakes and blood selenium concentrations.



Source: Adapted from Fairweather-Tait et al. (2011).

It is estimated that 15% of the world population is deficient in Se and its low intake is linked to cardiomyopathic diseases (e.g., Keshan's disease) and degenerative osteoarticular disorders (e.g., Kashin-Beck's disease). In general, these diseases are reported in areas where concentrations of the element in the soil are low such as in several regions of China, Tibet and Siberia (WHITE; BROADLEY, 2005; ZENG, 2009; RAYMAN, 2012). The geographical distribution of topsoil Se in China and its relationship to Keshan and Kashin-Beck diseases indicates that Se concentrations in the topsoil in affected areas are typically below 0.125 mg kg^{-1}. In contrast, the concentration in areas without disease is higher than 0.224 mg kg^{-1}, and the excessive level is above 3 mg kg^{-1} (TAN et al., 2002).

The increase of the intake by the population in regions where dietary Se intake is currently low can result in improvements in public health. Various strategies to increase Se intake can be employed, including education to increase consumption of foods richer in Se, individual supplementation, food fortification, livestock supplementation, Se fertilization, and plant cultivation to increase Se accumulation (COMBS JÚNIOR, 2001; RAYMAN, 2008). In order to select the best solution, one must consider that there are adverse health consequences for both deficient and excessive consumption. The ideal intake at the individual level depends on a large number of factors, such as the Se functions most relevant to a particular disease, the Se species most prominent in a given food source, the health condition considered, the adequacy of the intake of other nutrients, the presence of additional stressors and also whether the ability to produce selenoproteins can be compromised (RAYMAN, 2008).

Barium is considered a non-essential element for the human organism that is harmful to animals and plants. Its exposure can cause multiple deleterious effects, including damage to the cardiovascular, renal, respiratory, hematological, nervous, and endocrine systems and, eventually, result in metabolic, neurological, and mental disorders and even death. An oral toxicity assessment carried out by the US Environmental Protection Agency established an oral reference dose (RfD) of 0.2 mg kg^{-1} body weight/day for Ba (EPA, 2005). The toxicity of a Ba compound is significantly related to its solubility, and the more soluble the compound is, the more toxic it will be. Barium can enter the human body through the consumption of food and/or water. Thus, it is important to monitor the accumulation of Ba in soil and water as well as in food (LAMB et al., 2013; ÇANLI, 2018; LU et al., 2019).

The presence of As represents a major impact in the environment worldwide (soil, water and food) and often raises great concern due to its high toxicity to various systems of the human body (for example, cutaneous, nervous, respiratory, cardiovascular, hematopoietic, immunological, endocrine, hepatic, kidneys and reproductive), as well as its ability to cause different types of cancer (skin, lung and bladder) (MOHAMMED ABDUL et al., 2015; GAMBOA-LOIRA et al., 2017; CHATTERJEE et al., 2018). Also, As is considered a "Class I" human carcinogen established by the International Agency for Research on Cancer (IARC) (MINATEL et al., 2018). Since inorganic As is the most toxic fraction of this metalloid, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) initially recommended a provisional tolerable weekly intake (PTWI) of 15 μg inorganic As per kg^{-1} body weight (WHO, 2011). It is estimated that more than 200 million people are chronically exposed to concentrations of As at or above the WHO threshold worldwide (NAUJOKAS et al., 2013).

The key function of iodine is participation in the synthesis of thyroid hormones, which are essential for both pre- and postnatal brain development and for the lifelong metabolism of all cells. Recent studies show that I is also involved as an antioxidant agent protecting against bacterial and viral infections. This further increases the importance of adequate nutrition regarding this element (VILA et al., 2020). The body of a healthy adult contains 15–20 mg of iodine, of which 70-80% is in the thyroid. In chronic iodine deficiency, the iodine content of the thyroid might fall to less than 20 μg. Iodide is rapidly and almost completely absorbed (>90%) in the stomach and duodenum. Iodate, which is used in many countries in the iodization of salt, is reduced in the intestine and absorbed in the form of iodide (ZIMMERMANN, 2008; KNUST; LEUNG, 2016). The iodine concentration in most foods and beverages is low, and most consumed foods provide 3-75 μg per serving. The diet is the primary method of achieving adequate iodine nutrition, with dairy products, some slices of bread, seaweed, and other seafood. Iodized salt is the most common iodine-containing food. Adolescents and adults require iodine in amounts of 150 μg/day, with higher amounts required during pregnancy and lactation (250 and 290 μg, respectively) (KNUST; LEUNG, 2016).

2.3 Selenium, barium, arsenic, and iodine in plant nutrition

The absorption of Se by plants and its total level present in tissues are influenced by several factors, including the Se content in the soil, its chemical form, soil pH, redox potential (Eh), mineralogy, mineral fertilizers, and rain precipitation. Another factor participating in the total uptake of Se by plants is the atmosphere, which is in balance with its cycle (HAYGARTH et al., 1995). It is known that in terms of availability, the form of Se present in the soil is more important than the total amount to be absorbed by the plants (JEŽEK et al., 2012).

For some higher plants, Se is known to be a beneficial nutrient, but its essentiality for all crops has not yet been demonstrated, with discussions about its functions in plant metabolism and controversies regarding its essentiality (TERRY et al., 2000; PILON-SMITS; QUINN et al., 2010; LYONS et al., 2009). Based on some analyses, the hypothesis was raised that the essential role of Se is a primitive characteristic that was lost during the evolution of higher plants. It cannot be excluded, however, that some plants can fix Se, converting an aminoacid like serine to selenocysteine, and in the same way, producing selenoproteins by different routes. When it comes to absorption, Se is chemically similar to sulfur (S), and as a result, plants and other organisms readily absorb and metabolize Se via S transporters and at the same absorption sites (PILON-SMITS; QUINN, 2010). The chemical similarity shared by selenate and sulfate allows selenate to be absorbed by the plant through the same sulfate transporters, although the affinity that sulfate transporters have for selenate appears to vary between plant species. The absorption of selenite by the plant can occur through phosphate transporters (ZHU et al., 2009; LI et al., 2008).

Although Ba is reported as a common element in plants, as far as we know it is not an essential compost of plant tissue, and there is still a need for consolidated studies on the accumulation and toxic effects of Ba in plants. The average content of Ba in most plants ranges from 2 to 13 mg kg^{-1} and it is known that Ba can negatively affect plant growth depending on its availability in soil (KABATA-PENDIAS, 2011; LAMB et al., 2013). Specifically, negative effects have been reported for soybeans, with high concentrations of Ba (5000 µM in nutrient solution) inhibiting photosynthetic activity and plant growth, with the main effects being attributed to the closure of its stomata (SUWA et al., 2008). However, there are also plants such as vegetables, forage, Brazil nuts and mushrooms that can accumulate high levels of Ba. Bioconcentration factors of 2 to 20 have been reported for tomatoes and soybeans (CHOUDHURY; CAREY, 2009). Critical Ba concentrations in tissues of *Panicum maximum* (Tanzanian grass) have been reported in the range of 156 to 383 mg kg^{-1} when grown in nutrient culture (FRANCISCO et al., 2011). The highest levels of Ba are reported for Brazil nuts, depending on the location of the growth, from about 3000 to 4000 mg kg^{-1} (KABATA-PENDIAS; MUKHERJEE, 2007).

Toxicity of As is commonly observed in plants that grow in mine waste, soils treated with arsenic pesticides and soils with As added by the treatment of sewage sludge. Symptoms of As toxicity are described in various ways such as leaf wilting, violet staining (increased anthocyanin), root discoloration and cell plasmolysis. The most common symptom, however, is reduced growth (KABATA-PENDIAS, 2011). The toxicity of As species in plants follows the order As (III) > As (V) > MMA (monomethyl-arsenic) > DMA (dimethyl-arsenic) (TORRES et al., 2020). However, the response of As depends not only on the chemical form of the element, but also on the plant species. For example, it was found that arsenate is more toxic than arsenite for corn, but that the opposite response is observed in wheat, rice and lettuce (ABBAS; MEHARG, 2008). Simillarly, Yoon et al. (2015) demonstrated that the chemical status of arsenic compounds affects their phytotoxicity in different plant species. Besides, the toxicity of As compounds and plant responses to As can be modified by the physicochemical properties of the soil. The sensitivities of different plant species to As compounds vary according to their various response mechanisms, such as the rate of As absorption by the roots and their accumulation in the aerial part.

Iodine is not essential to plants, although the stimulating effect of I is observed at the concentration of 0.1 mg kg^{-1} in nutrient solutions, whereas toxic effects occur at the concentration of 0.5-1.0 mg kg^{-1}. In general, the phytoavailability of I is low, since most often, strongly bound I will not be either available to roots or easily volatilized from soils. Soluble forms of I seem to be easily available to plants; therefore, terrestrial plants contain much less I than do marine plants. Plants take up I relatively easily from the soil solution, and iodine (I^-) is a much more available species than iodate (IO_3^-). Both I species are more concentrated in roots than in shoots, which could be due to the absorption onto the root surface. Although I contents of individual plant species may vary considerably, the range in amount seems to be a species characteristic. Generally, vegetables and fleshy mushrooms contain more I than other land plants. Interesting to mention that plants are capable of absorbing I directly from the atmosphere, both through the cuticle and as adhesive particles on the surface of hairy leaves. Therefore, the atmospheric I can contribute significantly to the I content of plants. The I toxicity symptoms are similar to those caused by Br excess: margin chlorosis in the older leaves, while the younger leaves become dark green (KABATA-PENDIAS, 2011; FUGE, 2013).

Based on the current literature, with examples of hydroponic, pot, and field experiments, agronomic biofortification of crops with I seems to be a promising way to increase the intake of the population. Approximately 80% of the I in the human body and animals originates from plants, and nearly 99% of this I is bioavailable and can be easily assimilated. Therefore, plant-based products with increased I concentrations provide an attractive and cost-effective approach to reduce iodine deficiency in the population (GONZALI et al., 2017; DOBOSY et al., 2020).

2.4 Selenium, barium, arsenic, and iodine in the soil

Soil is a quite dynamic system composed by solid, liquid, and gaseous phases, which is subject to short-term variations in moisture status, temperature, pH, and redox conditions. In addition to the non-living mineral and organic constituents, the topsoil contains a highly diverse microbial and mesofauna population and a high density of roots of several plant species. These living organisms are all involved in the physical, chemical, and biological processes taking place in the soil. In addition to short-term changes, soils also undergo gradual alterations in response to changes in management and environmental factors. Examples of these longer-term changes in soil properties include a decrease in the content of organic matter with increasing cultivation and/or increasing temperatures and gradual acidification due to acid precipitation or lack of regular liming in areas of high precipitation relative to evaporation. These short- and long-term changes in soil properties can each have significant effects on the form and bioavailability of trace and major elements (of both indigenous geochemical and external pollution origin). Soils are also inherently heterogeneous at the micro (profile) and macro (field/catchment) scales (ALLOWAY, 2013).

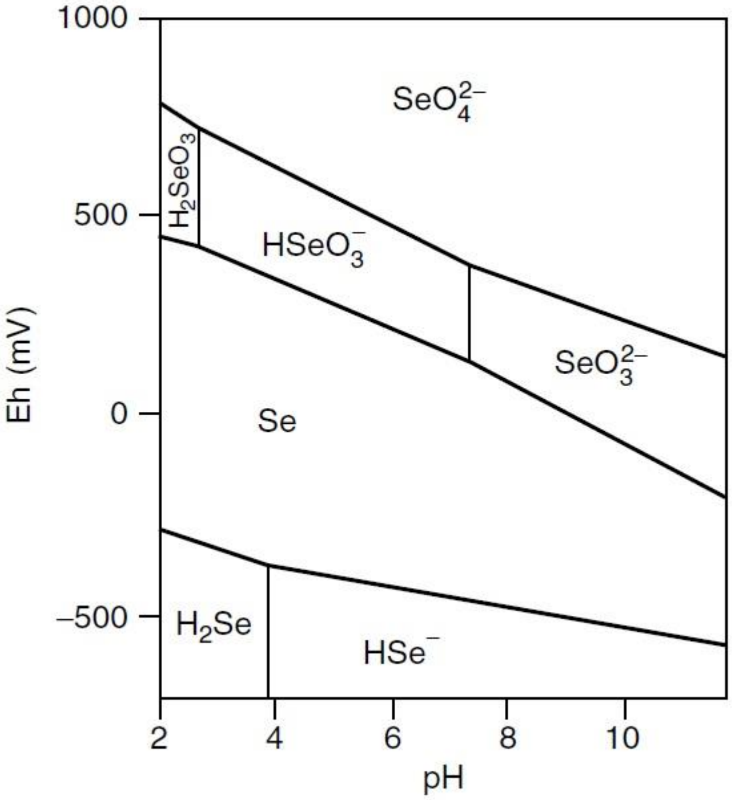
The study of Se distribution in soils is of extreme interest due to the narrow range between the levels of deficiency and toxicity of this element in living organisms. This critical line of distinction between its essentiality and toxicity differs among organisms and species, as well as it is dependent upon the applied level and Se speciation (HAWRYLAK-NOWAK et al., 2015). Selenium in the soil exists in several inorganic forms: elementary selenium (Se^0), selenide (Se^{2-}), selenite (SeO_3^{2-}), selenate (SeO_4^{2-}) and organic forms, such as selenocysteine and selenomethionine. In cultivated soils, the forms SeO_3^{2-} and SeO_4^{2-} predominate (KOPSELL; KOPSELL, 2007; ABREU et al., 2011).

Selenate is the most dominant anion because it is stable in oxidizing environments, is more mobile in the soil and is readily available to plants. Selenite has a lower proportion in the soil solution when compared with the anion selenate, as it can usually undergo specific adsorption processes with hematite and goethite, in the case of Brazilian oxisols, which can make it unavailable to plants (ROVIRA et al., 2008). Considered to be the form with the greatest toxicological potential, selenate preferably forms an external sphere complex, with a water molecule between the anion and the soil surface, whereas selenite preferably forms an internal sphere complex (MARTÍNEZ et al., 2006).

Although geology represents the primary control for the Se content present in soils, mobility, and absorption by plants and animals, known as availability, is determined by several biophysical and chemical parameters. These include the pH, redox potential, the chemical form or Se species, the texture and mineralogy of the soil, the content of organic matter, and the presence of competitive ions. Organic matter is a great influence on Se speciation in soils because the colloid-sized organic Se particles exhibit different mobility and availability than Se oxyanions (selenate and selenite) due to different chemical (e.g., functional groups, hydrophobicity) and physical (e.g., size) properties (TOLU et al., 2014). An understanding of the control of these parameters is essential for the prediction and remediation of the Se-related health risks. Even in soils that contain adequate total concentrations, Se may be deficient in animals and humans if the element is not in a readily available form (FORDYCE, 2007).

The mobility of Se in the soil and its presence in plants depends on the increase of its concentration in the soil solution, from where the element can be absorbed and translocated by plants or can be leached to underground aquifers. Besides, its persistence in the soil and its dynamics involve phenomena of sorption, desorption, precipitation, complexation, oxidation, and dissolution, so its presence and the possibility of toxicity in the environment depend on the soil management used and the behavior of Se specie (SPARKS, 1999; KABATA-PENDIAS, 2011). The dependence of Se forms on pH and redox potential is shown in Figure 6.

Figure 6 - Distribution of selenium in the soil depending on pH and redox potential (Eh).



Source: adapted from Kopsell and Kopsell (2007).

In areas where the natural availability of Se in the soil is low, a good alternative is supplementation via commercial fertilizers enriched with Se, so that there is an improvement in the nutritional quality of the food (ARO et al. 1995). And this is what is being done by current research, aiming to improve the mineral nutrition of plants in soils and environments where the low availability of Se ends up being reflected in the deficiency in human nutrition and health. The level of Se in agricultural products is highly dependent on the availability and mobility of this element in the soil and on regulation according to the plant species and genotype. However, due to the small number of studies carried out so far, there is currently a great need for research to evaluate the effect of genotypic variation and plant physiology on the accumulation of Se in plant products (LYONS et al., 2005; KOPSELL; KOPSELL, 2007).

The distribution of Se concentrations in Brazilian soils is quite heterogeneous and there is still a lack of comprehensive information to support future research aiming to provide optimal levels of Se to crops in the country. The focus is the application through fertilizer which is the aim of current research. An overview of these values in different regions was reported by Reis et al. (2017) for Brazilian soils, in which the values ranged from 10 to 670 µg kg^{-1}. Therefore, in many locations, Se can be considered deficient in soils.

Barium is a little-known trace element occurring in soils of the Amazon basin. Its content in soil surface is commonly similar to its distribution in the parent rock and ranges from 10 to 1500 mg kg^{-1}, with lower levels being found in organic soils and higher in clayey soils. The average reported range of Ba in soils on a global scale is 362 to 580 mg kg^{-1}. Although Ba is easily adsorbed, mainly by oxides and hydroxides, it is relatively mobile in soils, especially in acidic soils. Its great affinity for Mn nodules in the soil (up to 0.2%) and Mn minerals (up to more than 5%) is an effect of the easy displacement of other alkaline earth metals adsorbed to these phases. These metals, on the other hand, can also readily replace Ba in aluminosilicates (KABATA-PENDIAS, 2011). The high concentration of Ba in Brazil nuts grown in the Amazon basin of South America is probably due to the occurrence of a mineral-rich in Ba called hollandite ($Ba_2Mn_8O_{16}$) in the soils of the region (SECOR; LISK et al., 1989; CHANG et al., 1995).

Leaching guidelines and benchmarks have been developed for many potentially toxic elements, but Ba is generally not included. Only a few countries in the world (for example, Canada and the Netherlands) have adopted guidelines or benchmarks for Ba in soils. Therefore, research on Quality Reference Values (QRVs) for Ba in Amazonian soils is necessary to provide basic reference and knowledge for soil use and management (CAPPUYNS, 2018).

The global average value of total As for different soils worldwide is estimated at 6.83 mg kg^{-1} (KABATA-PENDIAS, 2011), very close to the value found for Brazilian Oxisols (5.92 mg kg^{-1}) (CAMPOS et al. Al., 2007). Still, As is found more abundantly in clay sediments (up to 13 mg kg^{-1}). The anionic forms, AsO^{2-}, $HAsO_4^{2-}$, and $H_2AsO_3^{2-}$, are predominant in the soil solution (KABATA-PENDIAS; MUKHERJEE, 2007). The soil physicochemical properties, such as pH, redox potential, clay content, organic matter (OM) and the presence of metal oxides (Fe, Mn and/or Al) and competing ions, such as phosphates, strongly influence the availability and mobility of arsenic in soils (SHARMA et al., 2011; BISWAS et al., 2014).

High levels of As has been detected in ground and surface water in 15 of the 20 countries in Latin America in 2011, and much of the abnormal As concentration observed in the region in the past 20 years have been published much later or never in the international literature (BUNDSCHUH et al., 2012). High levels of As originating from geogenic sources occur in many regions of the world, such as Bangladesh and India (ARCO-LÁZARO et al., 2018; RAHMAN et al., 2018). In addition to the natural occurrence of As, many other anthropogenic sources can increase the concentration of the element in the soil, for example, mining, smelting, application of residues, animal fertilizers, pesticides containing As, wood preservation, irrigation with contaminated water, ash coal, glass manufacturing, wastewater sludge and pharmaceutical waste (PUNSHON et al., 2017).

The mean I contents of worldwide soils is estimated as 2.8 mg kg^{-1}. However, its concentrations vary widely from <0.1 to 10 mg kg^{-1}. The association between I and SOM, hydrous oxides of Fe and Al, and clay of the chlorite-illite group are often observed. OM is most responsible for the I sorption in soil and therefore I is accumulated mainly in topsoil horizons. Soil microrganisms are believed to play a significant role in the I cycle, owing to their great capability to accumulate this element. Besides this, I level in soils is highly dependent on the atmospheric precipitation. The distance from the sea and recent glaciation's sediments (Pleistocene) also influence the soil I status (KABATA-PENDIAS, 2011).

Whereas little iodine is derived from the weathering of bedrock, the parent material governs the type of soil formed. In this context, the bedrock can exert a strong influence on the iodine retention capacity of soils. The other important feature of soil iodine geochemistry is the fact that the element can become strongly adsorbed by various soil composts, and thus its concentration and behavior in soils are going to depend on soil composition. Therefore, it is possible to predict iodine transformations in the soil as a function of pH, soil organic carbon content, oxide content, and temperature. As an example, the sorption of iodide by aluminum and iron oxides is strongly dependent on soil pH with greatest sorption in acid conditions, which is typical of anion adsorption (SHETAYA et al., 2012; FUGE, 2013).

2.5 *In vitro* oral bioaccessibility of selenium and barium

The bioaccessible fraction of an element can be defined as the part of the ingested nutrient or contaminant that is released from the food matrix in the lumen of the intestine, and that can thereby possibly be absorbed through the intestinal mucosa. The bioavailable fraction, on the other hand, is considered to be the part of the element that is effectively absorbed through the intestinal epithelium and that reaches the systemic bloodstream (THIRY et al., 2013; SUN et al., 2017). Bioavailability and bioaccessibility are influenced by different factors such as the type of food matrix, the presence of other elements that interact with the target element and the chemical form (species) present in the food (THIRY et al., 2013).

In recent years, the (potential) bioavailability of Se in different food sources has been demonstrated using various *in vitro* intestinal digestion procedures to assess bioaccessibility, with some studies already reporting differences in bioaccessibility between different sources of Se and stages of the digestion process. A complete characterization of Se compounds is also necessary to understand the significance of Se in metabolic processes, clinical chemistry, biology, toxicology, nutrition and the environment (MOREDA-PIÑEIRO et al., 2011; BRANDT-KJELSEN et al., 2012; LAVU et al., 2016).

Studies on barium bioaccessibility in foods are almost nonexistent in the literature. However, there is a study evaluating the bioaccessibility of minerals in blackberries, raspberries, blueberries and strawberries. In this research, it was observed that Ba was the element with the greatest bioaccessibility in raspberry (78%) and strawberry (69%) (PEREIRA et al., 2018). For soils, in a study assessing the oral bioaccessibility of Ba in various soil media affected by fugitive dust from mining operations where barite was generated as a waste byproduct, it was observed that the bioaccessibility of Ba via simulated gastric fluids using an *in vitro* test method for different materials of tundra and dust soils varied a lot. The range of variation was between 0.07 and 66%, depending on the sample location, grain size, solid/liquid ratio used in the experiments and the analytical method selected to determine the total Ba concentration in the sample substrates (SHOCK et al., 2007).

Although information on the total concentrations of Se, Ba and As in Brazil nuts is relatively common in the literature, there is still a need to study the bioaccessibility and bioavailability of these elements, since it is a complex food matrix (66- 67% fat, 14% protein and 13% carbohydrates) (DUMONT et al., 2006).

2.6 X-ray fluorescence imaging techniques for trace elements in plants

Most of the chemical analyses of trace elements in plant material are carried out using techniques that have high elemental sensitivity but do not have the necessary resolution to map the distribution of these elements on a subcellular scale and, therefore, require that the entire grain/vegetative organ be ground and digested, losing spatial distribution information. X-ray fluorescence (XRF) techniques have very high sensitivities and a resolution of a few micrometers, allowing the mapping of an entire grain of wheat or rice for example (MEHARG et al., 2008).

The X-ray micro fluorescence technique (μ-XRF) provides a direct and versatile means of investigating macro and microelements in situ in organisms and tissues. This is useful in a wide variety of sample conditions and requires very little or no pretreatment, which is important when studying delicate biological samples (DOLGOVA et al., 2018). Applications of this technique include phytoremediation, food security, and crop biofortification (SARRET et al., 2013).

µ-XRF is a valuable and sensitive tool for analyzing the distribution of elements in different regions of a plant (REIS et al., 2020). As X-rays can be focused on points smaller than 1 mm, the researchers apply this technique to identify, quantify and locate nutrients and contaminants such as arsenic (As) and cadmium (Cd) in plant tissue (VIJAYAN et al., 2015). In recent years, this technique has been used in combination with or often replaces conventional approaches (CAPOBIANCO et al., 2018).

Carey et al. (2012) studied the accumulation of Se species in rice grains, and the spatial distribution of Se in the grains was analyzed using synchrotron X-ray fluorescence microtomography. The authors observed that Se is very strongly located in the ovular vascular trace (OVT), the site of nutrient entry in the grain, for grains treated with selenite. On the other hand, images of grains treated with SeMet show the element dispersed throughout all the outer layers with some possible migration to the endosperm, whereas grains treated with SeMeSeCys also exhibit clear discharge in the pericarp/aleurone layer, but with significant accumulation within of the embryo. Freeman (2006) studied the spatial distribution of Se in hyperaccumulating species *Astragalus bisulcatus* and *Stanleya pinnata* using mapping with an X-ray fluorescence micro scanner (µ-SXRF) on new leaves, the results of which revealed that for *A. bisulcatus* the Se accumulated almost exclusively in leaf trichomes and for *S. pinnata,* the accumulation of Se was observed at the leaf margins and close to the tips.

3 JUSTIFICATION

The contents of Se, Ba, and As in Brazil nuts from different locations in the Amazon region depend on the local soil content, in addition to the plant's genotype, which should also influence the absorption capacity of these elements. It is known that there is a wide variation in terms of soil and Brazil nut populations across the Amazon.

Assuming that there is a reasonable variation in the concentrations of Se, Ba, As and I geographically, it is important to quantify their concentrations in different sites throughout the Amazon basin least affected by human activities in order to study their regional variation and since it is also expected different concentrations of these elements (mainly Se and Ba) in the nuts depending on the location where they were obtained. Therefore, it is crucial to quantify these elements in the soil and understand how they behave, to evaluate their transfer and accumulation in Brazil nuts. Brazil nuts have been consumed not only as an integral product of regional cuisine but also as a food supplement for people who wish to improve the status of Se and prevent its deficiency.

Knowing that the difference between the level of deficiency and toxicity of Se is very narrow in the human organism, and knowing that there are high levels of Ba in Brazil nuts, it is interesting to study the oral bioaccessibility *in vitro*, which provides relevant information about the potential amounts of Se, Ba or As being ingested and absorbed by the human organism. This investigation will constitute an important database, generating critical information for society and contributing greatly to future research. Such results aim to define, for example, the number of nuts that can be eaten daily to increase the natural intake of Se by the population, without leading to risks of toxicity of Ba, As, or even Se itself.

To achieve the objectives proposed in the present study, the thesis was compartmentalized into four articles, presented in the following sequence:

a) Article 1 - Natural variation of arsenic fractions in soils of the Brazilian Amazon;

b) Article 2 - Distribution of selenium, barium, and iodine in soils of the Brazilian Amazon;

c) Article 3 - Selenium and barium concentration and distribution in Brazil nuts (*Bertholletia excelsa*) seeds using µ-XRF;

d) Article 4 - Occurrence and bioaccessibility of essential and toxic trace elements in Brazil nuts from Amazonian agroecosystems.

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SECOND PART: ARTICLES

ARTICLE 1 - Natural variation of arsenic fractions in soils of the Brazilian Amazon

Paper published in the journal Science of The Total Environment (STOTEN)

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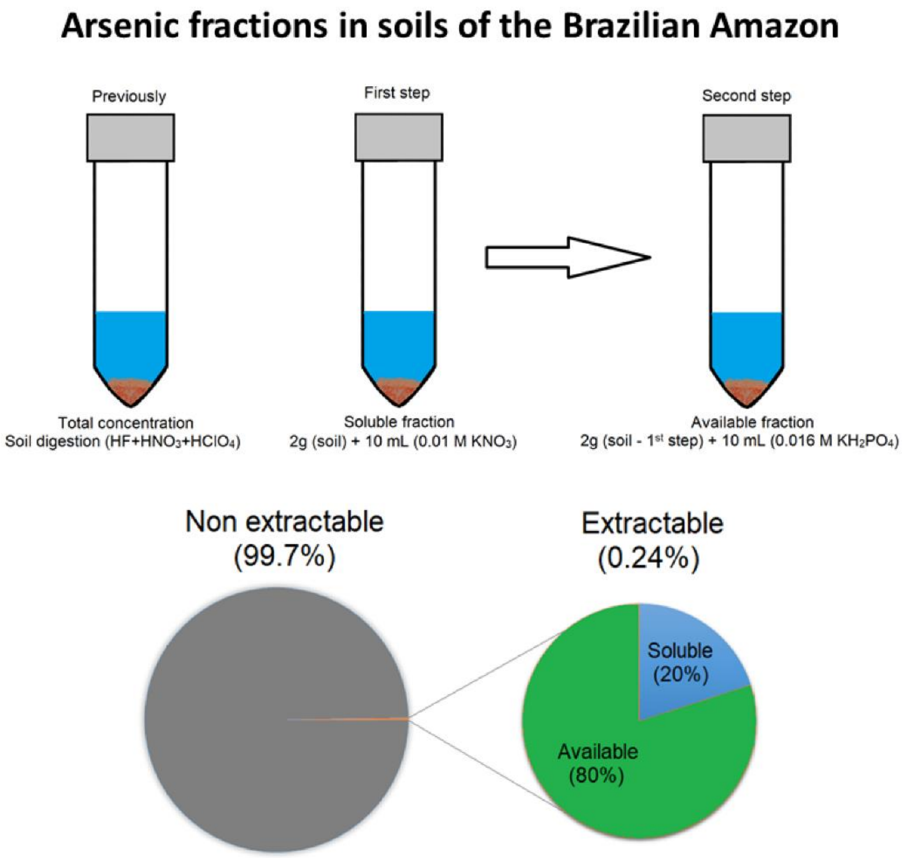
Graphical Abstract



Highlights

- Sena Madureira presents As concentration in soils higher than the prevention level;
- Average extractable As (soluble + available) corresponds to 0.24% of the total;
- Available As represents more than 70% of the extractable As, on average;
- Arsenic fractions are mainly influenced by soil pH and are highly correlated with Al^{3+}.

Abstract

Arsenic (As) in native soils of the Amazon rainforest is a concern due to its likely origin from the Andean rivers, which transport loads of sediments containing substantial amounts of trace elements coming from the cordilleras. Yet, unveiling soil As baseline concentrations in the Amazon basin is still a need because most studies in Brazil have been performed in areas with predominantly high concentrations and cannot express a real baseline value for the region. In this study, 414 soil samples (0-20, 20-40 and 40-60 cm layers) were collected from different sites throughout the Amazon basin - including native Amazon rainforest and minimally disturbed areas - and used to determine total and extractable (soluble + available) As concentrations along with relevant soil physicochemical properties. Descriptive statistics of the data were performed and Pearson correlation supported by a Principal Component Analysis (PCA) provided an improved understanding of where and how As concentrations are influenced by soil attributes. Total As concentration ranged from 0.98 to 41.71 mg kg^{-1} with values usually increasing from the topsoil (0-20 cm) to the deepest layer (40-60 cm) in all sites studied. Considering the proportional contribution given by each fraction (soluble and available) on extractable As concentration, it is noticeable that KH_2PO_4-extractable As represents the most important fraction, with more than 70% of the As extracted on average in all the sites studied. Still, the extractable fractions (soluble + available) correspond to ~0.24% of the total As, on average. Total, available, and soluble As fractions were strongly and positively correlated with soil Al^{3+}. The PCA indicated that soil pH in combination with CEC might be the key factors controlling soil As concentrations and the occurrence of each arsenic fraction in the soil layers.

Keywords: arsenic; extractable As; prevention level; carcinogenic; baseline; Amazon.

1 INTRODUCTION

Arsenic (As) occurs naturally in rocks, soil and water, with higher levels usually found close to sites with anthropogenic pollution, with special attention to groundwater in several countries in the world, with concentration levels exceeding the WHO drinking water guideline value of 10 µg L^{-1} (WHO, 2001; Chowdhury et al., 2018). Contamination has been detected in ground and surface water in 15 of the 20 Latin American countries in 2011, and much of the As contamination described during the last 20 years in this region were published much later or never in the international literature (Bundschuh et al., 2012).

The overall mean value of total As for different soils worldwide is estimated as 6.83 mg kg^{-1} (Kabata-Pendias, 2011), which is very close to the mean value found for Brazilian oxisols (i.e., 5.92 mg kg^{-1}) (Campos et al., 2007). Yet, As is found more abundantly in argillaceous sediments (up to 13 mg kg^{-1}). Anionic forms, AsO_2^-, $HAsO_4^{2-}$, and $H_2AsO_3^{2-}$, predominate in soil solution (Kabata-Pendias and Mukherjee, 2007).

Exposure to As has a great impact on human health across the world (Kapaj et al., 2006; Meliker et al., 2007; Chatterjee et al., 2018). In the environment As often raises a great concern because of its high toxicity to diverse human body systems (e.g. integumentary, nervous, respiratory, cardiovascular, hematopoietic, immune, endocrine, hepatic, renal, reproductive) as well as to its capability of causing different types of cancer (skin, lung, and bladder) (Abdul et al., 2015; Gamboa-Loira et al., 2017). In fact, this naturally occurring metalloid is considered a well-established “Class I” human carcinogen by the International Agency of Research on Cancer (IARC) (Minatel et al., 2018).

Arsenic contamination originating from geogenic sources occurs in many regions worldwide, such as Bangladesh and India (Arco-Lázaro et al., 2018; Rahman et al., 2018). It is estimated that over 200 million people are chronically exposed to As concentrations at or above the World Health Organization (WHO) threshold across the world (Naujokas et al., 2013). In addition to the natural occurrence of arsenic, many other anthropogenic sources may increase soil arsenic concentration, for example, mining, smelting, application of wastes, animal manures and As-bearing pesticides, wood preservation, irrigation using water contaminated, coal fly ash, glass manufacturing, wastewater sludge, pharmaceutical waste, livestock dips and smelting activities to phosphate fertilizers (Punshon et al., 2017).

Several studies have clearly shown that the adverse effects of arsenic are not only dependent on the total concentration of this element, but rather on its reactive fractions (Wang et al., 2018). For As, this fractionation may include soluble and exchangeable, bound to carbonates, iron and manganese oxides, organic matter, sulfides, and residual fraction among others. Those fractions govern arsenic availability and mobility in soils (Ho et al., 2013; Shahmoradi et al., 2017; Liu et al., 2018). Additionally, soil physicochemical properties such as pH, redox potential, clay and organic matter (OM) content and the presence of metal (Fe, Mn and/or Al) oxides and/or competing ions such as phosphates highly influence arsenic availability and mobility (Sharma et al., 2010; Biswas et al., 2014).

Many sequential extraction procedures (SEP) can be used to extract chemical fractions of trace elements in soil (Wan et al., 2017; He et al., 2018). These methods classify trace elements in soil into different operationally-defined fractions with increasing metal-binding strength (Wan et al., 2017). Soil chemical extractions for soluble and available As can help understand the element’s dynamic chemical behavior and its possible entrance into the food chain.

Many extractants can be used for this purpose, for example, the soluble fraction can be extracted using water (water-soluble) (Wenzel et al., 2001; Baroni et al., 2004; Jiang et al., 2014; Wan et al., 2017). This fraction contains water-soluble species such as free arsenate, and weakly adsorbed species, representing the most mobile and potentially the most bioavailable As species in the environment (Wan et al., 2017). Additionally, the soluble fraction can be also extracted using inorganic salts such as KCl (Kim et al., 2003) and KNO_3 (Alam et al., 2007).

Another relevant fraction of As - the available fraction - is not well defined in the literature in terms of method of extraction, but would be better classified as the As retained in the solid phase interacting with the soil solution and occupying exchangeable sites as diffuse ion or as outer-sphere complexes (Van Herreweghe et al., 2003) and can be extracted by using Na_2HPO_4 (Kim et al., 2003), $MgCl_2$ (Jiang et al., 2014), CH_3COOH (Wan et al., 2017), $(NH_4)_2SO_4$ (Wenzel et al., 2001) and KH_2PO_4 (Thinh et al., 2018) among others.

In Brazil, soil quality criteria for some contaminants were defined by CONAMA in Normative 420/2009, which sets screening values based on the total concentration of selected elements. For arsenic, the established threshold for a so-called "prevention level" in soil was 15 mg As kg^{-1} and is primarily based on phytotoxicity tests and ecological risk assessment. Besides this, investigation/intervention values derived from human health risk assessments were set as 35 mg As kg^{-1} for agricultural, 55 mg As kg^{-1} for residential, and 150 mg As kg^{-1} for industrial areas (Conama, 2009). These thresholds were established for total concentrations and in some situations can be very restrictive, especially in soils with low availability. This justifies investing more effort to assess As availability in soils, especially in tropical ecosystems, which are known for their oxidic characteristics that render arsenic less bioavailable (Campos et al., 2007; 2013a).

Increased As levels have been observed in soils worldwide and also in Brazil, especially due to mining activities (Bundschuh et al., 2012). Hypothetically, we could expect high concentrations of As in soils nearby the Amazon basin because of the sediments coming through the Amazon river (and its tributaries) from the Andes, which are expected to be naturally enriched in selected volatile trace elements (e.g., Se and Hg) (Fadini and Jardim, 2001; Silva Junior et al., 2017). However, there is a lack of comprehensive information about background levels of As in soils from the Amazon basin to prove this assumption since the more recent studies have focused only on specific states of Eastern Amazon (Fernandes et al. 2018). Therefore it is necessary to perform a more inclusive study concerning the occurrence/distribution of As in different Amazon agroecosystems to provide information for the local population about possible health issues involved in the use of soil and water resources for living, building and growing crops in this region.

To the best of our knowledge, no research data regarding the determination of soluble and available soil As in the Brazilian Amazon region have been published so far. This is relevant, as such information could support public policies for decision-makers and help guide future research in these sites in order to provide better planning for sustainable use of land and natural resources by the local population while ensuring also human health and food security.

In this context, this study aims to: i) characterize and compare baseline levels of As in different sites in the Amazon region; ii) study the dynamics of As fractions (soluble and available fractions) with depth, i.e., in three different layers (0-20, 20-40 and 40-60 cm) of soils occurring in agroecosystems with Brazil nuts plantations; and, iii) assess the influence of the main soil physicochemical attributes for determining the soil As concentrations and the occurrence of each arsenic fraction in the soils.

2 MATERIALS AND METHODS

2.1 Study approach

This study involved the following steps: (a) soil survey to collect baseline data for As in 9 target sites; (b) description of the natural variation of As and soil physicochemical attributes in each site; and, (c) correlation and PCA analysis to support the inference of soil environmental controls of As variability in the soil. The methods used to reach our goals are described in the following sections.

2.2 Sampling sites and characterization of the study area

Soil samples were collected in nine sites belonging to the Amazon Biome in Brazil. Each site correspond to a Brazil nut stand, identified by: SM (Sena Madureira, state of Acre); XP (Xapuri, state of Acre); ANO (Anori, state of Amazonas); AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); MT (Itaúba, state of Mato Grosso); PA (Santarém, state of Pará); RO (Porto Velho, State of Rondônia) and RR (Caracaraí, state of Roraima). In each site, samples of 15 collection points were sampled in the native Amazon rainforest, except for AR (Itacoatiara), where samples of 18 spots were collected in the Aruanã farm, which is a large Brazil nut plantation. Samples were collected between 2014 and 2017. The chosen target sites for this study were the same ones used by Silva Junior et al. (2017) in their survey assessing the natural variation of selenium in Brazil nuts and soils from the Amazon region, to which we have added four additional areas. Sampling sites and detailed geographic information are provided in **Figure 1** and **Table S1**.

Soil samples were collected at the depths of 0-20, 20-40, and 40-60 cm at a distance of 3 m from the trunk of selected Brazil nuts trees. These samples were collected at four points and mixed to compose a single sample for each depth. The sampling method is represented schematically according to Silva Junior et al. (2017).

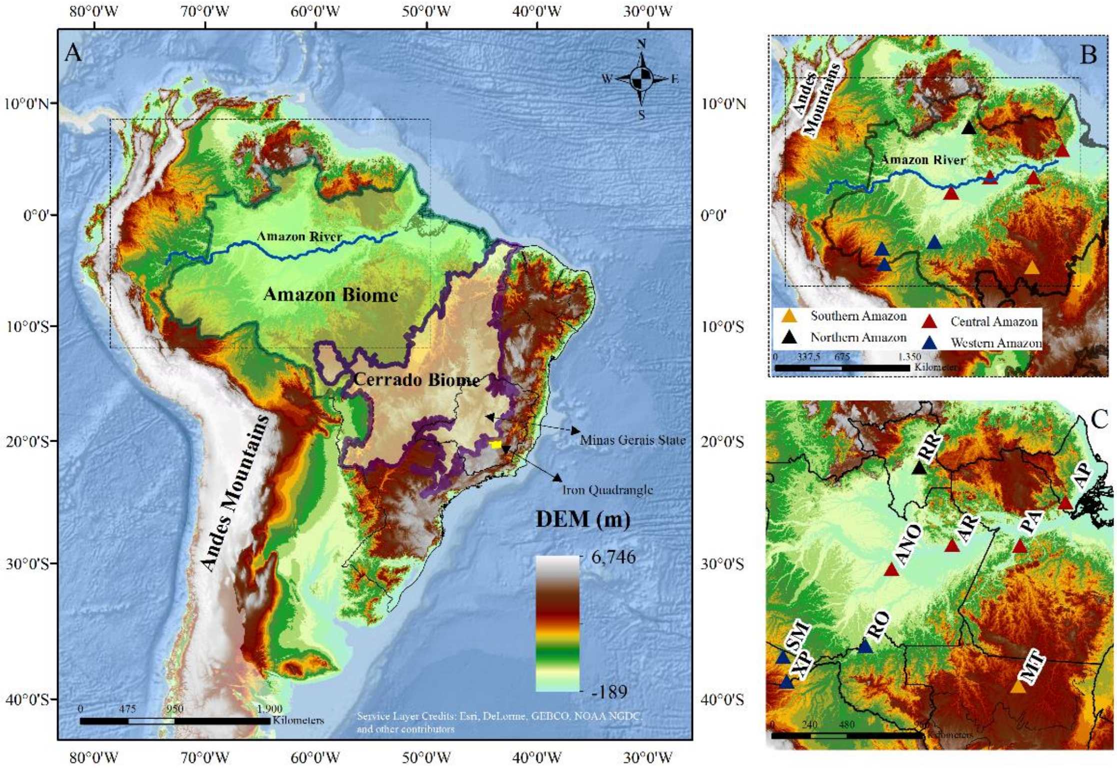


Figure 1. Map of the Brazilian Amazon (within South America) (A) with visualization of the Andes mountain range and Amazon sub-regions (B) as well as indicating the location of the nine studied sites (C) identified by: Sena Madureira (SM) and Xapuri (XP)-state of Acre; Porto Velho (RO)-state of Rondônia; Anori (ANO) and Itacoatiara (AR)-state of Amazonas; Itaúba (MT)-state of Mato Grosso; Caracaraí (RR)-state of Roraima; Santarém (PA)-state of Pará and Laranjal do Jari (AP)-state of Amapá. Relevant locations referred to in the manuscript for data comparison (e.g., the Cerrado biome as well as the State of Minas Gerais) are also shown on the map. DEM - Digital elevation model. Source: U.S. Geological Survey's Center for Earth Resources Observation and Science (EROS).

2.3 Soil physicochemical characterization

Soil samples were taken to the Soil Science Department of the Federal University of Lavras, Minas Gerais-Brazil, where they were ground, sieved at < 2 mm, dried at room temperature, and subjected to chemical and physical (texture) analysis. The soil pH was determined potentiometrically in water (pH H_2O) and KCl (1 mol L^{-1}) in ratio 1:2.5 soil/solution. The exchangeable cations (Ca^{2+}, Mg^{2+} and Al^{3+}) were obtained by 1 mol L^{-1} KCl extractants. Sulfur ($S\text{-}SO_4^{2-}$) was extracted as sulfate by monocalcium phosphate with acetic acid and the result converted to S. Available P, Na, K, and micronutrients (Fe, Zn, Mn, and Cu) were obtained by Mehlich-1 (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4; in ratio 1:10 soil/solution). Potential acidity (H + Al) was estimated indirectly through SMP pH (Shoemaker et al., 1961). Exchangeable aluminum (Al^{3+}) was determined by titration with sodium hydroxide (0.025 mol L^{-1} NaOH). Phosphorus (P) was determined colorimetrically, K by flame emission photometry, $S\text{-}SO_4^{2-}$ by turbidimetry, and Ca, Mg, Zn, Fe, Mn, and Cu were determined by atomic absorption spectrometry (AAS) (Raij and Quaggio, 1983). The organic matter content was determined by oxidation of the organic matter in the soil with a potassium dichromate solution in the presence of sulfuric acid (Carter and Gregorich, 2006). Sum of bases (SB), cation exchange capacity at pH 7 (T), effective cation exchange capacity (t), base saturation (V%), and aluminum saturation (m) were calculated based on the results of the chemical analysis. The particle size analysis (clay, silt, and sand) was performed by the “Bouyoucos” method, as described by Embrapa (1997).

2.4 Total arsenic determination

Total As analyses were performed in air-dried and < 2 mm sieved soil samples. A portion of each soil sample was finely ground (< 0.45 mm) using an agate ball mill (Retch PM 400 mill). Soil samples (~0.2 g DW) were fully digested in 70% HF, 70% HNO_3 and 60% $HClO_4$ (TAG; Fisher Scientific UK Ltd), using perfluoroalkoxy (PFA) digestion vessels and a 48-place Teflon-coated graphite digester block (Model A3, Analysco Ltd, Chipping Norton, UK) (Chilimba et al., 2011).

The digester block runs with two different programs, a mix of reagents and a few steps for 3 days as can be observed with further details in Table 1. After the digestion, the block was turned off and the tubes were left to cool down completely for about 30 minutes. Digested samples were diluted to 50 mL into volumetric flasks using Milli-Q water and stored in 5% HNO_3 at room temperature in universal sample bottles, pending elemental analysis. Before the analysis in the ICP-MS, the extracts were diluted using 1 mL + 9 mL of Milli-Q water.

Table 1. HF digestion steps with digester block

Day	**program**	**reagent**	**volume (mL)**	**steps**
1°	1[a]	HNO_3 $HClO_4$	2 1	1) 80 °C, 8 h; 2) 100 °C, 2 h.
2°	2	HF	2.5	1) 120 °C, 1h; 2) 140 °C, 3 h; 3) 160 °C, 4 h; 4) 50 °C, 1h.
3°	-	HNO_3 Milli-Q H_2O	2.5 2.5	

[a]Left overnight with the program turned on.

Each digestion batch (n = 70) included a standard reference material (n = 3) (NIST 2711a; Montana Soil II, National Institute of Standards and Technology, Gaithersburg, MD, USA), which contains 107 ± 5 mg kg^{-1} As. The average recovery in the standard reference material was 101% (n = 21, SD = 6.61) for As. In addition, 3 blank samples were used in each batch for quality control purposes and for calculating limits of detection. The limit of detection (LOD) was established using 21 blank extracts for soil samples following the overall procedure. The values were calculated with three times the standard deviation (SD) of the blanks used in each batch (n = 3) using the formula: SD (3 blanks) x 3 x final volume extract (mL) ÷ sample weight (g). The average LOD was 0.029 mg kg^{-1} for total soil As.

2.5 Soluble and available As in soil (extractable As)

Soluble and available As analyses were performed in sequence, according to the modified method described by Cai et al. (2002). First, for the soluble fraction, 2 g of air-dried and < 2 mm sieved soil was added into a 50 mL polycarbonate centrifuge tube. Next 10 mL of 0.01 mol L^{-1} KNO_3 were added to each tube and the suspensions were shaken for 2 hours using a rotary shaker (20 rpm). Soil suspensions were then centrifuged for 30 min at 3500 rpm, filtered to < 0.22 µm using a Millex syringe driven filter unit (Millipore, Cork, Ireland) and stored at room temperature prior to analysis of As by ICP-MS. In the second step, for the available fraction, an extraction with 10 mL of 0.016 M KH_2PO_4 was performed in the same tubes from the previous step. The suspensions were shaken for 1 hour in the rotary shaker (20 rpm) and then centrifuged for 30 minutes at 3500 rpm. The supernatant was filtered using a < 0.22 µm syringe filter and put in a universal tube (used for analysis in the ICP-MS). For the ICP-MS analysis, 4.8 mL of the supernatant collected from KNO_3 and KH_2PO_4 extractions were added into an ICP tube containing 0.2 mL of 50% HNO_3 for As analysis. The limits of detection (LOD) for soluble and available soil As were calculated as previously mentioned, and corresponded to 0.047 and 0.056 µg kg^{-1}, respectively.

2.5 Analytical determination of As

The analytical determinations of As were performed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, X-Series II, Thermo Fisher Scientific) in the Environmental geochemistry analytical suite from the University of Nottingham-UK. Samples were introduced at 1 mL min^{-1} through a concentric glass venturi nebulizer and Peltier-cooled (3 °C) spray chamber. Instrumental drift was corrected using internal standards (^{72}Ge, ^{103}Rh

and ^{185}Re). Data for total As concentration in soils are reported on a dry weight basis (DW) and expressed in mg kg^{-1}. Data for soluble and available As are expressed in µg kg^{-1}.

2.6 Statistical analysis

A descriptive analysis of the concentrations of total As, as well as for soluble and available As was carried out using Box plots to display data distribution. The data were subjected to Pearson's correlation analysis, which was performed between total, soluble and available As concentrations and other physicochemical soil properties. All the graphics were performed using the software R 3.4.4 (R Development Core Team, 2018).

Analysis of variance was performed to assess how the log-transformed As concentration in soils varied across different sites. After verifying the significance through ANOVA, the mean values for As concentration were compared among sites for the three layers studied using Tukey's HSD test with the package emmeans v 2.23 (Lenth, 2016) in R 3.4.4 (R Development Core Team, 2018). The same approach was used to compare soluble and available As in soils, but in this case using generalized least squares (GLS) for the data log-transformed, to allow the variance to be estimated separately for each site (Cleasby and Nakagawa, 2011).

A principal component analysis (PCA) was performed to find out the main soil attributes influencing As concentration in the soil according to the degree of variance explained and to confirm the previous relationships obtained by Pearson correlation analyses. Because the distribution of As total concentrations and fractions was not normal, the data were log-transformed, before analysis to meet the assumption of normality required for the Pearson correlation and PCA, except for soil pH, because it follows a normal distribution in soil data.

3 RESULTS AND DISCUSSION

3.1 Soil physicochemical properties, element contents and associations

The chemical and textural attributes of soil varied widely among the sites studied, reflecting a great variability in soil characteristics throughout the Amazon basin that may be related to the diversity of parent material and soil types. Most of the collected samples show acidic soils (mean pH ranging from 3.75 to 5.5) which reflects the well-known weathered soils from this region according to Alvarez et al. (1999). The OM content in the topsoil is classified as moderate in most sites (average OM content ranging from 1.16% to 2.63%) according to Alvarez et al. (1999) (**Table 2**). The moderate contents of OM can be explained by the high temperature and precipitation levels (humidity) in the region, i.e., the most important climatic factors controlling the dynamics of ecological processes (da Silva et al., 2018), which reflects not only in the biomass production but also in decomposition rates of the litter in the soil.

The dominant soil texture was clay (AR, RO and PA) and sandy loam (MT and AP). Such high percentage of clay content facilitates As retention, especially onto kaolinite, as well as Fe and Al oxyhydroxide surfaces that predominate in the clay fraction of the highly developed soils of the Amazon region, which is subjected to strong weathering conditions (de Souza et al., 2018) (**Table 2**).

Soil cation exchange capacity (CEC) ranged from 2.96 to 25.2 cmol$_c$ dm^{-3} demonstrating a great variability among the sites studied. The soils from ANO showed the highest mean values of CEC (22.6 cmol$_c$ dm^{-3}). For the other sampled sites, CEC values were all below 13 cmol$_c$ dm^{-3} with the lowest values observed in the sites RR and AP (5.33 and 4.28 cmol$_c$ dm^{-3}, respectively). This wide variation in soil CEC indicates that besides the predominance of dystrophic and highly weathered soils with secondary minerals, there are still soils with 2:1 clay minerals, composing less weathered soils with high activity in the clay fraction in this region (**Table 2**).

Extractable S and P in the present study exhibited considerable variation among the sites with mean values ranging from 5.29 to 47.9 mg kg^{-1} (S) and from 0.52 to 3.72 mg kg^{-1} (P). In general, the content of available P was moderate in most sites, where also the OM contents are usually higher than in deeper layers, except in the sites MT and RO in southern Amazon, which contained available P values below 2 mg kg^{-1} (**Table 2**). The content of extractable S on the other hand presented higher values in the sites AR and RO (**Table 2**).

Iron (Fe) usually plays an important role in controlling the concentration of trace elements in soil solution because of its involvement in adsorption. The variation of available Fe among the sites was also quite outstanding with mean values ranging from 58.5 mg kg^{-1} (AP) to 489.7 mg kg^{-1} (ANO) (**Table 2**). We observed that soil pH is the major factor explaining the concentration of this element in soil solution, since the site with the lowest Fe content present also the highest pH (site AP). On the other hand, available Fe might not reflect the concentration of iron (hydr)oxides that explains the retention of anions in soil.

Table 2. Physicochemical properties of the topsoil (0-20 cm) from samples collected at different sites in the Amazon region.

Site	n	pH (H_2O)	OM (%)	CEC ($cmol_c$ dm^{-3})	S (mg kg^{-1})	P (mg kg^{-1})	Fe (mg kg^{-1})	Clay	Silt	Sand
								(%)		
SM	15	4.53±0.34	1.42±0.35	6.92±1.74	5.29±1.17	3.72±1.27	174.9±86.3	21.6±4.70	22.5±2.38	55.9±4.85
XP	15	4.36±0.32	1.62±0.55	7.21±2.25	8.09±5.16	3.08±0.75	190.1±83.9	18.4±5.37	32.3±10.2	49.3±10.5
MT	15	4.19±0.16	2.32±0.40	7.10±2.52	7.32±2.00	1.46±0.32	324.3±69.6	19.5±2.61	3.86±1.85	76.6±2.82
AR	18	4.26±0.09	2.63±0.32	7.97±1.34	16.5±7.06	2.56±0.61	183.0±48.6	77.6±3.46	10.1±2.37	12.3±2.56
ANO	15	4.00±0.35	2.03±0.70	22.6±10.9	9.55±4.97	2.53±0.85	489.7±134.1	27.2±5.82	56.7±8.05	16.1±11.9
AP	15	5.49±0.34	1.16±0.34	4.28±0.67	9.49±1.60	2.25±0.72	58.5±22.6	19.9±2.60	3.40±1.92	76.7±3.24
RR	15	4.46±0.16	2.43±0.46	5.33±0.73	10.1±1.64	2.38±0.28	177.2±50.2	44.1±5.97	8.13±3.54	47.7±6.12
RO	15	3.75±0.22	2.40±0.37	12.6±3.63	18.0±8.88	1.87±0.37	196.2±70.8	51.7±9.78	28.1±12.5	20.2±7.70
PA†	15	4.41±0.26	2.20±0.42	9.24±2.24	10.6±3.51	2.25±0.43	242.4±62.5	52.0	8.00	40.0

The values of soil physicochemical attributes are mean ± standard deviation (SD).

Each site is identified by: SM (Sena Madureira, state of Acre); XP (Xapuri, state of Acre); ANO (Anori, state of Amazonas); AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); MT (Itaúba, state of Mato Grosso); PA (Santarém, state of Pará); RO (Porto Velho, state of Rondônia) and RR (Caracaraí, state of Roraima).

[a] Texture in PA was derived from one single analysis performed by a composite sample resulted of subsamples from 5 spots in the site.

3.2 Total As concentration in soil

Total As concentrations ranged from 0.98 to 41.7 mg kg^{-1}, with 75% of the soil samples showing values lower than 6.93 mg kg^{-1}, which is close to the mean value of total As reported for different soils worldwide (i.e., 6.83 mg kg^{-1}) (Kabata-Pendias, 2011). The sites with the highest baseline As concentration were SM followed by RO and XP. On the other hand, the sites with lowest As concentrations were AP and RR, according to the Tukey HSD test (**Figure 2**).

Interestingly, total As concentration in the western Amazon (SM, XP and RO) presented the highest values and a wide variation not only among the layers but also within the three layers evaluated. The total As concentration in the western Amazon showed median concentrations increasing from the topsoil to the deepest layers as follow: SM (15.9 < 19.0 < 26.5 mg kg^{-1}) and XP (5.96 < 7.20 < 9.34 mg kg^{-1}) for the layers 0-20, 20-40 and 40-60 cm, respectively. A wide variation for arsenic concentration within layers was observed especially at the deepest layer (40-60 cm), which also presented the highest As concentrations for each site studied in the western Amazon: SM (17.3 to 41.7 mg kg^{-1}), XP (5.69 to 17.8 mg kg^{-1}), and RO (5.83 to 23.6 mg kg^{-1}) (**Figure 2**).

There is no nationwide As baseline level established by the Brazilian legislation to be used in terms of quality reference values (QRVs) for soils. This is also true for the soils of the Brazilian Amazon region. However, a QRV for As in soils from the Eastern Amazon was proposed by Fernandes et al. (2018). These authors observed total As concentration ranging from 0.07 to 7.75 mg kg^{-1} in the 0-20 cm layer and, considering the 90th percentile of the sample population after eliminating anomalies (cumulative frequency distribution), 2.7 mg kg^{-1} was suggested as the QRV. Given the heterogeneity in the pedogeoclimatic conditions in the region, the 90th percentile seems to best represent the QRVs because of the wide variances for the values observed, as suggested by Conama (2009).

In the present study 57% of the samples from the 0-20 cm soil layer show As concentrations higher than the QRV for the 90th percentile proposed by Fernandes et al. (2018), which reinforces the difficulty of establishing a single QRV for a very large region as the Brazilian Amazon. Besides, the medians for total As concentrations in the three layers (0-20, 20-40 and 40-60 cm) from the site SM are higher than the As prevention level (15 mg kg^{-1}) established by the Brazilian Normative CONAMA 420, for soils nationwide (Conama, 2009) (**Figure 2**). This is indicative that the current Brazilian prevention values need to be validated using representative soils from the Amazon basin since this region may present soils that may be naturally enriched with As. All other studied sites presented median As concentrations below 6 mg kg^{-1} - a value close to the average As content reported by Campos et al. (2007) for 17 Brazilian oxisols (i.e., 5.92 mg kg^{-1}) - with the lowest median concentrations observed in the site AP (1.30, 1.53 and 1.71 mg kg^{-1} for 0-20, 20-40 and 40-60 cm respectively) (**Figure 2**).

The sites from the central and eastern portion of the Amazon basin (ANO, AR, PA and AP) presented low As concentrations in all the three layers evaluated. They also presented low variation among and within the layers, as observed in the median's concentrations in the site AR (5.07, 5.33 and 5.41 mg kg^{-1} for 0-20, 20-40 and 40-60 cm respectively) (**Figure 2**).

The sites located in the northern and southern portion of the Amazon, which are RR (medians of 1.39, 1.70 and 1.80 mg kg^{-1}) and MT (medians of 2.56, 2.99 and 3.03 mg kg^{-1}) also presented low total As concentration for the three layers evaluated compared to the other sites studied, and no meaningful variation among and within the layers was noticed (**Figure 2**).

Campos et al. (2013b) determined background levels of As in representative soils from the states of Goiás and Minas Gerais (from 2000 to 2500 km far away from the studied sites), which belongs to Cerrado biome (Brazil) in three sub-regions and observed concentrations similar to those found in the southern Amazon in the present study, with mean concentrations in decreasing order as follow: East of state of Goiás (3.29 mg kg^{-1}) > Triângulo Mineiro (state of Minas Gerais) (2.18 mg kg^{-1}) > Northeast of Minas Gerais (0.62 mg kg^{-1}). The highest As concentrations were observed in profiles of Plintosols and Cambisols from the East of Goiás. In another study, Campos et al. (2013a) observed levels of As ranging between 0.14 mg kg^{-1} (for a gley soil) and 9.3 mg kg^{-1} (for an oxisol) in soils from Minas Gerais. Those concentrations are usually found in non-contaminated soils (< 10 mg kg^{-1}) (Adriano, 2001; Campos et al., 2007, 2013a; Bundschuh et al., 2012) and are similar to the ones reported in the present study.

In a different scenario far away from the Amazon region, in the Iron Quadrangle, southeast region of Brazil, within the state of Minas Gerais, the presence of As is associated with primary gold deposits where it is contained predominantly in S-bearing minerals such as arsenopyrite and pyrite (Borba et al., 2003). Also in the iron quadrangle, Alves and Rietzler (2015) observed high As concentrations in soils from mining areas in Nova Lima (13.2 in the control site and 489 mg kg^{-1} in the mine stream) and Santa Bárbara (82.3 in the control site and 1329 mg kg^{-1} nearby the mine plant). Out of the Iron Quadrangle, but still in Minas Gerais, natural soil As concentrations found in a private natural reserve (native Cerrado vegetation) located nearby a gold-mining area ranged from 25 to 35 mg kg^{-1} (Ono et al., 2012), which is also greater than the prevention level established by Normative 420, for Brazilian soils (Conama, 2009). These studies emphasize the importance of the parent material in total arsenic concentration in soils and make us believe that the main source of arsenic, in the present study, is geogenic.

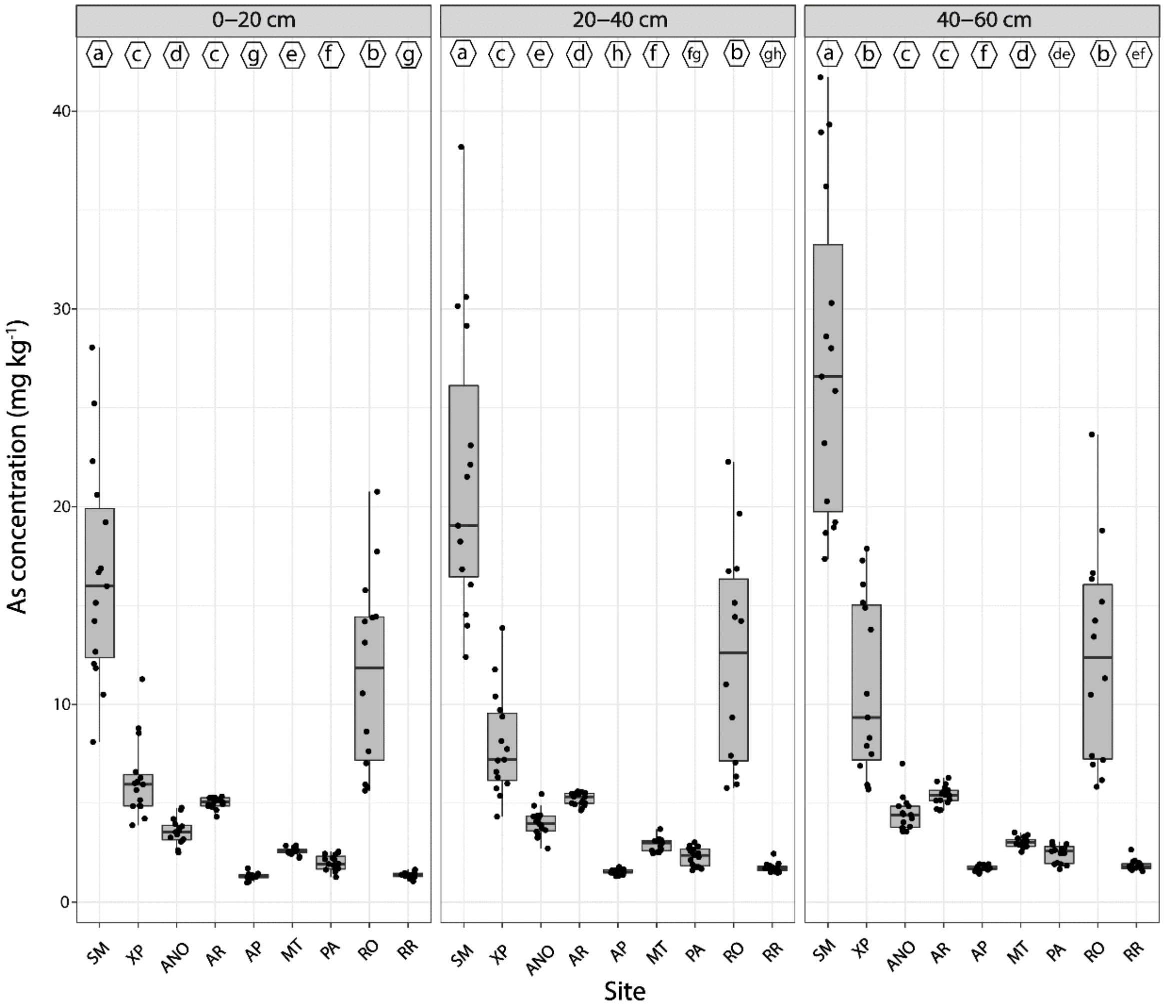


Figure 2. Raw data for total As concentrations in soils from different sites in the Amazon region for the layers 0-20, 20-40 and 40-60 cm. Each site correspond to a Brazil nut stand, identified by: SM (Sena Madureira, state of Acre); XP (Xapuri, state of Acre); ANO (Anori, state of Amazonas); AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); MT (Itaúba, state of Mato Grosso); PA (Santarém, state of Pará); RO (Porto Velho, state of Rondônia) and RR (Caracaraí, state of Roraima). The letter on top of each state indicates the result of Tukey's HSD test (p = 0.05) on the log-transformed As concentration, following a significant ANOVA ($p < 0.001$).

3.3 Extractable soil As (soluble and available)

Extractions for soluble and available As showed the highest concentrations for the site SM, which is in accordance with the results observed for total As. For the soluble As the concentrations ranged from < 0.047 (MT) to 16 µg kg^{-1} (SM). Available As varied from 2.14 (AR) to 236.5 µg kg^{-1} (SM) (**Figure 3**).

Observing the mean concentrations for soluble As, it is noteworthy that in SM the values are increasing from the topsoil to the deepest layers. On the other hand, in the sites AR, ANO, PA, RO and AP, soluble As decreases from the topsoil (0-20 cm) to the deepest layers (20-40 and 40-60 cm). The sites RR and AP presented the lowest average concentration for soluble As (**Figure 3**). The site with the highest pH (AP) also presented the lowest median values for soluble As (**Figure 3**). This effect is confirmed by the negative correlation between soluble As and pH in all the three layers studied (**Table 3**).

The average concentration for available As in SM in the layers 0-20, 20-40 and 40-60 cm (45.3, 68.6 and 117 µg kg^{-1}) is at least 4-fold higher than those observed in all other sites studied. There was no meaningful variation among the sites for available As.

The observed behavior of soluble and available As in these soils may be partly explained by their interactions with available P (**Table 2**), which showed a positive correlation with both fractions in all the three layers studied (**Table 3**). This is expected as a greater content of P in oxidic soils (which retain phosphate strongly) make As much more available and soluble due to competitive adsorption on positively-charged sites (Campos et al., 2013a).

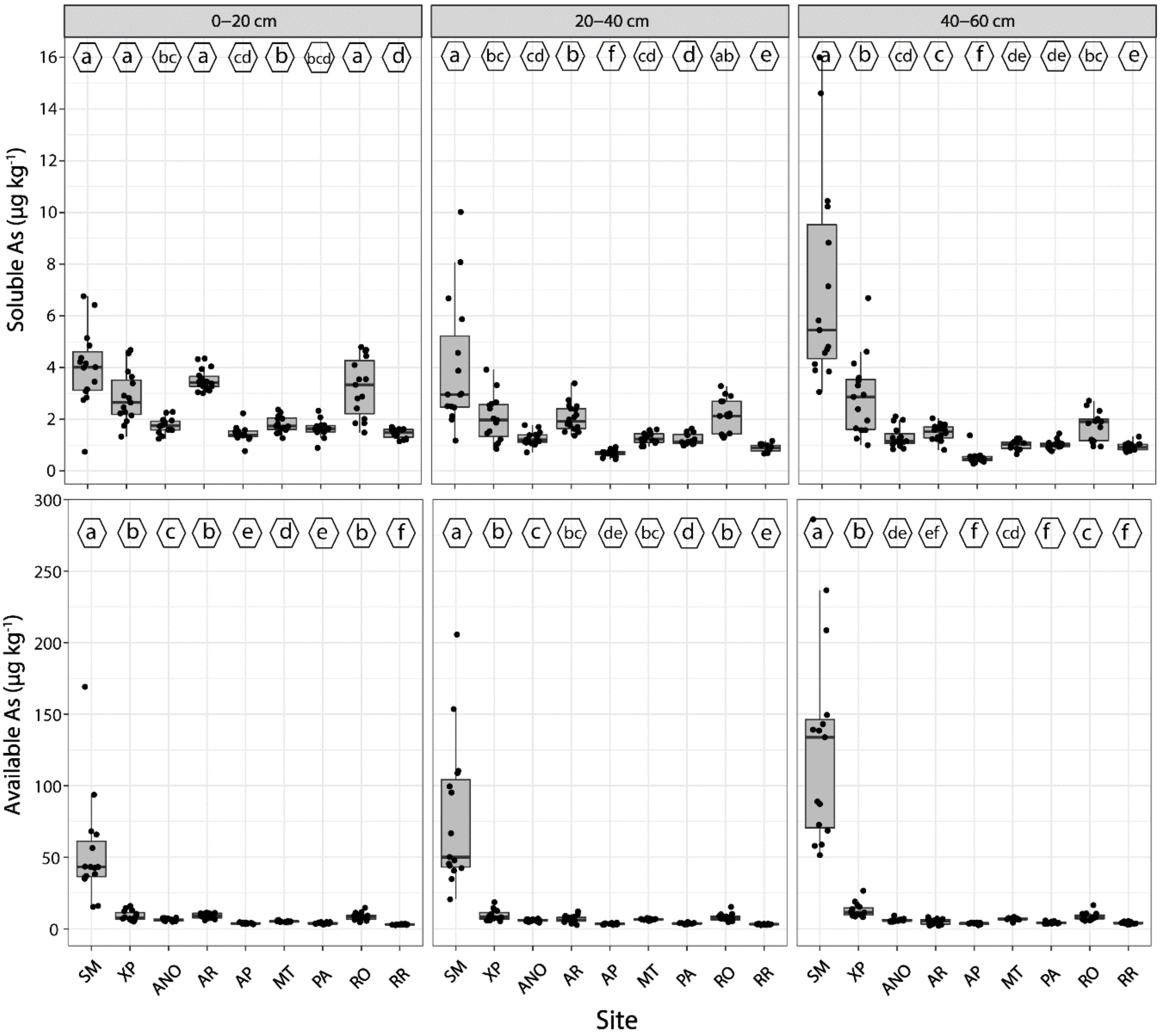


Figure 3. Raw data for concentration of As available and soluble for 9 sites in the Amazon region. Each site correspond to a Brazil nut stand, identified by: SM (Sena Madureira, state of Acre); XP (Xapuri, state of Acre); ANO (Anori, state of Amazonas); AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); MT (Itaúba, state of Mato Grosso); PA (Santarém, state of Pará); RO (Porto Velho, state of Rondônia) and RR (Caracaraí, state of Roraima). The letter on top of each state indicates the result of Tukey's HSD test ($p = 0.05$) on the log-transformed As concentration, following a significant ANOVA ($p < 0.001$) using generalized least squares.

Considering the proportional contribution given by each fraction (soluble and available) to the extractable As concentration, it is noticeable that available As represents the major fraction with more than 70% of the As being extracted by KH_2PO_4 on average in all the sites studied. Generally, the contribution given by the soluble fraction in all the sites reduces from the topsoil to the deepest layer, as follows: 25% > 18% > 16% (0-20, 20-40 and 40-60 cm respectively) (**Figure 4**). The site with the greatest contribution given by available As was SM with 92%, 94% and 94% for the 0-20, 20-40 and 40-60 soil layers. On the other hand, the sites with the smallest contribution given by available As were RR in the layer 0-20 cm (67.7%), PA in the layer 20-40 cm (75.7%) and AR in the layer 40-60 cm (77.5%) (**Figure 4**).

Arsenic availability was also assessed using tri-distilled water, ammonium sulfate, ammonium phosphate, ammonium oxalate + oxalic acid, a mixture of organic acids, Mehlich-1, and USEPA 3051A in artificially contaminated soils (entisol and oxisol) by Melo et al. (2012). The extracting solutions tested in the experiment were equally efficient in assessing plant availability of As in the soils, however, there was a greater concentration of As extracted from the clayey entisol when compared with the medium-textured oxisol due to differences between the adsorptive capacities of these soils. Besides validating extractants for plant-available As, these authors emphasized the capacity of tropical soils, with variable charge, to adsorb As, especially those soils rich in clay (Melo et al., 2012).

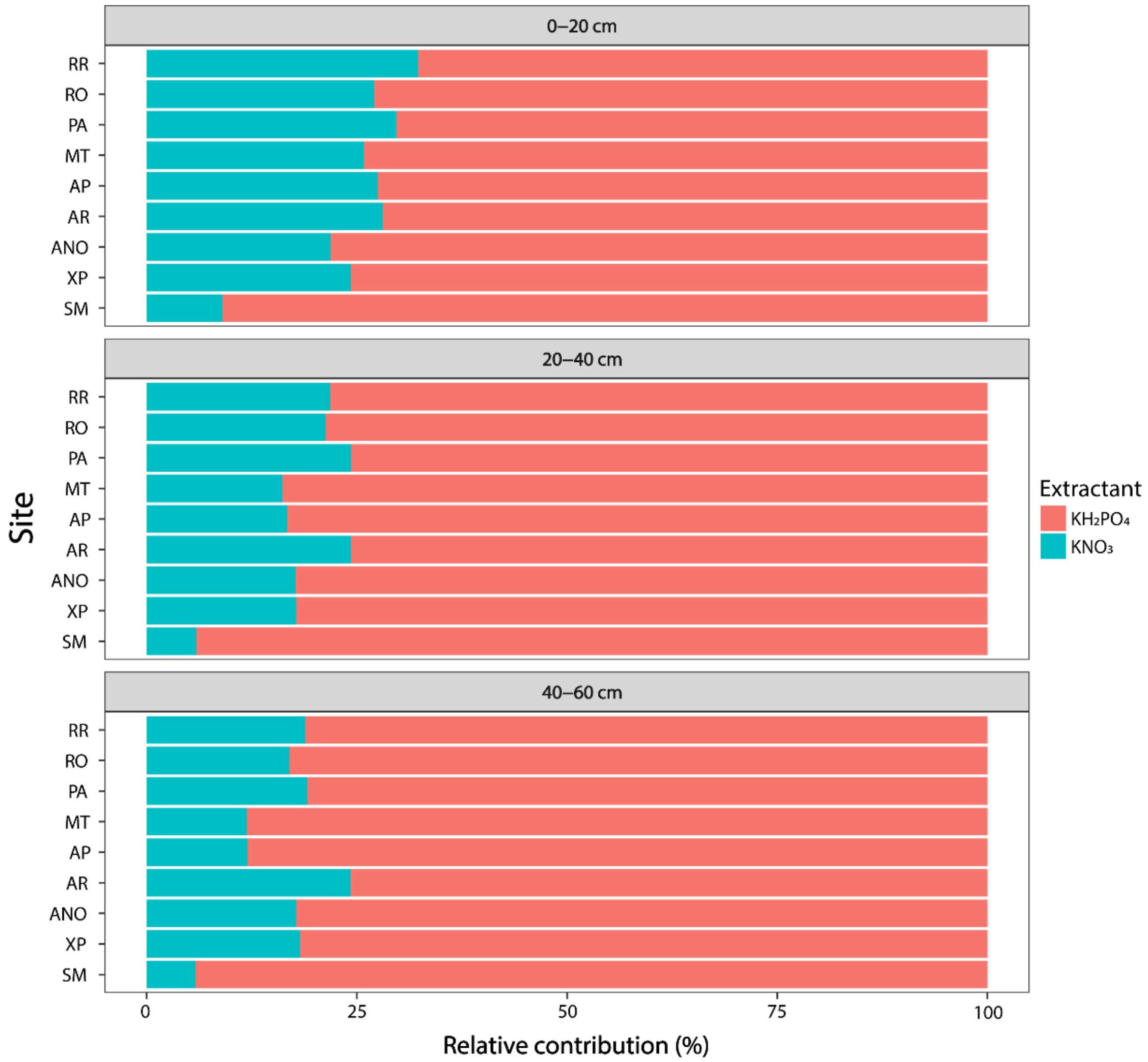


Figure 4. Relative contribution in % from soluble and available As in soils from different sites in the Amazon region. Each site correspond to a Brazil nut stand identified by: SM (Sena Madureira, state of Acre); XP (Xapuri, state of Acre); ANO (Anori, state of Amazonas); AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); MT (Itaúba, state of Mato Grosso); PA (Santarém, state of Pará); RO (Porto Velho, state of Rondônia) and RR (Caracaraí, state of Roraima).

3.4 Relationship between soil As and physicochemical attributes

Total As concentration (total As) correlated positively ($p < 0.05$) with CEC, available P, K, exchangeable Al, and silt in the three layers studied and also with available Zn in the 20-

40 and 40-60 cm layers. Total As correlated negatively ($p < 0.05$) with pH, exchangeable Ca, available Cu in the three layers studied and also with exchangeable Mg and available Mn in the 0-20 cm layer and with available B and sand in the 0-20 and 20-40 cm layers. These results indicate that, in tropical soil conditions, increasing pH values will decrease total As content either directly because it influences colloidal charges (i.e., causing a decrease in positive charges) or indirectly influencing the availability and retention of competing anions such as phosphate and sulfate (considering that soils are open systems and available forms can be removed, thereby reducing total levels) (**Table 3**).

Soluble As correlated positively ($p < 0.05$) with CEC, available P, exchangeable Al and silt in the three layers evaluated and also with OM and clay in the 0-20 cm layer and with available Zn and K in the 40-60 cm layer. Soluble As correlated negatively ($p < 0.05$) with pH, exchangeable Ca and available Cu in the three layers studied and also with exchangeable Mg, available B and sand in the 0-20 and 20-40 cm layers following by Fe in the 40-60 cm and available Mn in the 0-20 and 40-60 cm layers (**Table 3**).

On the other hand, available As correlated positively ($p < 0.05$) with available P, K, Zn, exchangeable Al and silt in all the three layers studied and also with CEC in the 20-40 and 40-60 cm layers and with available Mn in the 20-40 cm layer following by sand in the 40-60 cm layer. Available As correlated negatively ($p < 0.05$) with OM and clay in the 20-40 and 40-60 cm, and also with pH in the 20-40 cm, with extractable S in the 0-20 and 40-60 cm layers, Fe in the 40-60 cm layer and available Cu in the 0-20 cm layer (**Table 3**).

In the present study, an important factor influencing As availability that negatively affected total and soluble As was soil pH as can be observed in **Figure 5**. As pointed out by Zhang et al. (2017), numerous studies have shown that soil pH is an important factor controlling arsenic geochemistry, with adsorption of As decreasing as pH increases. This is attributed to the higher amounts of negative charges on soil composts that generate variable charges, such as 1:1 clays, along with fewer amounts of positive charges on Fe and Al oxides. Such pH influence on charge generation is pronounced in tropical soils since these are variable charge soils (Fontes et al., 2001).

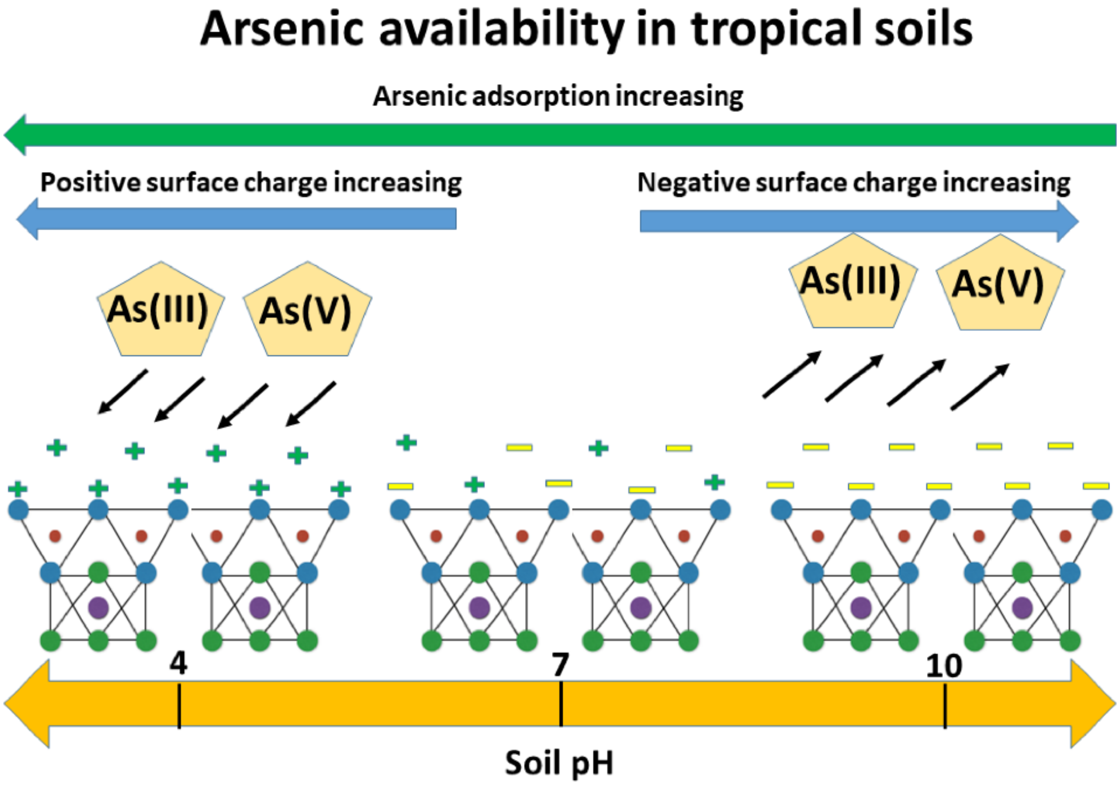


Figure 5. Major mechanism controlling arsenic availability in tropical soils.

According to Huang et al. (2006), NaH_2PO_4-extractable As correlates well with total As concentrations found in edible parts of various crops and soils collected from paddy rice fields in suburban areas of Fukan province in southeast China, showing the usefulness of this extractant to assess the availability of As in soils. These authors also reported that As availability decreased significantly with increasing silt size (0.02-0.002 mm) and free iron content, but increased significantly with soil pH and organic matter content. Those findings are not supported by results observed in the present study for the silt fraction, which showed a positive correlation with total, soluble, and available As in soil samples of all the three layers studied (**Table 3**).

Similarly to what was observed in the present study (20-40 and 40-60 cm layers), Melo et al. (2012) observed that As availability (expressed by the concentration of As in plants) was negatively correlated with clay content. The main reason for finding negative correlations between available As and clay content, especially in the subsurface, is because of the particular composition and characteristics of the clay fraction in tropical soils, which presents significant amounts of Fe and Al oxides and oxyhydroxides as well as high PZC (Fontes et al., 2001). According to McBride (1994), chemical adsorption of As occurs mainly in Fe and Al oxides, as well as in amorphous aluminosilicates, with small quantities being retained by clay silicates and organic substances. For this reason, the mobility and availability of As are generally lower in clayey soils with variable charge.

It is noteworthy that the highest correlation coefficients were observed between exchangeable Al (Al^{3+}) and total, available, and soluble As in all the three layers studied, which were all positive ($p < 0.05$) (**Table 3**). The mechanism by which high Al^{3+} is associated with high As concentrations may be related to the preferential retention of Al in the negative sites of variable-charge minerals (including clay minerals, Fe and Al oxides and oxyhydroxides), thus creating a bridge for binding As through formation of surface ternary complexes, which has been previously reported (Oliveira et al., 2015).

Given that As (as arsenate) and P (as phosphate) present similar chemical behavior in soils, available P has an antagonistic effect upon As retention, as P can compete with As for anionic sorption sites (Sharma et al., 2011). This explains the positive correlation observed between the soluble and available As fractions and the available P in soil samples of all the three layers studied (**Table 3**). Several studies show evidence supporting a strong relationship between As availability in soil and soil available P as a consequence of the chemical and structural similarities between their anionic forms (Punshon et al., 2017; Arco-Lázaro et al., 2018; Chowdhury et al., 2018). Jiang et al. (2014) observed a significant positive correlation between soil available P and exchangeable As. The authors also stated that the main factors influencing As uptake by rice in that study included soil pH, total organic carbon, as well as soil available P and Fe. In this case, soil pH affected As uptake indirectly via impact on available P and Fe. Wang et al. (2018) conducted a pot experiment to study the effect of P application on the lability and phytoavailability of As in the soil and observed that application of exogenous phosphate induced soil arsenic mobilization, thereby increasing arsenic lability due to a competition effect. Finally, a positive correlation found between available P and total As in all soil layers is probably related to the fact that positively-charged colloidal surfaces that adsorb P in tropical agroecosystems are also relevant for retaining As in the soil system.

Extractable sulfur in this study affected negatively the amount of available As in the soil, in the layers 0-20 and 40-60 cm. The competition between S and As, mainly in the 40-60 cm layer, where sulfur could have more affinity with the binding sites in tropical soils is relevant if we consider that As in soil solution cannot overcome S in the sites available, due to a concentration effect, i.e., S in solution is generally higher than As in solution in most soils. Therefore, the decreasing of available As in the long term is certain when the concentration of S is higher. But this is not the behavior often showed in experiments of shorter-term where the addition of S displaces As from the binding sites and increase its concentration in soil solution. Song et al. (2015) studying the effect of sulfate on As mobility in contaminated soils observed that the addition of sulfate significantly enhanced As availability. Moreover, in a study aiming to evaluate the competitive adsorption of arsenate/phosphate and arsenate/sulfate in Brazilian soils, Campos et al. (2013a) observed that the addition of phosphate and sulfate decreased arsenate adsorption and consequently increased the concentration of As in soil solution and its availability.

The negative correlation between OM and available As in the 20-40 and 40-60 cm layers observed in the present study (**Table 3**), suggests that OM can be considered trap and should be able to alleviate As mobility in soil by the formation of covalent bindings, especially in OM-rich soils which are the case of some points sampled in these sites studied (Langner et al., 2012).

The adsorption capacity of As by soils indicates the system's buffer power since many soil attributes (such as texture, Fe and Al oxide content, among others) interfere with the buffer capacity and, consequently, influence leaching losses (Campos et al., 2013a). Although we did not find any relevant correlation among Fe and As fractions in soils from the present study, a great number of studies in the literature show otherwise. In fact, a close coupling between the biogeochemical cycles of iron and arsenic in both oxidizing and reducing environment has been well established (Dixit and Hering, 2003). The mechanism that is presumably involved in this interaction is that both As(III) and As(V) adsorb to iron (hydr) oxides through, in part, the formation of inner-sphere complexes (Tufano et al., 2008). Wang et al. (2012), studying As retention in muddy sediments observed that As retention was strongly coupled to Fe and S, and correlation analysis showed that total and extractable As correlated positively and well with total Fe and HCl-extractable Fe.

Table 3. Pearson's correlation coefficients (*r*) for log-transformed physicochemical variables versus total, soluble, and available arsenic concentrations from different sites in the Amazon region in the layers 0-20 cm, 20-40, and 40-60 cm.

Soil attributes	**-----0-20 cm-----**			**-----20-40 cm-----**			**-----40-60 cm-----**		
	Total As	**As-KNO_3**	**As- KH_2PO_4**	**Total As**	**As-KNO_3**	**As- KH_2PO_4**	**Total As**	**As-KNO_3**	**As- KH_2PO_4**
pH	-0.43**	-0.33**	-0.14	-0.44**	-0.48**	-0.17*	-0.46**	-0.36**	-0.17
OM	0.06	0.27**	-0.12	-0.02	0.11	-0.19*	-0.12	-0.15	-0.23**
CEC	0.34**	0.26**	0.15	0.33**	0.35**	0.22*	0.39**	0.39**	0.30**
P	0.27**	0.39**	0.43**	0.31**	0.25**	0.32**	0.29**	0.23**	0.31**
S	0.03	0.08	-0.23*	0.14	0.10	-0.14	0.06	-0.05	-0.19*
Al	0.53**	0.40**	0.41**	0.52**	0.55**	0.44**	0.56**	0.60**	0.47**
Fe	0.08	0.09	-0.01	-0.06	0.09	-0.15	-0.15	-0.19*	-0.29**
Clay	0.15	0.26**	-0.06	0.04	0.10	-0.21*	0.01	0.0	-0.29**
Silt	0.55**	0.31**	0.40**	0.57**	0.45**	0.37**	0.56**	0.51**	0.40**

Soil attributes: total As (HF digestion); soluble arsenic (KNO_3-extractable As); available arsenic (KH_2PO_4-extractable As); pH (pH in H_2O); P (available phosphate); S (extractable S-SO_4^{2-}); CEC (cation exchange capacity); OM (organic matter); Al (exchangeable aluminum); Fe (available iron); clay and silt (%). * Significant correlation ($p < 0.05$); ** significant correlation ($p < 0.01$). The correlation includes all the sites studied except the site "Santarém/PA" because the data for texture was obtained in only one point.

Figure 6 shows the gradient of influence of the soil chemical variables and correlation groups of the samples for each site studied in the Amazon region. The model including the two principal components accounted for 52.7% of the total variance explained in the three layers studied (29.8% for PC1 and 22.9% for PC2).

The first component (PC1) was negatively dominated by P, S, K, total As, available As, soluble As, CEC, OM, Al and Fe and positively dominated by Zn, Mn, Cu, B, Mg, Ca and pH, which accounted for 29.8% of the variance. The second (PC2) was negatively dominated by S and positively dominated by Fe, OM, Al, AS, CEC, soluble As, available As, K and P, representing 22.9% of the total variance in the data (**Figure 6**).

The soil attribute with the biggest contribution for PC1 is soil pH, followed by Ca and Mg, i.e., in terms of acidity and the presence of basic cations, soils from the Amazon are very diverse, reflecting heterogeneous mineralogy and capacity to adsorb anions as can be observed by the groupings of sites formed along the axis of these attributes. The soil attribute that contributed the most for PC2 is Zn, followed by K and P, which are influencing the grouping of the samples from the site XP.

Total, soluble, and available As are positively correlated with K, P, CEC, OM, Al and Fe and also present a major influence in the samples of the sites ANO, XP and SM. The close correlation between the As fractions and CEC and OM suggests that these soil properties are important factors to retain these As forms in the soil. Concerning CEC, unless there is a formation of a ternary complex in the interface soil-solution with an intermediate cation in the system, we believe that the correlation between CEC and As fractions is indirectly influenced by soil organic matter. The extractable S was separated from the other soil chemical attributes in the third quadrant and showed no relationship with total and soluble As, and only a weak relationship with available As (negative correlation in the 0-20 and 40-60 cm layers) (**Table**

3) and therefore represented a major influence for the samples from the sites AR and PA (**Figure 6**).

The site AP forms a separated group in the border of the first and fourth quadrant, which indicates that this site presents its chemistry distinct from the others, mainly because of the higher values of pH, Ca, Mg, B, Mn, and Cu. On the other hand, this site receives a lower impact from the total, soluble and available As fractions because of its lower As concentrations compared with the other sites (**Figure 6**). In summary, soil pH in combination with CEC and OM might be the key factors that influence As concentrations in soil as well as the concentrations of soluble and available As in distinct soil layers (depths) for the conditions of the present study.

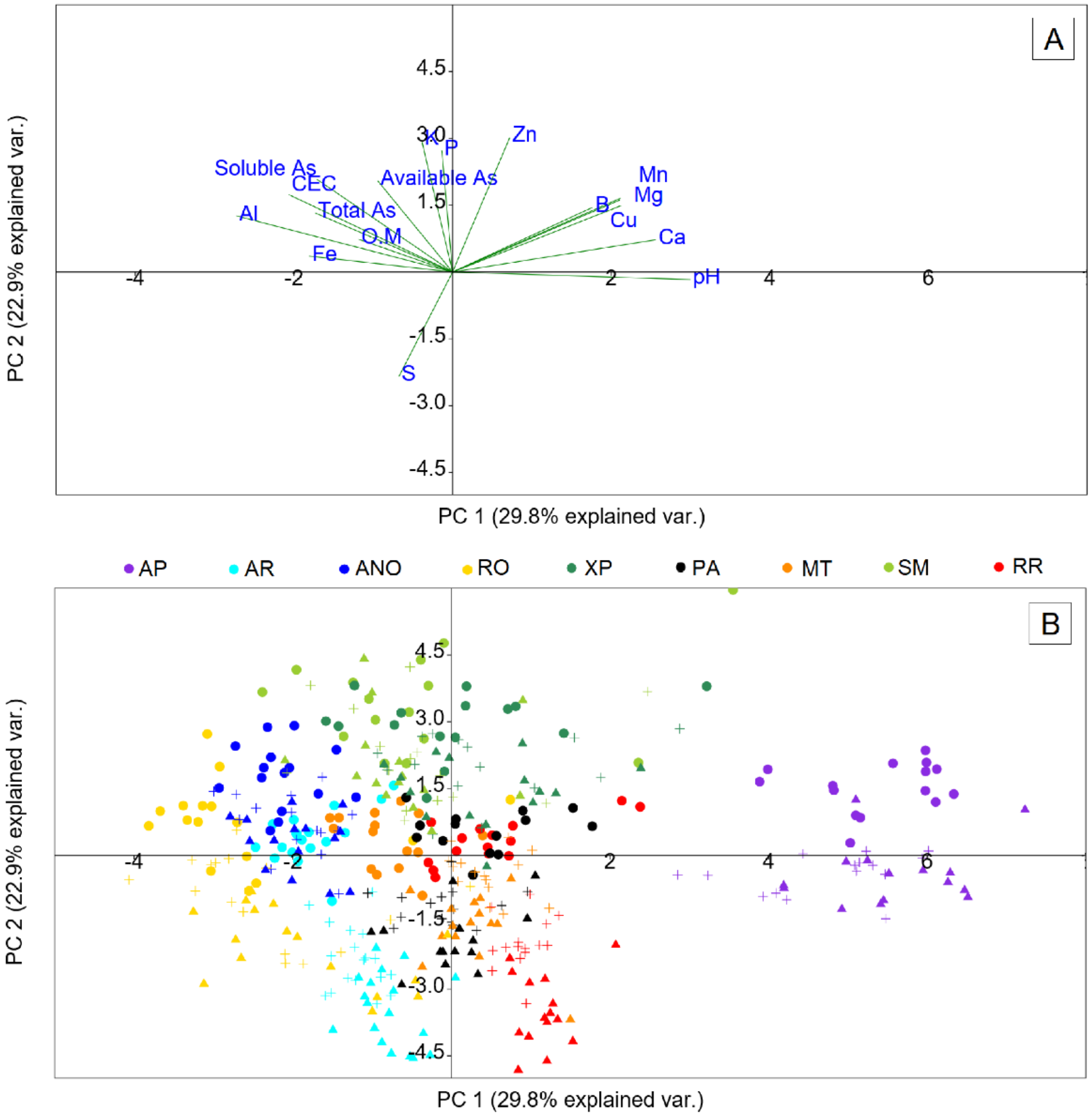


Figure 6. Principal Component Analysis (PCA) of log-transformed data for A) Vectors with soil chemical attributes and total, soluble and available soil As concentrations and B) Sampling points distributed from 9 different sites of the Amazon region identified by: SM (Sena Madureira, state of Acre); XP (Xapuri, state of Acre); ANO (Anori, state of Amazonas); AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); MT (Itaúba, state of Mato Grosso); PA (Santarém, state of Pará); RO (Porto Velho, State of Rondônia) and RR (Caracaraí, state of Roraima). The symbols represent each soil depth as follows – circle: 0-20 cm; cross: 20-40 cm and triangle: 40-60 cm. Soil chemical attributes: S (extractable S-SO_4^{2-} in mg kg^{-1}); available Mn, Zn, Fe, Cu, B, K, and P (mg kg^{-1});

exchangeable Mg, Ca, and Al in $\text{cmol}_c\ \text{dm}^{-3}$; CEC (cation exchange capacity in $\text{cmol}_c\ \text{dm}^{-3}$); OM (organic matter in %); pH (pH in H_2O); total As (mg kg^{-1}); soluble As ($\mu\text{g kg}^{-1}$); available As ($\mu\text{g kg}^{-1}$).

4 CONCLUSIONS

Soil pH and CEC indirectly are the main chemical attributes influencing the dynamic of As fractions in soils from the sites studied in the Amazon basin. In a smaller magnitude but not less important, the competing anions P and S affect soluble as well as available As in the soil.

The highest variable correlating with total, soluble and available As is soil Al^{3+} in the three layers studied. The mechanisms by which this element affect As mobility and retention needs further investigation, but might be related to the formation of ternary complexes.

Our study raises the need to complement the information about the background levels of As in soils from the Amazon region using representative soils that reflects the natural concentrations of each sub-region in order to provide guideline values for better use of soil and water resources in the region.

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Supplementary material

Table S1. Information about the sampling sites of soil samples in the Amazon region.

Municipality/state	GPS coordinates		Altitude (m)	Climate: Köppen class.
SM	9°25’54.59’’S	68°35’42.98’’W	232	Am
XP	10°50’02.02’’S	68°23’23.51’’W	262	Am
MT	11°06’00.32’’S	55°02’06.78’’W	387	Am
AR	3°01’05.59’’S	58°49’55.60’’W	92	Af
ANO	3°50’10.01’’S	61°36’11.64’’W	29	Af
AP	0°33’50.61’’S	52°18’23.43’’W	135	Am
RR	1°28’10.09’’N	60°44’16.96’’W	107	Am
RO	8°48’30.13’’S	63°50’47.17’’W	103	Am
PA	3°03’15.18’’S	54°55’37.79’’W	92	Am

Table S2. Summary statistics of some soil physicochemical properties in different sites in the Amazon region.

Site	**n**	**Depth (cm)**	**K (mg kg^{-1})**	**Ca (cmol$_c$ kg^{-1})**	**Mg (cmol$_c$ kg^{-1})**	**Al (cmol$_c$ kg^{-1})**	**Zn (mg kg^{-1})**	**Fe (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Cu (mg kg^{-1})**	**B (mg kg^{-1})**
SM	15	0-20	50.4±23.7	0.38± 0.64	0.23±0.31	2.43±0.82	1.37±1.66	174.9±86.3	28.5±30.1	1.32±0.49	0.16±0.04
	15	20-40	26.7±9.2	0.27± 0.40	0.17±0.23	2.77±0.81	1.31±2.27	83.1±51.6	17.8±14.7	1.17±0.45	0.13±0.04
	15	40-60	21.8±7.3	0.22± 0.26	0.17±0.18	3.33±0.79	1.22±1.88	35.2±11.0	12.3±8.3	1.01±0.28	0.14±0.03
XP	15	0-20	75.3±18.5	0.20± 0.29	0.38±0.21	2.16±0.69	1.66±1.09	190.1±83.9	18.5±13.2	1.53±0.53	0.14±0.08
	15	20-40	48.0±14.4	0.16± 0.21	0.35±0.19	2.53±0.88	1.45±1.63	142.4±62.9	15.1±13.6	1.52±0.50	0.13±0.05
	15	40-60	35.2±12.4	0.13± 0.13	0.35±0.26	2.97±0.92	1.32±1.13	62.6±26.1	11.4±9.3	1.31±0.34	0.12±0.05
MT	15	0-20	38.3±7.6	0.15± 0.06	0.11±0.03	1.34±0.22	0.36±0.42	324.3±69.6	3.0±1.0	0.88±0.33	0.15±0.05
	15	20-40	26.7±4.7	0.11± 0.03	0.10±0	0.95±0.15	0.18±0.07	349.1±104.6	3.4±1.5	0.89±0.30	0.18±0.19
	15	40-60	21.1±3.8	0.11± 0.03	0.10±0	0.79±0.09	0.14±0.04	297.1±61.7	4.7±1.7	0.63±0.28	0.16±0.14
AR	18	0-20	20.0±6.9	0.16± 0.10	0.12±0.04	1.51±0.13	0.56±0.20	183.0±48.6	2.2±1.3	0.18±0.06	0.08±0.04
	18	20-40	8.4±3.5	0.11± 0.05	0.10±0.02	1.22±0.10	0.20±0.10	132.5±37.4	1.1±0.4	0.11±0.04	0.10±0.09
	18	40-60	6.0±2.8	0.12± 0.05	0.10±0.02	1.14±0.14	0.18±0.10	94.5±17.8	0.9±0.3	0.11±0.04	0.08±0.04
ANO	15	0-20	33.1±6.3	0.10±0	0.13±0.05	5.94±1.65	1.05±0.49	489.7±134.1	2.9±1.0	1.03±0.41	0.16±0.12
	15	20-40	23.2±4.71	0.10±0	0.11±0.03	6.85±1.85	0.75±0.36	332.6±127.4	2.0±0.9	0.78±0.31	0.11±0.07
	15	40-60	24.0±8.9	0.10±0	0.10±0	7.61±1.72	0.65±0.19	243.6±117.3	1.7±0.9	0.55±0.25	0.10±0.06
AP	15	0-20	29.8±8.5	1.55± 0.61	0.48±0.14	0.13±0.08	1.52±1.12	58.5±22.6	109.5±22.9	14.62±26.7	0.35±0.07
	15	20-40	15.2±2.91	1.11± 0.56	0.38±0.15	0.17±0.11	0.70±0.30	54.7±10.4	99.1±35.8	16.8±31.4	0.30±0.07
	15	40-60	12.4±4.7	0.88± 0.38	0.35±0.12	0.19±0.11	1.43±2.45	43.9±10.5	88.0±25.3	26.6±39.3	0.25±0.07
RR	15	0-20	36.5±5.2	0.23± 0.24	0.23±0.12	0.89±0.17	0.43±0.09	177.2±50.2	9.1±10.2	1.89±0.30	0.04±0.02
	15	20-40	17.5±5.4	0.11± 0.03	0.11±0.03	0.75±0.10	0.23±0.23	90.3±32.1	5.2±6.8	1.44±0.24	0.04±0.02
	15	40-60	10.9±3.1	0.10±0	0.11±0.02	0.59±0.15	0.12±0.08	41.3±16.5	4.2±6.5	1.19±0.21	0.03±0.01
RO	15	0-20	76.2±131.7	0.15± 0.15	0.17±0.10	1.88±0.74	0.42±0.20	196.2±70.8	1.6±1.1	0.71±0.38	0.01±0.006
	15	20-40	28.6±8.5	0.1± 0.05	0.12±0.07	1.53±0.71	0.19±0.14	173.6±86.2	1.3±0.8	0.62±0.33	0.01±0.003
	15	40-60	17.8±8.2	0.11± 0.05	0.12±0.05	1.37±0.75	0.17±0.14	134.3±73.4	1.7±1.4	0.39±0.34	0.01±0
PA	15	0-20	26.4±7.6	0.63± 0.29	0.34±0.10	1.22±0.38	0.36±0.14	242.4±62.5	7.5±5.0	0.55±0.33	0.08±0.01
	15	20-40	13.4±3.4	0.28± 0.13	0.17±0.07	1.39±0.15	0.25±0.14	218.8±60.7	2.9±0.9	0.41±0.25	0.08±0.01
	15	40-60	12.0±8.3	0.21± 0.10	0.14±0.05	1.33±0.15	0.24±0.10	171.17±46.3	2.5±1.1	0.41±0.25	0.10±0.02

The values of soil physicochemical attributes are mean ± standard deviation (SD).

Table S3. Descriptive statistics for total, soluble and available arsenic concentrations from different sites in the Amazon region in the layers 0-20 cm, 20-40 and 40-60 cm.

Site/Layer	**Total As (mg kg^{-1})**					**Soluble As (μg kg^{-1})**					**Available As (μg kg^{-1})**				
	Mean	Median	Min.	Max.	SD	Mean	Median	Min.	Max.	SD	Mean	Median	Min.	Max.	SD
SM (0-20 cm)	16.630	15.979	8.106	28.052	5.589	3.996	4.011	0.740	6.759	1.491	45.330	42.942	15.261	93.695	20.576
SM (20-40 cm)	21.665	19.041	12.404	38.195	7.363	4.046	2.955	1.171	10.015	2.528	68.597	48.971	20.658	153.563	38.304
SM (40-60 cm)	27.548	26.586	17.358	41.714	8.255	7.169	5.447	3.057	15.996	4.029	116.774	111.410	51.475	236.526	57.375
XP (0-20 cm)	6.208	5.957	3.889	11.282	1.971	2.839	2.651	1.328	4.677	0.998	9.053	7.601	5.060	15.933	3.410
XP (20-40 cm)	7.984	7.204	4.325	13.861	2.603	2.029	1.970	0.845	3.922	0.886	9.397	8.093	5.324	18.516	3.741
XP (40-60 cm)	10.865	9.340	5.694	17.879	4.490	2.870	2.865	1.000	6.689	1.522	12.895	11.592	8.183	26.606	4.888
AR (0-20 cm)	5.031	5.075	4.312	5.341	0.273	3.505	3.409	3.003	4.351	0.411	9.138	9.247	5.906	11.216	1.776
AR (20-40 cm)	5.228	5.328	4.645	5.601	0.312	2.051	1.918	1.362	3.393	0.527	6.724	6.225	2.680	12.152	2.315
AR (40-60 cm)	5.398	5.405	4.649	6.288	0.472	1.487	1.514	0.810	2.038	0.299	5.111	5.284	2.137	8.163	1.903
AN (0-20 cm)	3.571	3.546	2.516	4.776	0.659	1.749	1.747	1.249	2.281	0.295	6.243	6.035	4.918	7.768	0.878
AN (20-40 cm)	3.998	3.963	2.712	5.466	0.672	1.258	1.201	0.719	1.772	0.270	5.830	5.819	4.237	7.158	0.806
AN (40-60 cm)	4.472	4.410	3.566	7.009	0.890	1.312	1.147	0.839	2.104	0.405	6.073	5.723	4.903	9.254	1.078
MT (0-20 cm)	2.579	2.564	2.240	2.872	0.177	1.815	1.740	1.270	2.373	0.318	5.175	5.185	4.591	6.182	0.446
MT (20-40 cm)	2.901	2.995	2.464	3.691	0.338	1.273	1.231	0.943	1.605	0.217	6.579	6.516	5.553	7.527	0.508
MT (40-60 cm)	3.021	3.031	2.548	3.521	0.264	0.942	0.993	0.020	1.262	0.313	6.928	6.889	4.237	8.326	0.934
RR (0-20 cm)	1.382	1.389	1.056	1.646	0.154	1.448	1.479	1.142	1.696	0.181	3.032	3.019	2.557	3.513	0.292
RR (20-40 cm)	1.743	1.704	1.482	2.453	0.242	0.890	0.901	0.667	1.164	0.146	3.179	3.222	2.580	3.732	0.345
RR (40-60 cm)	1.866	1.801	1.564	2.652	0.267	0.936	0.911	0.731	1.324	0.153	4.048	3.891	2.935	5.302	0.655
AP (0-20 cm)	1.301	1.297	0.989	1.711	0.171	1.448	1.388	0.768	2.232	0.304	3.807	3.701	3.218	4.639	0.456
AP (20-40 cm)	1.535	1.525	1.313	1.777	0.130	0.685	0.700	0.451	0.925	0.125	3.435	3.488	2.680	4.247	0.523
AP (40-60 cm)	1.728	1.712	1.440	1.924	0.136	0.516	0.458	0.274	1.375	0.257	3.730	3.894	2.531	4.423	0.553
RO (0-20 cm)	11.549	11.848	5.637	20.76	4.893	3.224	3.330	1.487	4.797	1.143	8.527	8.199	4.612	14.711	2.611
RO (20-40 cm)	12.302	12.616	5.773	22.273	5.479	2.141	2.128	1.284	3.278	0.668	7.934	7.113	4.745	15.333	2.629
RO (40-60 cm)	12.405	12.374	5.835	23.657	5.420	1.751	1.905	0.944	2.713	0.563	8.579	8.109	5.539	16.510	2.728
PA (0-20 cm)	2.001	1.930	1.264	2.557	0.396	1.646	1.635	0.892	2.328	0.324	3.892	3.721	3.087	4.773	0.540
PA (20-40 cm)	2.314	2.373	1.615	3.027	0.473	1.224	1.126	0.976	1.641	0.214	3.805	3.736	3.056	4.680	0.426
PA (40-60 cm)	2.452	2.577	1.665	3.057	0.465	1.022	0.999	0.767	1.450	0.168	4.372	4.151	3.432	5.743	0.684

Table S4. Pearson's correlation coefficients (*r*) for log-transformed physicochemical variables versus total, soluble and available arsenic concentrations from different sites in the Amazon region in the layers 0-20 cm, 20-40 and 40-60 cm.

Soil attribute	**-----0-20 cm-----**			**-----20-40 cm-----**			**-----40-60 cm-----**		
	Total As	**Soluble As**	**Available As**	**Total As**	**Soluble As**	**Available As**	**Total As**	**Soluble As**	**Available As**
K	0.24**	0.13	0.21*	0.30**	0.17	0.27**	0.30**	0.28**	0.40**
Ca	-0.33**	-0.26**	-0.12	-0.29**	-0.41**	-0.10	-0.28**	-0.30**	-0.10
Mg	-0.24**	-0.26**	-0.14	-0.12	-0.26**	-0.07	-0.04	-0.07	0.04
Mn	-0.26**	-0.26**	0.04	0.0	0.0	0.28**	-0.13	-0.19*	0.15
Zn	0.07	0.05	0.23**	0.19*	0.15	0.31**	0.26**	0.25**	0.34**
Cu	-0.39**	-0.45**	-0.22*	-0.29**	-0.34**	-0.08	-0.29**	-0.20*	0.02
B	-0.29**	-0.20*	0.05	-0.27**	-0.19*	0.07	-0.16	-0.03	0.15
Sand	-0.27**	-0.26**	-0.06	-0.19*	-0.21*	0.09	-0.14	-0.11	0.20*

Soil attributes: total As (HF digestion); soluble arsenic (KNO_3-extractable As); available arsenic (KH_2PO_4-extractable As); K (available potassium); Ca (exchangeable calcium); Mg (exchangeable magnesium); Mn (available manganese); Zn (available zinc); Cu (available copper); B (available boron); and sand (%). * significant correlation ($p < 0.05$); ** significant correlation ($p < 0.01$). The correlation includes all the sites studied except the site "Santarém/PA" because the data for sand was obtained in only one point.

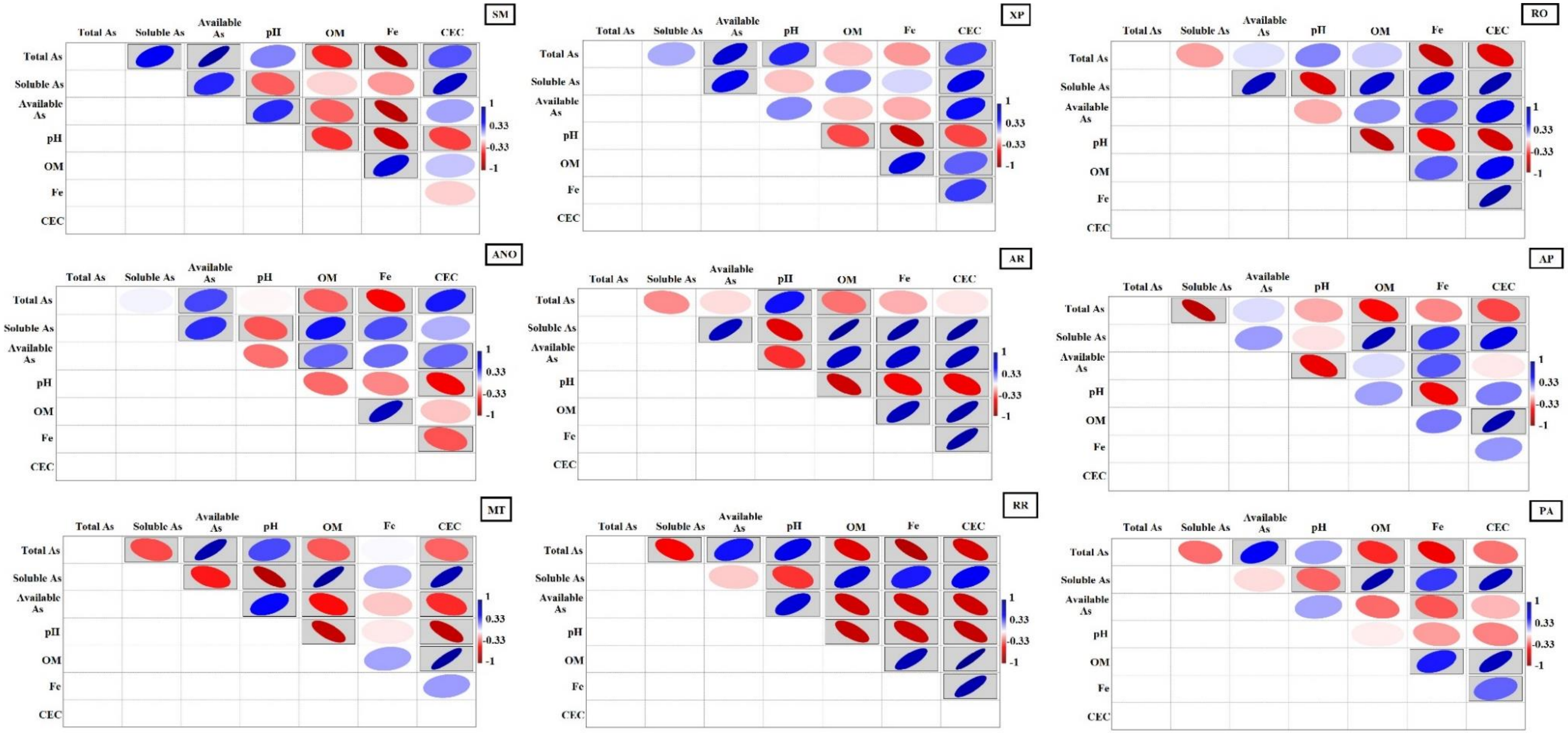

Figure S1. Spearman correlation matrix among total, soluble and available arsenic and the soil attributes pH, OM, Fe and CEC for each of the 9 sites studied being represented by: Sena Madureira (SM) and Xapuri (XP)-state of Acre; Porto Velho (RO)-state of Rondônia; Anori (ANO) and Itacoatiara (AR)-state of Amazonas; Itaúba (MT)-state of Mato Grosso; Caracaraí (RR)-state of Roraima; Santarém (PA)-state of Pará and Laranjal do Jari (AP)-state of Amapá. Ellipses with squares marked in grey are significative ($p < 0.05$). Graphics were designed using the software PAST (Hamme et al., 2001).

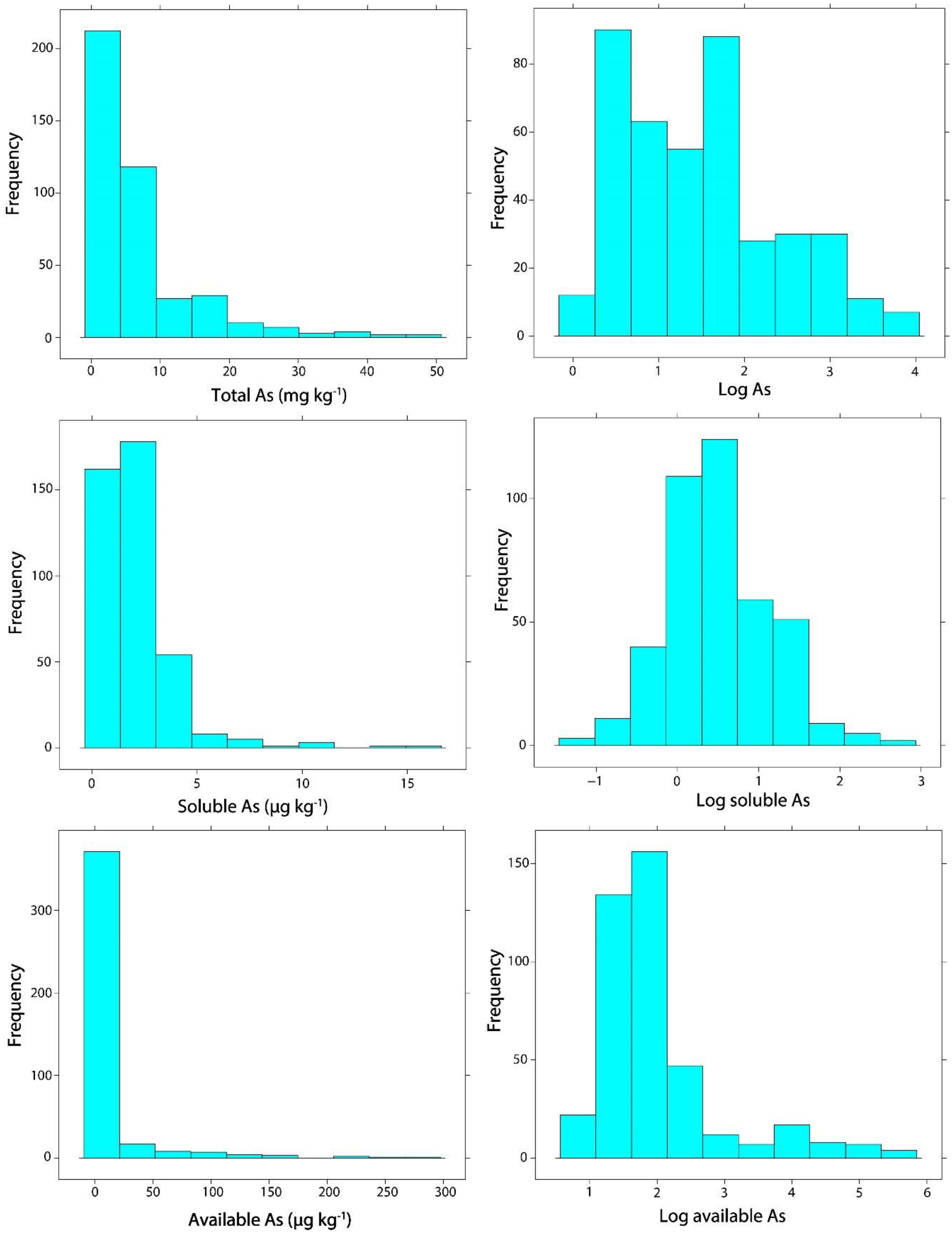

Figure S2. Frequency histograms for non- (left) and log- (right) transformed data for total, soluble and available arsenic concentrations.

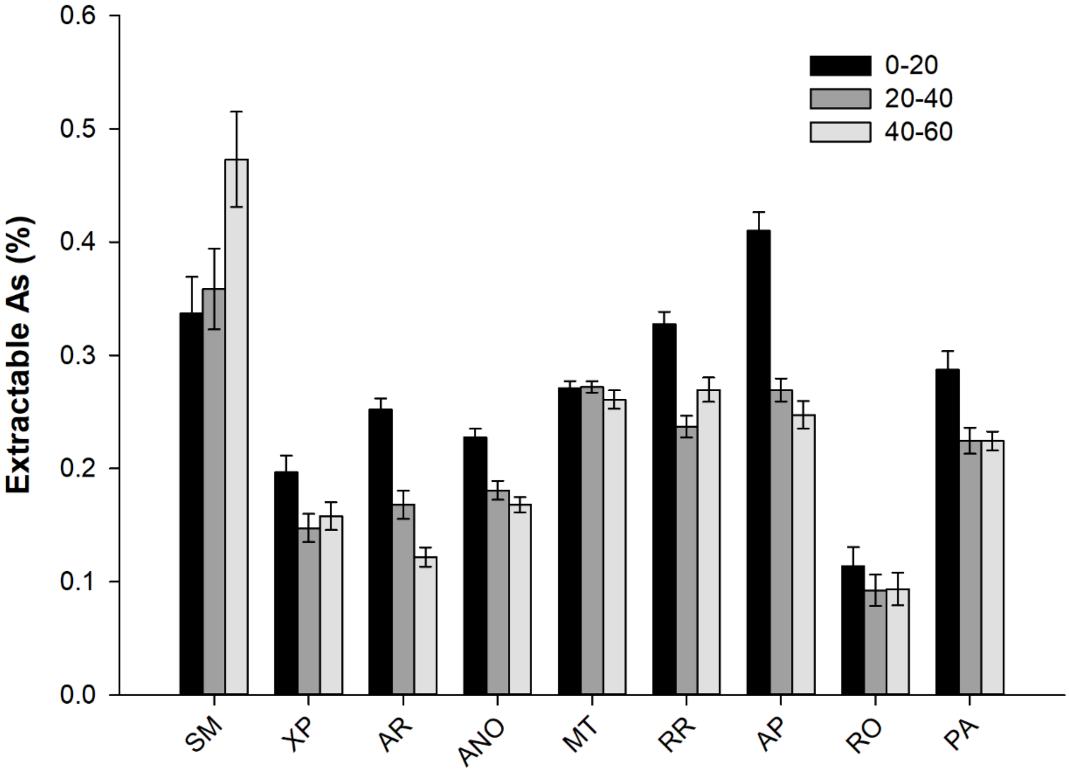

Figure S3. Percentage of As extractable (soluble + available) in relation to the total As analyzed in each site studied. Error bars represent the standard error of the mean (SEM).

Table S5. Soil physicochemical attributes calculated for different sites in the Amazon region. Values are average ± SD.

Site	**n**	**Depth (cm)**	**SB (cmol$_c$ kg^{-1})**	**t (cmol$_c$ kg^{-1})**	**V (%)**	**m (%)**	**P-rem (mg kg^{-1})**
SM	15	0-20	0.75±0.98	3.17±0.83	11.53±15.41	78.44±19.51	25.09±4.45
	15	20-40	0.51±0.63	3.28±0.76	8.02±10.11	85.14±15.04	23.20±3.58
	15	40-60	0.45±0.43	3.78±0.77	6.00±5.94	88.23±10.04	19.00±3.51
XP	15	0-20	0.78±0.42	2.94±0.48	12.77±11.24	72.51±16.41	27.36±4.69
	15	20-40	0.64±0.33	3.17±0.77	10.85±8.55	78.03±16.90	23.40±4.69
	15	40-60	0.58±0.33	3.55±0.92	9.24±7.26	82.99±11.99	20.63±4.01
MT	15	0-20	0.36±0.08	1.71±0.21	5.68±2.28	78.53±5.13	23.98±3.54
	15	20-40	0.28±0.03	1.23±0.16	6.70±1.49	76.74±3.68	16.54±3.90
	15	40-60	0.27±0.03	1.06±0.10	6.91±1.87	74.69±2.57	15.68±2.05
AM	18	0-20	0.34±0.12	1.84±0.10	4.32±1.83	81.88±6.35	9.71±2.36
	18	20-40	0.24±0.05	1.45±0.12	5.01±1.43	83.57±3.08	8.95±1.67
	18	40-60	0.24±0.06	1.39±0.14	6.23±2.14	82.26±4.28	8.29±1.67
ANO	15	0-20	0.32±0.05	6.25±1.67	1.59±0.52	94.65±1.31	5.15±1.65
	15	20-40	0.27±0.03	7.11±1.86	1.30±0.48	96.01±0.98	4.34±1.54
	15	40-60	0.26±0.02	7.87±1.73	1.18±0.41	96.60±0.62	4.03±1.36
AP	15	0-20	2.11±0.74	2.24±0.69	48.59±11.94	6.80±5.89	35.16±2.74
	15	20-40	1.54±0.70	1.71±0.63	41.05±14.45	12.45±12.10	32.11±2.43
	15	40-60	1.26±0.48	1.45±0.42	36.70±9.41	15.25±11.25	31.01±1.61
RR	15	0-20	0.55±0.35	1.44±0.24	10.42±6.43	63.75±15.20	17.34±2.61
	15	20-40	0.26±0.06	1.02±0.11	7.54±1.77	73.89±4.67	14.85±2.08
	15	40-60	0.23±0.03	0.83±0.15	8.11±1.67	70.78±5.74	12.71±2.01
RO	15	0-20	0.51±0.45	2.39±0.92	4.21±3.17	78.40±13.00	18.08±3.71
	15	20-40	0.29±0.12	1.82±0.70	3.55±2.14	81.64±9.80	15.17±3.08
	15	40-60	0.28±0.10	1.65±0.78	4.19±2.52	80.02±11.00	12.50±3.11
PA	15	0-20	1.04±0.36	2.26±0.26	11.80±5.13	54.06±15.16	38.35±1.94
	15	20-40	0.49±0.19	1.88±0.16	7.69±3.37	74.24±8.43	34.94±3.82
	15	40-60	0.38±0.15	1.70±0.12	6.90±2.69	77.79±8.48	34.16±3.41

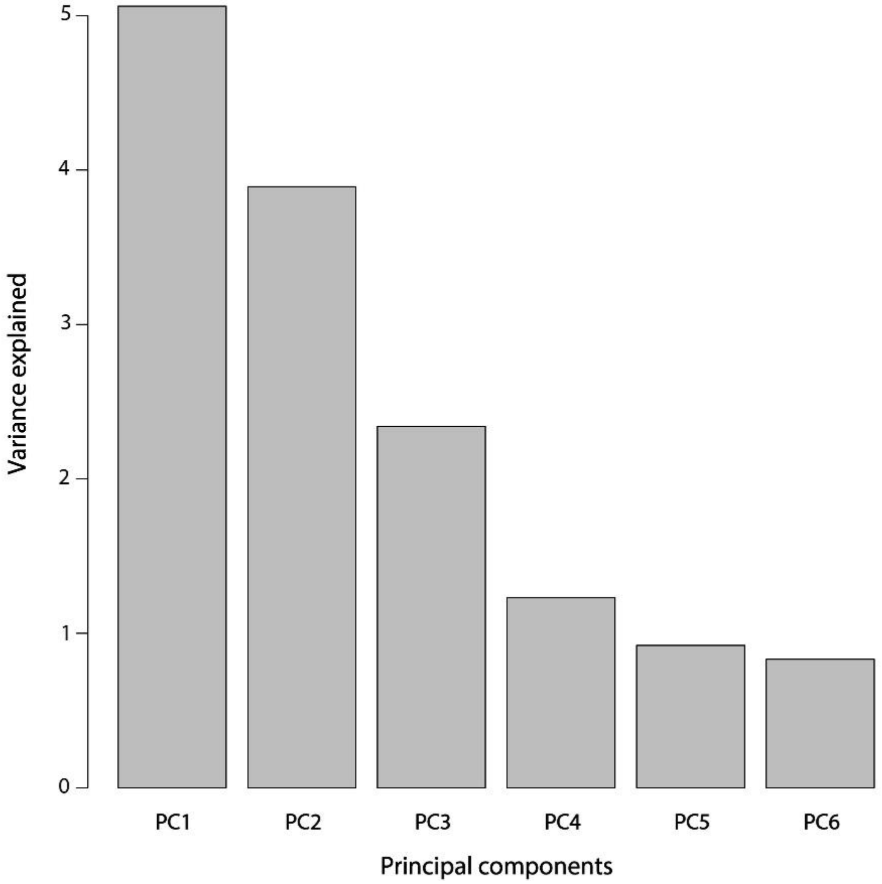

Figure S4. Variance explained for each of the principal components corresponding to the PCA.

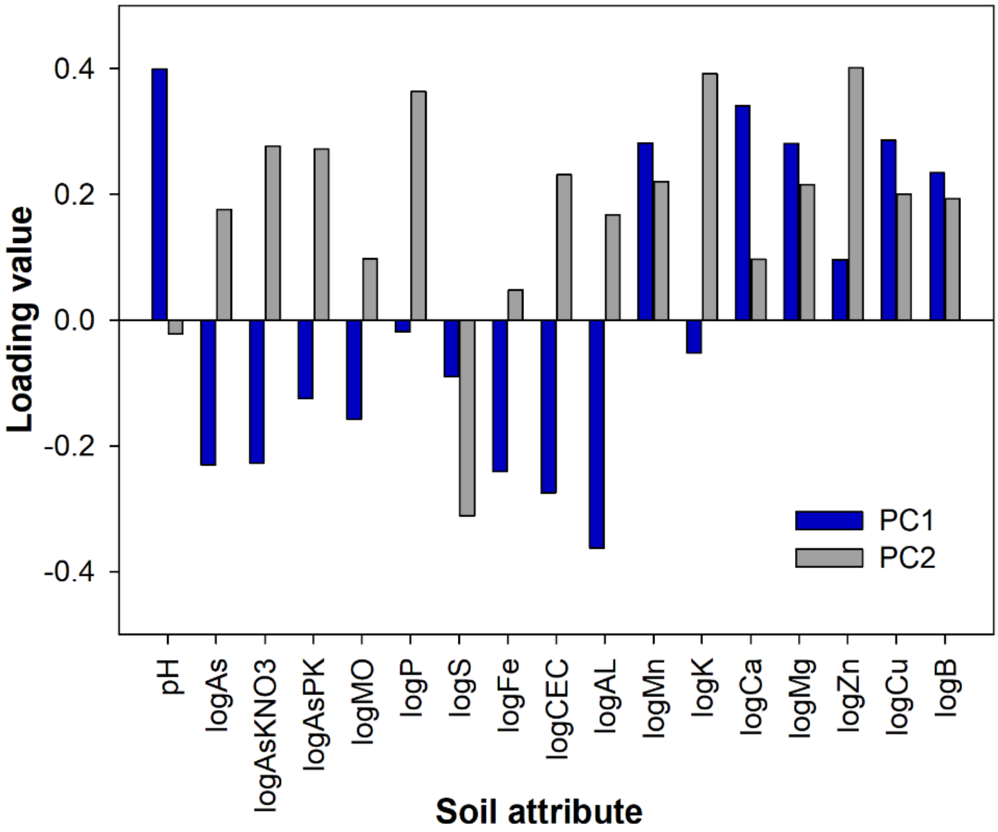

Fig. S5. Loading values corresponding to the soil attributes used to perform the PCA in the first two PCs.

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ARTICLE 2 - Distribution of selenium, barium and iodine in soils of the Brazilian Amazon

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Abstract

Amazonian agroecosystems are heterogeneous, which makes their soils present geographically variable behavior in terms of trace elements distribution. Few studies have addressed the concentrations of selenium (Se) and barium (Ba) in soils in a more comprehensive approach, and so far, no publication on soil iodine (I) exists yet, mainly because of the considerable size of the Amazon, which makes soil sampling more complex when going in expeditions inside the native forest through sites with difficult access. In this study, relevant physicochemical soil attributes and the concentrations of total selenium (SeT), total barium (BaT), soluble Se (SSe), and exchangeable Se (ESe) in 3 different soil layers (0-20, 20-40, and 40-60 cm), besides organic bound-Se and iodine (I) (TMAH-extractable) status in the surface layer (0-20 cm) were characterized and compared for 9 different sites in the Brazilian Amazon. The main results obtained showed that the collected concentrations of Se and Ba can be used as a base to refine the current Quality Reference Values established in the Brazilian legislation. Selenium levels are considered safe but Ba in the western Amazon (state of Acre) and central Amazon (site Anori, state of Amazonas) is found above legislation prevention levels (PVs). Available Se (soluble + exchangeable fractions) accounts for only 4.45% of the total concentration on average. The average I-TMAH in the topsoil is 5.43 mg kg^{-1}, which is above the worldwide mean (2.8 mg kg^{-1}). Multiple regression models supported by Spearman correlation analyses showed that: a) Se and Ba in soil present inverse relationship; b) Se fractions (soluble, exchangeable and organic), despite representing different pools, share common soil factors that contribute to explain their respective regression models; c) Iodine is somehow affected by Se although, the mechanisms that link Se and I in topsoil are still unknown.

Keywords: selenium; iodine; barium; Amazon environment; soil status; soil dynamics.

Highlights

- Soil Se concentrations are considered safe on the sites studied (< 5 mg kg^{-1});
- Extractable Se is below 10% of the total concentration;
- Barium reaches levels higher than the national prevention level (150 mg kg^{-1});
- Average I in topsoil (5.43 mg kg^{-1}) is above the world average (2.8 mg kg^{-1});
- Selenium concentration is inversely related to Ba in the soil surface layer.

1 INTRODUCTION

The Amazon region in Brazil comprises approximately 4,500,000 km^2 of tropical forest in a hot and humid climate. This vast ecosystem is highly diverse not only in terms of biota but also in morphology, soil types, lithology, and rainfall patterns and it is known that limited representative pedogeochemical data exist to characterize the region in its entirety (Lima et al., 2014; Gardi et al., 2015; Matschullat et al., 2020). Amazon soils have been formed from both ancient and recent parent materials under one of the most severe weathering conditions in the world. The inheritance of trace elements contents derived from parent material can be modified by pedogenesis. Therefore, pedogenetic processes contribute to the enrichment, depletion, and mobilization of elements in the soil profile, and consequently to soil diversity in the Amazon (Souza et al., 2018).

Selenium (Se) is an essential micronutrient for both humans and animals but not for plants, even though they can take up and assimilate Se, and beneficial effects for some specific groups of superior plants can occur. In turn, soil, water, plants, and livestock are the major sources of Se in the food chain, which are fundamentally determined by geology (Dinh et al., 2018; White, 2018; Reynolds et al., 2020). The mean total Se content in worldwide soils is estimated as 0.44 mg kg^{-1}, however, concentration in soils is highly variable and dependent on site-specific characteristics, which reflects soil-forming processes and also atmospheric deposition that can be influenced by human activities (Kabata-Pendias, 2011; Plant et al., 2014).

Selenium concentrations in Brazilian soils are reported to range from <0.089 to 5.97 mg kg^{-1} (Gabos et al., 2014; Matos et al., 2017; Reis et al., 2017). The deficiency range for Se in soils worldwide is considered to vary from 100 to 600 µg kg^{-1} (Lyons et al., 2003). Therefore for certain regions of the country, the supplementation with Se in the soils starts to become important for agricultural practices.

Total Se concentration in soil is an inaccurate predictor of its availability for crops. Therefore, to obtain valuable information on its mobility and potential for accumulation by plants and other organisms, a chemical extraction procedure has to be applied to understand the possible impacts on the soil ecosystem and the general environment. Selenium in different fractions displays various degrees of mobility and availability, based on its binding strength and reflected in its extractability from the soil solid phase. The main extractable Se fractions in soil include soluble, exchangeable, organically-bound, and oxides-bound (Sharma et al., 2014; Li et al., 2017; Feudis et al., 2019).

Iodine (I) is another essential trace element for the human body and just like Se, an important compost of thyroid hormone synthesis, but not essential to plant growth, despite being important for grazing animals. There are various effects of iodine on human health, and it is closely related not only to normal thyroid function but also to the physical and brain development of children (Bowley et al., 2019; Wang et al., 2020a). The average I content of worldwide soils is estimated as 2.8 mg kg^{-1}. Its accumulation in surface and subsurface soil horizons is affected by aerial deposition and biogenic concentration and it has been stated that the atmosphere is a basic source of I in soils. The ocean is the reservoir from which all of the atmosphere I is drawn (Kabata-Pendias, 2011; Fuge and Johnson, 2015). The recommended daily allowance (RDA) for iodine is 150 µg/day for adults (> 14 years old) (Hays et al., 2018). Limited data are available on I distribution in tropical soils under rainforest, and since forest soils, in general, contain large amounts of OM, they could be an important terrestrial reservoir of I (Roulier et al., 2018), especially for this great and complex ecosystem represented by the Amazon.

Barium (Ba) is a dense alkaline earth metal considered to be a non-essential element for organisms in general. Its exposure may cause multiple deleterious effects in animals, including damage to the cardiovascular, renal, respiratory, hematological, nervous, and endocrine systems and eventually resulting in death (Kravchenko et al., 2014; Lu et al., 2019). Barium toxicity is significantly related to its solubility, and the more soluble the compound is, the more toxic it is (Lamb et al., 2013; Çanlı, 2018). An oral toxicity assessment of the US Environmental Protection Agency for Ba established an oral reference dose (RfD) of 0.2 mg kg body weight^{-1} day^{-1} (EPA, 2005). During weathering, Ba is not very mobile in the soil because it is easily precipitated as sulfates and carbonates, and also strongly adsorbed by clays. Its contents in surface soils vary from 10 to 1500 mg kg^{-1} (Coscione and Berton, 2009; Kabata-Pendias, 2011). There are evidences in southwestern Amazonia, suggesting the presence of hollandite ($Ba_2Mn_8O_{16}$) in the soils of the region, which is the possible cause of the origin of soils with high Ba concentrations and the reason for the high accumulation of this element in Brazil nuts (Lisk et al., 1988; Chang et al., 1995; Kabata-Pendias and Pendias, 2001).

The Brazilian National Environment Council established Prevention Values (PVs) in the soil for Se as 5 mg kg^{-1} and Ba as 150 mg kg^{-1}. For I there is no reference value established by the national legislation, but it is believed so far that I does not present a health risk for the population at the natural levels occurring in Brazilian soils. For Se and Ba, on the other hand, there are reports showing levels that exceed the prevention value in certain locations, despite toxicity for organisms was not reported (CONAMA, 2013).

The Brazilian Amazon, having the largest rainforest coverage in the world, presents still incipient research about the distribution of Se, I, and Ba in soils. The main question to be answered with the present study is which of the soil physicochemical attributes (pH, OM, CEC and texture (clay, silt, and sand) are the major factors controlling the abundance of Se (total and bioavailable), Ba and I on different sites and in different soil layers in the Amazon region. Therefore, the present work provides a novel dataset with interpretation on total concentrations and distribution of different Se fractions, TMAH-extracted I and total content of Ba in soils of the Brazilian Amazon.

2 MATERIALS AND METHODS

2.2 Study design

This study involved a regional soil survey to collect background data for Se, Ba and I on 9 target sites and different soil layers (0-20, 20-40, and 40-60 cm) to describe the distribution of these elements as well as soil physicochemical attributes on each site. A total of 414 samples (0-20, 20-40, and 40-60 cm) were used for the physicochemical soil characterization, the analyses of total Se, total Ba, the soluble and exchangeable Se fractions. For the Se and I (TMAH extractions) in the topsoil (0-20 cm), a total of 138 samples were used. Correlation and regression analysis are performed to make inferences about soil environmental controls and relationships with Se, Ba and I in soil.

2.2 Characterization of the study area and soil sampling

The sites selected for this study were based on our former study (Silva Junior et al., 2019) assessing the natural variation of arsenic fractions in soils of the Brazilian Amazon. Soil samples were collected on 9 sites belonging to the Amazon Biome in Brazil. Each site corresponds to a Brazil nut stand (forested area and plantation), identified by AC:SM (Sena Madureira, state of Acre); AC:XP (Xapuri, state of Acre); AM:AN (Anori, state of Amazonas); AM:AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); MT (Itaúba, state of Mato Grosso); PA (Santarém, state of Pará); RO (Porto Velho, state of Rondônia) and RR (Caracaraí, state of Roraima). In order to better understand the position of each site in the Amazon region, they were grouped taking the Amazon river mainstream as a reference, being: Northern Amazon: RR and AP; Southern Amazon: MT and

RO; Western Amazon: AC:SM and AC:XP; and Central Amazon: AM:AN, AM:AR and PA. Information about the sampling site coordinates, altitude and Köppen climate classification is presented in **Table S1** of the supplementary material.

On each site, samples were taken at 15 collection points in the native Amazon rainforest, except for AM:AR, where 18 points were sampled in the Aruanã farm (Itacoatiara), a big Brazil nut plantation. Soil samples were collected between 2014 and 2017. The soil was sampled manually at each site with a soil auger (Sondaterra, Piracicaba, São Paulo State, Brazil) at three different depths (0–20, 20–40, and 40–60 cm) after removing surface litter material. Spots were located at a distance of 3 m from the trunk of selected Brazil nuts trees and all sampling spots were geo-referenced. These samples were collected at four points (North, South, East, and West taking the tree trunk as a reference) and mixed to compose a single sample for each depth. The sampling method is represented schematically according to Silva Junior et al. (2017).

2.3 Soil physicochemical characterization

Soil samples were ground, sieved, dried at room temperature, and subjected to chemical and physical (texture) analysis. The soil pH was determined potentiometrically in water (pH H_2O) in the ratio 1:2.5 soil/solution. The exchangeable cations (Ca^{2+}, $Mg^{2+,}$ and Al^{3+}) were obtained by 1 mol L^{-1} KCl extractor. Sulfur was extracted (as S-sulfate) by monocalcium phosphate with acetic acid. Available P, Na, K, and micronutrients (Fe, Zn, Mn, and Cu) were obtained by Mehlich-1 (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4; in ratio 1:10 soil/solution). Potential acidity (H + Al) was estimated indirectly through the SMP pH method (Shoemaker et al., 1961). Exchangeable aluminum (Al^{3+}) was determined by titration with sodium hydroxide (0.025 mol L^{-1} NaOH). Phosphorus (P) was determined colorimetrically, potassium (K) by flame emission photometry, sulfur (S) by turbidimetry, and Ca, Mg, Zn, Fe, Mn, and Cu were determined by atomic absorption spectrometry (AAS). The organic matter content was determined by oxidation of the organic matter in the soil with a potassium dichromate solution in the presence of sulfuric acid (Carter and Gregorich, 2006). The Sum of bases (SB), cation exchange capacity at pH 7 (T), effective cation exchange capacity (t), base saturation (V%), and aluminum saturation (m) were calculated based on the results of the chemical analysis. The particle size analysis (clay, silt, and sand) was performed according to the “Bouyoucos” method, as described by Embrapa (1997).

2.4 Total selenium and barium determination

Total Se and Ba analyses were performed in air-dried and < 2 mm sieved soil samples. Next, a portion of each soil sample was finely ground (< 0.45 mm) using an agate ball mill (Retch PM 400 mill). Soil samples (~0.2 g DW) were fully digested in 70% HF, 70% HNO_3, and 60% $HClO_4$ (TAG; Fisher Scientific UK Ltd), using perfluoroalkoxy (PFA) digestion vessels and a 48-place Teflon-coated graphite digester block (Model A3, Analysco Ltd, Chipping Norton, UK) (Chilimba et al., 2011).

The digestion of the samples was processed in two different programs, as detailed in **Table 1**. Briefly, during the first day, 2 mL HNO_3 and 1 mL of $HClO_4$ were added to the PFA digestion vessels. Next, the tubes were placed in the block digester and left overnight with program 1 turned on. On the second day, 2.5 mL of HF was added to each tube, and program 2 was turned on, with the process being regularly monitored during the day. On the third day, 2.5 mL of HNO_3 and 2.5 mL of Milli-Q water were added to each tube and then the extracts were left at 50°C for 1 hour. After that, the block was turned off and the tubes were left to cool down completely for about 30 minutes. Digested samples were diluted to 50 mL into volumetric flasks using Milli-Q water and stored in 5% HNO_3 at room temperature in universal sample bottles, pending elemental analysis. Before the analysis in the ICP-MS (see Section 2.6), the extracts were diluted using 1 mL + 9 mL of Milli-Q water.

Table 1. HF digestion with digester block: programs

Steps	Program 1	Program 2
1	80 °C, 8 h	120 °C, 1h
2	100 °C, 2 h, End	140 °C, 3 h
3		160 °C, 4 h
4		50 °C, End

Each digestion batch (n = 70) included a standard reference material (NIST 2711a; Montana Soil II, National Institute of Standards and Technology, Gaithersburg, MD, USA), which contains 2 mg kg^{-1} Se (information value) and 730 ± 15 mg kg^{-1} Ba. The average recovery rate in the standard reference material was 95.4% (n = 7, SD = 8.84) for Se and 93.3% (n = 7, SD = 8.56) for Ba. In addition, 3 blank solutions were used in each batch for quality control purposes and for calculating limits of detection. The method detection limit (MDL) was established using 21 blank extracts for soil samples following the overall procedure. The values were calculated with three times the standard deviation (SD) of the blanks used in each bath (n = 3) using the formula: SD (3 blanks) x 3 x final volume extract (mL) ÷ sample weight (g). The average MDL was 10.7 µg kg^{-1} for Se and 767 µg kg^{-1} for Ba (n = 21).

2.5 Soluble and exchangeable Se in soil

Soluble and exchangeable Se were analyzed in a sequential extraction. First, for the soluble fraction, 2 g of air-dried and < 2 mm sieved soil was added into a 50 mL polycarbonate centrifuge tube. Next, 10 mL of 0.01 mol L^{-1} KNO_3 were added to each tube and the suspensions were shaken for 2 hours using a rotary shaker (20 rpm). Soil suspensions were then centrifuged for 30 min at 3500 rpm, filtered to < 0.22 µm using a Millex syringe driven filter unit (Millipore, Cork, Ireland), and stored at room temperature before analysis of Se by ICP-MS (see Section 2.6).

In the second step, for the exchangeable fraction, an extraction with 10 mL of 0.016 M KH_2PO_4 was performed in the same tubes with soil samples from the previous step. The suspensions were shaken for 1 hour in the rotary shaker (20 rpm) and then centrifuged for 30 minutes at 3500 rpm. The supernatant was filtered using a < 0.22 µm syringe filter and put in a universal tube (used for analysis in the ICP-MS). For the ICP-MS analysis (see Section 2.6), 4.8 mL of the supernatant collected from KNO_3 and KH_2PO_4 extractions were added into an ICP tube containing 0.2 mL of 50% HNO_3.

The method detection limit (MDL) for soluble and exchangeable soil Se were calculated as previously mentioned, i.e., with three times the standard deviation (SD) of blanks extractant solution used in each batch (n = 3) using the formula: SD (3 blanks) x 3 x final volume extract (mL) ÷ sample weight (g), and corresponded to 0.51 µg kg^{-1} (n = 18) and 0.60 µg kg^{-1} (n = 21), for soluble and exchangeable Se respectively.

2.6 Analytical determination of selenium and barium

The analytical determinations of total Se and Ba were performed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, X-Series II, Thermo Fisher Scientific). Samples were introduced at 1 mL min^{-1} through a concentric glass venturi nebulizer and Peltier-cooled (3 °C) spray chamber. Instrumental drift was corrected using internal standards (^{72}Ge, ^{103}Rh, and ^{185}Re, 10 µg L^{-1}). Analysis was performed in standard mode for Ba and in ‘hydrogen cell mode’ for Se. Hydrogen (4 mL min^{-1}) was used as the reaction gas to eliminate

Ar polyatomic interferences for Se quantification. Internal standards were diluted in 1% HNO_3 matrix for Ba and with 2% methanol to increase sensitivity for Se analysis (Darrouzès et al., 2007). Data for total Se and Ba concentration in soils are reported on a dry weight basis (DW) and expressed in mg kg^{-1}. Data for soluble and available Se in the soil are expressed in µg kg^{-1}.

2.7 TMAH extraction for selenium and iodine

The tetramethylammonium hydroxide (TMAH) extraction of soil to fractionate Se (organic fraction) and I was performed as a "stand-alone" method, and therefore, performed in a single step of preparation. Iodine and Se concentration in the organic matter were measured following TMAH extraction (Watts and Mitchell, 2009). Samples from topsoil (0-20 cm) were selected because of the expected higher organic matter content and presence of Se and I linked to this layer. Then, 2 grams of air-dried soil (< 2 mm sieved and milled in a ball mill, finely ground, < 0.45 mm) was added into a 50 mL polypropylene centrifuge tube. Then 10 mL of 10% TMAH (Sigma-Aldrich, 25 wt. % in H_2O) was added to each tube. The tubes were placed in an oven (Memmert, beschickung - loading model 100-800) at 70 °C for 3 hours with lids loosened. The tubes lids were gently shaken after 1.5 hours and at the end of the heating process (after 3 hours). Then, the tubes were left at room temperature to cool down for about 30 minutes. Afterward, the lids were tightened and the tubes were centrifuged for 30 minutes at 3500 rpm. The supernatant of each tube was transferred to new falcon tubes and the soil discarded. For the ICP-MS analyses of Se and I in the extracts, 1 mL of the supernatant was added in an ICP tube containing 9 mL of Milli-Q water.

Analyses of all TMAH extracts were performed by ICP-MS (Thermo-Fisher Scientific X-series II) in standard mode for iodine and in 'hydrogen cell mode' for selenium. Hydrogen (4 mL min^{-1}) was used as the reaction gas to eliminate Ar polyatomic interferences for Se quantification. Internal standards were Rh, Re and In (10 µg L^{-1}) prepared in a 1% TMAH matrix (for iodine) and with 2% methanol to increase sensitivity for Se analysis (Darrouzès et al., 2007).

2.8 Statistical analysis

A descriptive analysis of the concentrations of Se and Ba (total), soluble and available Se (0-20, 20-40 and 40-60 cm layers), and TMAH-extracted Se and I (0-20 cm layer) was carried out. Box plots were used to display data distribution and compare sites and layers. After verifying the significance of effects through ANOVA, the mean values for Se and Ba concentration in soils were compared among layers using Tukey's HSD test with the package emmeans v 2.23 (Lenth, 2019) in R 3.6.2 (R Development Core Team, 2020). Logarithmic transformations were used whenever data did not meet statistical assumptions for the ANOVA.

Generalized least squares (GLS) was used for the log-transforming of the TMAH-I data, in order to allow the variance to be estimated separately for each site and avoid heteroscedasticity of the residues, before proceeding to ANOVA (Cleasby and Nakagawa, 2011).

The data from the 0-20 cm layer was log-transformed before subjecting to Pearson's correlation analysis, which was performed for all 6 main variables studied (total, soluble and available Se, total Ba, Se and I TMAH-extracted concentrations, denoted as response variables) and other relevant soil physicochemical properties (denoted as explanatory variables). The following procedure was applied for each response variable: 1) Select explanatory variables to obtain a list of candidates for the multiple linear regression model based on the significance ($p \leq 0.05$) and correlation coefficient ($|r| \geq 0.4$) of each candidate in the Pearson correlation matrix (**Fig. S1** of the supplementary material); 2) For the multiple linear regression model, the raw data was also submitted to log-transformation in order to attend parametric statistical assumptions (normality and homoscedasticity) whenever necessary, and the best combination of explanatory variables was obtained with regression by leaps and bounds approach using the branch and bound algorithm (Furnival and Wilson Junior, 2000; Zhang et al., 2014) and the criteria used for the selection of best explanatory variables combination was the minimum Mallow’s Cp (equivalent to Akaike information criteria - AIC) (Darnius and Tarigan, 2018). The non-multicollinearity of the explanatory variables was checked with the variance inflation factor (VIF < 5) and removed from the regression equation whenever necessary. The Pearson correlation and multiple regression models analyses were performed using R 3.6.2 (R Development Core Team, 2020).

3 RESULTS AND DISCUSSION

3.1 Relevant soil physicochemical properties and associations

Summary characteristics of the 0-20 cm soil layer are presented in **Figure 1**. Presented attributes were selected according to their relevance in explaining the dynamics of Se, I and Ba in soil. As expected from the vastness and diversity in the region, there is a considerable variation for most attributes studied. Many types of soil have developed under various conditions of soil genesis. The Amazon region is located in the humid tropical zone, where soils develop under the drastic conditions of high temperature and heavy rainfall. About 70% of the Brazilian Amazon region is covered with lateritic soil according to Falesi (1972).

The topsoil samples showed acidic pH levels mostly and covered a range of pH from 3.2 to 6.10, with the highest average pH observed in the site AP (5.5) and lowest in the site RO (3.75). Organic matter concentration ranged from 0.75 to 3.8% with the highest average percentage observed in the site AM:AR (2.63%) and lowest in the site AP (1.16%). For the cation exchange capacity of the soil (T) the highest average value by far was observed in the site AM:AN with 22.6 cmol_c dm^{-3} and ranging from 3.45 to 52.4 cmol_c dm^{-3} in all sites studied. The available elements P (ranging from 0.84 to 6.2 mg kg^{-1}) and S (ranging from 2.28 to 37.9 mg kg^{-1}) also presented a wide variation among the sites studied. Manganese (ranging from 0.09 to 152 mg kg^{-1}) presented notably the highest average value in the site AP (110 mg kg^{-1}) (**Fig. 1**).

The texture, as an important physical factor used to distinguish soil classes, also reflected the great variability amongst the soils in the present work. With clay contents ranging from 12 to 85% and sand from 4 to 82%, the particle size analyses demonstrated that the dominant soil texture was clay (AM:AR, RO, and PA) and sandy loam (MT and AP) (**Fig. 1**). However, more important than particle size in the soil is the clay mineralogy of the soil. The typical soils from the Brazilian Amazon are dominated by kaolinite, gibbsite, iron oxides, and small amounts of 2:1 clay mineral, given that the lowland soils tend to be less uniform in the clay mineral composition than the upland soils (Kitagawa and Moller, 1979).

Matschullat et al. (2020) had also noticed such variability in Amazon basin soils and observed that soils from the Central Amazon basin seem to be slightly more weathered than in the South and the higher precipitation in the first region likely explains this finding. Also, higher precipitation in the Central part of Amazon may impact plant-soil interaction and relative enrichment/depletion of elements.

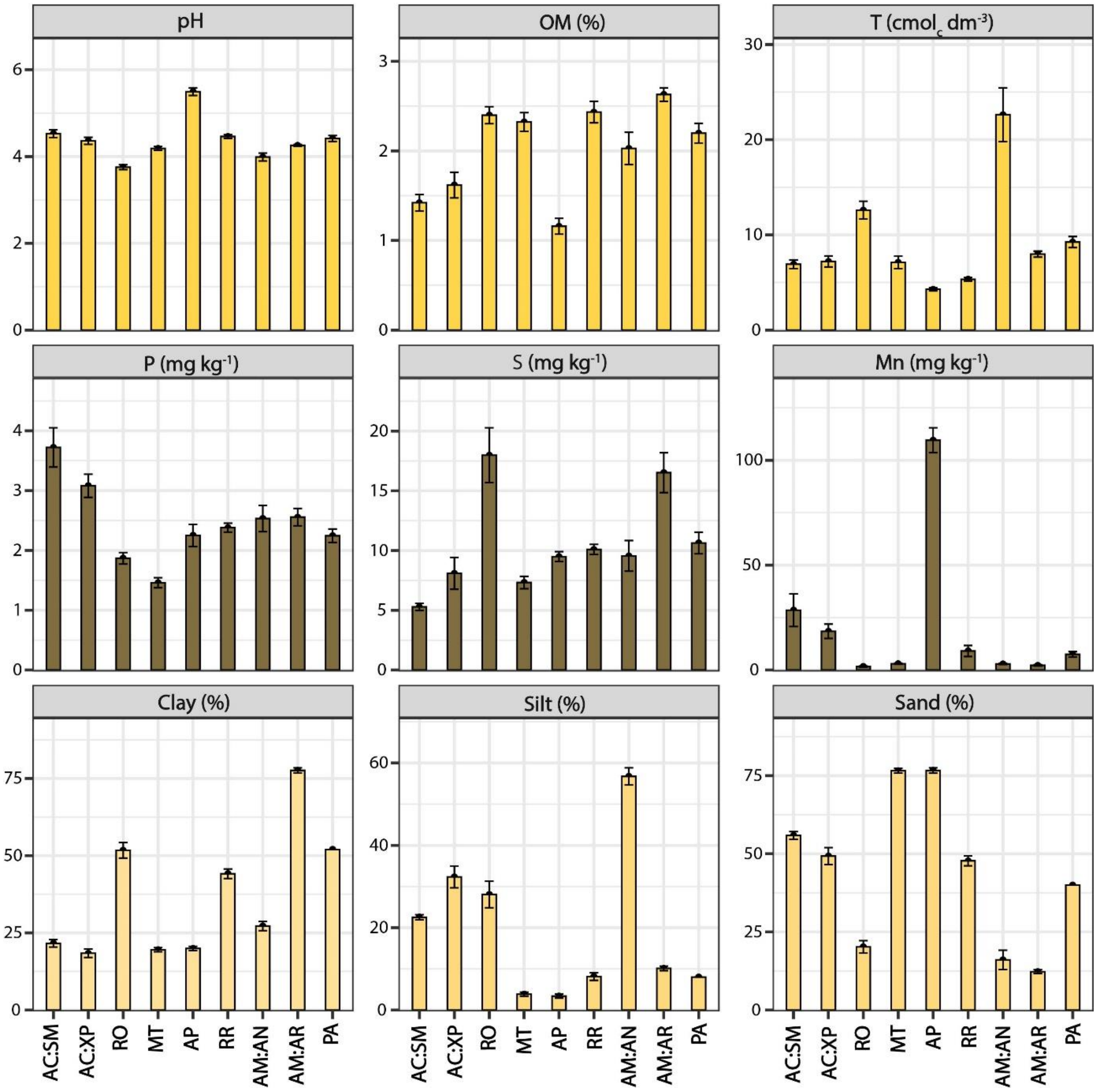

Fig. 1. Relevant physicochemical soil attributes in the topsoil (0-20 cm). The sites are identified as AC:SM = Acre-Sena Madureira; AC:XP = Acre-Xapuri; RO = Porto Velho-Rondônia; MT = Itaúba-Mato Grosso; AP = Laranjal do Jari-Amapá; RR = Caracaraí-Roraima; AM:AN = Amazonas-Anori; AM:AR = Amazonas-Aruanã farm (Itacoatiara); PA = Santarém-Pará. Bar plots contain average values for each site, and error bars represent the standard error of the mean (SEM, n=15; AM:AR, n = 18).

3.2 Total selenium and barium concentration in soil

Total Se in soils ranged from 0.05 to 2.48 mg kg^{-1} (average of 0.81 mg kg^{-1}), which represents a considerable variation among the sites studied, with the highest median concentrations observed in the site AM:AR for the layers 0-20 and 20-40 cm (2.08 and 1.87 mg kg^{-1} respectively). For the layer 40-60 cm, the site RO presented the highest median value (1.44 mg kg^{-1}). The sites with the lowest Se median concentrations (located in western and southern Amazon) are AC:SM, AC:XP, and MT, being below 0.5 mg kg^{-1}. Another noticeable characteristic of the Se distribution in these soils is that the 3 sites with the highest median values (located in southern and central Amazon) also present the widest variation, being RO (0.65 to 1.70 mg kg^{-1}), AM:AR (0.81 to 2.48 mg kg^{-1}) and PA (0.73 to 1.68 mg kg^{-1}) (**Fig. 2**).

The site AM:AR was the only one in which the Se concentration decreased from layer 20-40 cm to the layer 40-60 cm. For the sites AC:XP and RR an increasing average Se concentration from the topsoil to the bottom was seen and for the sites AC:SM, AM:AN and MT the Se concentration increased from the topsoil until the 20-40 cm layer. On the other hand, for the sites RO, PA and AP there was no significant difference in Se concentration among the layers studied (**Fig. 2**).

In soils from Scotland, Shand et al. (2012) observed a large difference in Se concentrations between the A horizon (average 0.64 mg kg^{-1}) and the C horizon (mean 0.26 mg kg^{-1}), which means that increasing Se from the top to the bottom soil layer in the profile is not a standard for Se, and the Se profile may differ according to origin and properties of the soil. It should be emphasized that samples from the superficial layer (0-20 cm) are influenced to a greater extent by external factors, such as weathering and atmospheric deposition, compared to subsurface samples. Additionally, the heterogeneous distribution of carbon compounds results in different Se contents in different soil layers (Gabos et al., 2014).

The general average Se concentration observed in the present study (0.81 mg kg^{-1}) is almost twice as high as the estimated mean total Se content in worldwide soils (0.44 mg kg^{-1})

described by Kabata-Pendias (2011). Although, given the values of total Se observed, even the highest concentrations are still lower than the prevention value (PV) of 5 mg kg^{-1} established by the Brazilian National Environmental Council (Conama, 2013). This value is considered as the concentration of a substance in the soil above which changes in soil quality may occur. Therefore, Se levels can be considered safe on these studied sites.

Apart from the Amazon region, the natural concentrations of Se in another study, with samples representative of the state of São Paulo, Southeast Brazil, a range of Se concentrations more similar to the ones obtained in the present study (< 0.09 to 1.61 mg kg^{-1}) was observed, despite having a much lower mean concentration (0.19 mg kg^{-1}) (Gabos et al., 2014). On the other hand, on a site also located in Southeast Brazil, more precisely in the state of Minas Gerais, a wider range of Se concentration was reported (< 0.30 to 5.97 mg kg^{-1}). The authors also noticed that the average concentration slightly increased from the A horizon (1.09 mg kg^{-1}) to the B horizon (1.20 mg kg^{-1}) (Matos et al., 2017), which was also a behavior observed for Se in some sites in the present study.

In order to establish a comparison and have an idea about the status of Se in terms of deficiency, Tan et al. (2002) studied the relationships between soil Se and endemic human diseases (such as Keshan, Kashin-Back, and selenosis), and they considered 0.12-0.18 mg kg^{-1} as reference threshold values for assessing the risk for potential Se deficiency in soils. If we compare the concentrations observed in the present study in the topsoil (0-20 cm) we would have ~20% of soil samples (from a total of 138) below this threshold, which could be considered deficient.

Samples collected at experimental farms of Embrapa (Brazilian enterprise for research in agronomy and cattle breeding) or similar institutions from Minas Gerais, Santa Catarina, Mato Grosso do Sul, Amazônia and Rio Grande do Sul were compared by using HG-AAS for the soil analysis. The results showed that samples from the states of Amazonia (AM) (5.5 to

19 mg kg^{-1}) and Minas Gerais (MG) (9.5 to 12.5 mg kg^{-1}) have the highest total Se content, and those from Santa Catarina (SC) (0.10 to 4.70 mg kg^{-1}) in the South of Brazil, the lowest ones (Schneider et al., 2016).

An important finding worth mentioning on the behavior of Se in tropical soils is that soil management affects Se adsorption capacity, i.e., adsorbed Se amounts are higher for uncultivated soils (non-anthropic areas) when compared to cultivated ones. This fact is attributed mainly to the presence of greater amounts of competing anions such as phosphate and sulfate, in cultivated soils, due to fertilizer application. These anions may compete with selenate for adsorption sites, decreasing Se retention. Additionally, Se adsorption tends to be greater in clayey soils compared to sandy ones (Lessa et al., 2016).

For total Ba in soils, with exception of the site AC:XP where the concentrations decreased from the top to the bottom, all other sites did not present significant difference between the layers studied, which reflects a quite homogenous distribution of Ba as a function of soil depth. Barium presented overall concentrations ranging from 8 to 765 mg kg^{-1}, with a relatively low global average (106.54 mg kg^{-1}), which indicates the presence of possible abnormally high concentrations in the sites from western Amazon (AC:SM and AC:XP). The site AC:XP presents the highest median concentrations (456, 267, and 231 mg kg^{-1} for 0-20, 20-40, and 40-60 cm respectively). The sites with the lowest median concentrations are represented by MT (southern Amazon), AM:AR, and PA (Central Amazon), having all median concentrations below 25 mg kg^{-1}. Similar to Se behavior in soil, the widest variations for Ba were observed in the sites with the highest median values, being AC:SM (103 to 592 mg kg^{-1}), AC:XP (119 to 765 mg kg^{-1}) and AM:AN (120 to 323 mg kg^{-1}) (**Fig. 3**).

Different than observed in the present study, Jeske (2013) who studied the mobility and distribution of Ba in profiles of podzolic soils from Poland, observed that Ba is transported downward the soil profile or accumulated in the subsurface soil horizons. The authors also stated that this process is particularly common in acidic conditions, but it may concern soils that are much less weathered than the ones from the Amazon basin.

Considering the Brazilian legal reference values established for Ba, there are not regulations for guiding values established specifically for soils from the Amazon region. Thereby, they are mostly based on criteria defined for the southeast region and do not consider data from the north of the country which comprises the Amazon region. The only available regulation published was the one adopted by the Environmental Agency of the state of São Paulo (CETESB, 2014), which establishes the value of 75 mg kg^{-1} as a reference value.

It is important to notice that average Ba concentrations in soils of the Amazon region, in some cases, such as the sampled soils from the state of Acre (AC:SM and AC:XP) and the state of Amazonas (AM:AN), are higher than the prevention level established by the Brazilian Legislation (150 mg kg^{-1}). This demonstrates the necessity to update or even create new reference values being more representative for the region, based on studies considering properly the pedology and geochemical specificities of the soils from the Amazon region.

Background levels of Ba in soils above the guiding values of the Brazilian legislation have also been previously reported for the state of Pernambuco in the northeast region of the country, which corroborates the need for a more comprehensive knowledge of regional diversities when elaborating national standards in such a big country like Brazil (Biondi et al., 2011). Legislations in other countries, based on risk analysis, suggest much broader intervention values for Ba in soils, as is the case in Canada, where soil quality guidelines range between 750 and 96,000 mg kg^{-1} (Canadian Council of Ministers of the Environment, 2013). In the Netherlands, the intervention value of 920 mg kg^{-1} has also temporarily been suspended from the legislation on soil quality because this value was often lower than the natural background concentration (Soil Remediation Circular, 2006).

High concentrations of Ba found in soils from the Amazon region are presumably not harmful to the native plants or other organisms, since they may present mechanisms providing adaptation to such high levels. Besides this, the current legislation does not consider the availability of Ba in the soil. Although it is important to notice that concentrations above 600 mg kg^{-1} have been reported to reduce slightly the production of soybean in an Entisol according to Melo et al. (2011). In summary, Ba dynamics in those soils should be well understood before starting any agricultural practices.

Wide variations of Ba were observed by Biondi et al (2011), who studied natural levels in reference soils from the state of Pernambuco for the establishment of quality reference values. The values observed for the three sub-regions of the state were in the Zona da Mata 129 mg kg^{-1}, Sertão 111 mg $kg^{-1,}$ and in the Agreste 52.8 mg kg^{-1}, in superficial horizons. In this study, Ba levels were higher than the Prevention and Investigation Values established by Conama (2013), leading to the need to increase knowledge on these values at regional levels to update the national standards.

Despite that total Ba concentrations can serve as a reference for general Ba status in soils, correlations of total Ba in soil with plant uptake seem to be poor because Ba solubility in soils is low and dependent on several soil properties. Therefore, total Ba will not be able to predict the accumulation of Ba in plants so efficiently. Additionally, Ba distribution in soils seems to be not homogeneous but rather concentrated in "nuggets", which also might explain the wide range of concentrations within the sites studied (Lamb et al., 2013; McBride et al., 2014).

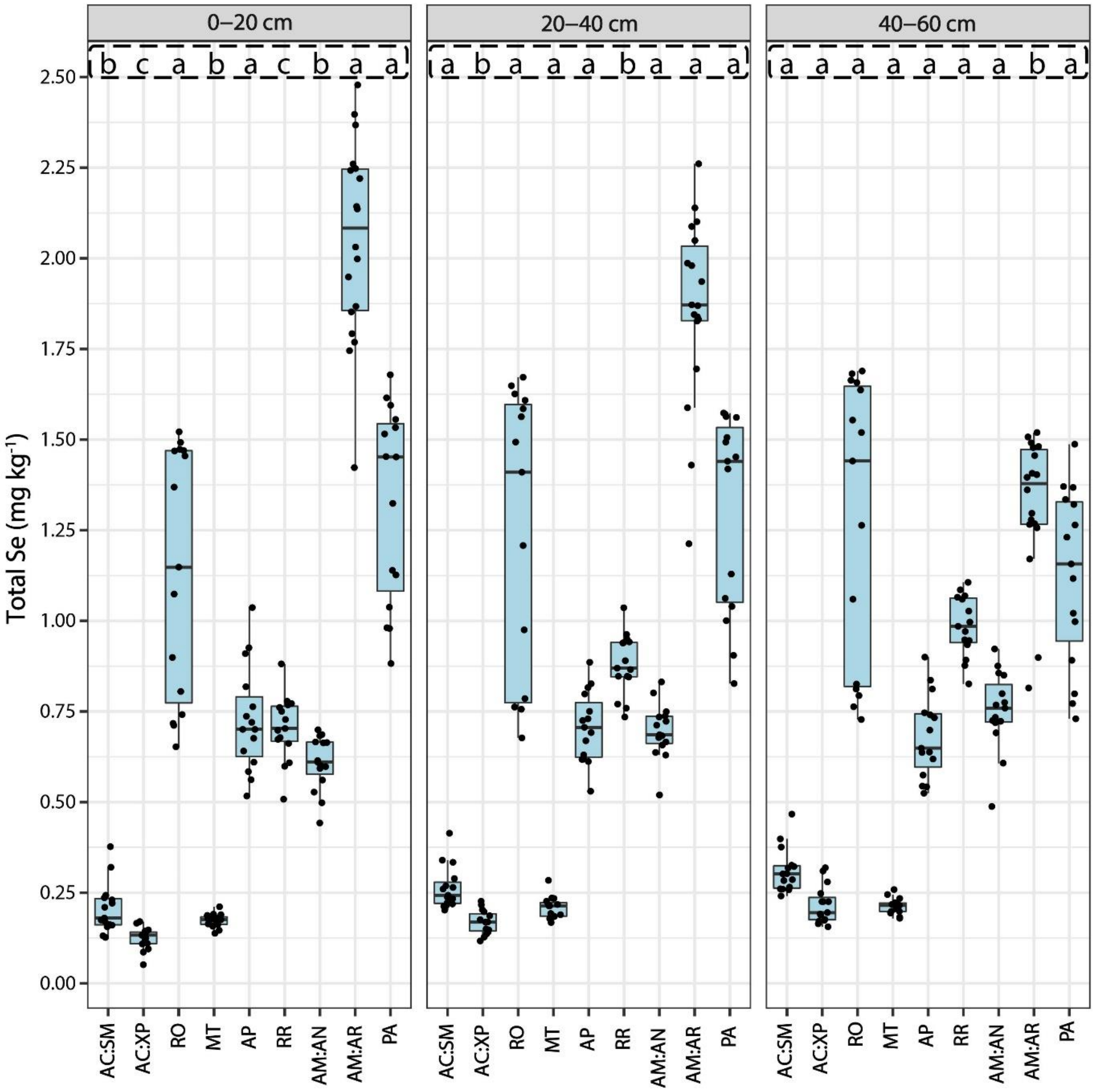

Fig. 2. Raw data of total soil Se concentration distribution in samples collected at three different depths (0-20, 20-40, and 40-60 cm) on different sites of the Amazon region (AC:SM = Acre-Sena Madureira; AC:XP = Acre-Xapuri; RO = Porto Velho-Rondônia; MT = Itaúba-Mato Grosso; AP = Laranjal do Jari-Amapá; RR = Caracaraí-Roraima; AM:AN = Amazonas-Anori; AM:AR = Amazonas-Aruanã farm; PA = Santarém-Pará. Random horizontal noise was added to the points (raw observations) to facilitate visualization. The letter on top of each site indicates the result of Tukey's HSD test (p = 0.05) following a significant ANOVA (p < 0.001) for Se concentration, comparing the different layers for each site. Data for the site AC:SM were log-transformed before ANOVA to attend statistical assumptions.

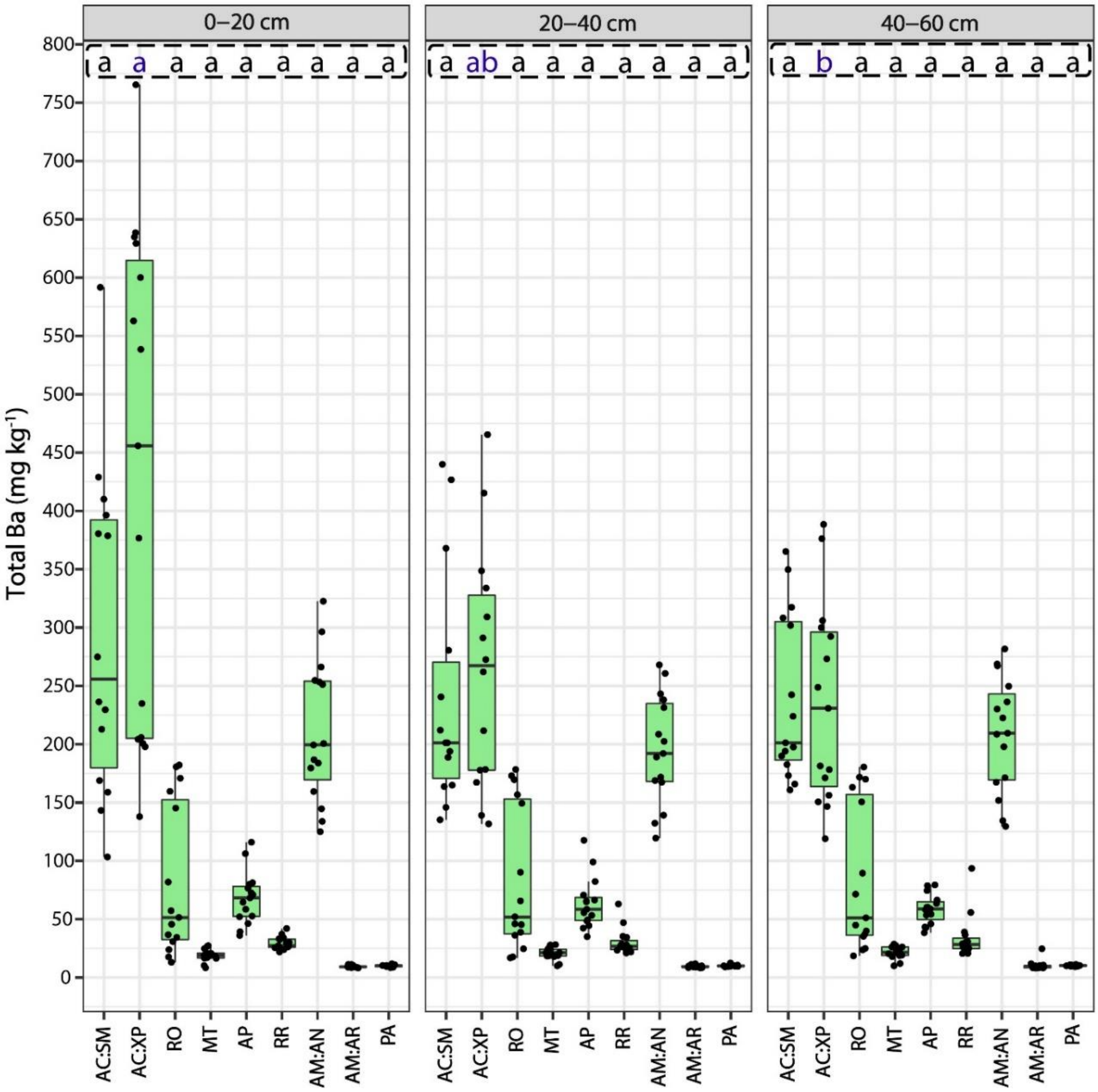

Fig. 3. Raw data of total soil Ba concentration distribution in samples collected at three depths (0-20, 20-40, and 40-60 cm) in different sites of the Amazon region (AC:SM = Acre-Sena Madureira; AC:XP = Acre-Xapuri; RO = Porto Velho-Rondônia; MT = Itaúba-Mato Grosso; AP = Laranjal do Jari-Amapá; RR = Caracaraí-Roraima; AM:AN = Amazonas-Anori; AM:AR = Amazonas-Aruanã farm; PA = Santarém-Pará. Random horizontal noise was added to the points (raw observations) to facilitate visualization. Data for the site AC:XP were log-transformed before ANOVA to attend statistical assumptions. The letter on top of the site AC:XP indicates the result of Tukey's HSD test (*p* = 0.05) following a significant

ANOVA ($p < 0.001$) for Se concentration, comparing the different layers for each site. For all other sites, there was no significant difference between the layers studied.

3.3 Soluble and exchangeable soil selenium

Figure 4 shows the results of extractions with two extracting agents predicting bioavailable Se in soil, namely soluble (KNO_3) and exchangeable (KH_2PO_4). Extractable Se in the soil decreases from the topsoil (0-20 cm) to the deepest layer (40-60 cm), with a few exceptions, and also presents a reasonable variation among sites and layers studied. For the soluble Se, the absolute concentrations ranged from 1.72 (MT) to 20.3 µg kg^{-1} (AP) with an average value of 6.7 µg kg^{-1}. Exchangeable Se ranged from 4.40 (AM:AN) to 50.8 µg kg^{-1} (AP) with an average value of 17.3 µg kg^{-1}. The results confirm that the exchangeable fraction of Se in the soil is higher than the soluble fraction in the sites studied.

The sites with the highest average soluble concentrations are AC:SM, RR and AP in the topsoil (0-20 cm layer), with concentrations of 10.1, 13.2, and 14.5 µg kg^{-1}, respectively. However, the site with the highest relative concentration extracted by KNO_3 was AC:SM with a relative concentration of 52, 50, and 53% (0-20, 20-40, and 40-60 cm) when extracting with KNO_3. The highest average exchangeable concentrations were observed in the site AP for all three layers studied, with 35, 34.4, and 33.3 µg kg^{-1} for 0-20, 20-40, and 40-60 cm, respectively. The highest relative concentration extracted by KH_2PO_4 is observed on the sites AP (71-84%) and RR (61-87%) (**Fig. 4**).

The relative concentrations extracted by KNO_3 and KH_2PO_4 were quite variable among sites and layers studied. Moreover, it is important to mention that while the relative occurrence in the soluble (KNO_3) fraction decreased from the topsoil to deepest layer (35.9% > 28.6% > 25.7%), the relative occurrence in the exchangeable (KH_2PO_4) fraction increased from the 0-20 cm to the 40-60 cm layer (64.1% < 71.4% < 74.3%). Such results reflect the behavior of Se in tropical soils and its relationship with the soil charges. It is possible to deduce that because of the lower amount of negative charges in soil colloids in deeper layers, Se may be transferred from the soluble pool to the exchangeable/adsorbed pool. The overall relative concentration extracted by KH_2PO_4 was higher for all sites studied, but it was noticeable that for the site AC:SM the percentage was $\leq 50\%$ in the 3 layers studied.

Zhang et al. (2019) presented available soil Se as the sum of soluble and exchangeable Se fractions. Moreover, they showed significant correlations between Se in soil and Se in plant tissues (grain, leaf, and roots) in their experiment. The bioavailable form of Se in soil (soluble and exchangeable/adsorbed) is the most dynamic and important pool of Se in terms of environmental cycle. Even though soluble and exchangeable Se are the most available species, metallic oxi-hydroxides and organic matter represent the major binding sites of Se in the soil, even though Se sorption on organic particles is indirect, mainly resulting from association with surface Fe oxides or clays (Coppin et al., 2009; Feudis et al., 2019).

The Se represented by the more mobile fractions (soluble and exchangeable) in the present study demonstrates a pool that can be lost more easily through leaching for example, than when compared with fractions more strongly bound to clay such as oxide-bound or residual fractions. This was demonstrated in an experiment performed by Zhai et al. (2019), studying the leaching behavior and chemical fraction distribution of Se through simulated rainfall. Before leaching, the study showed Se predominantly being found in the soluble fraction, followed by the exchangeable, residual, and organic matter-bound fractions; the least one was the iron/manganese oxide-bound fraction. After leaching, Se mainly existed in the residual fraction, followed by the iron/manganese oxide-bound and organic matter-bound fractions. We hypothesize that during the rainy season in the Amazon (which varies in time of the year from one state to another), Se fractions may change completely and Se could become more abundant in a more stable soil fraction.

Figure 5 presents complementary information on Se availability in the soils with details about the relative Se extractability (available = soluble + exchangeable fractions) in soils from the Amazon when compared to total concentrations (HF-extracted Se). The overall average percentage of extractability ranged from 1.05% to 10.6% (mean = 4.54%). The extractability tends to be higher for the topsoil (0-20 cm) than for the deeper layers studied (20-40 and 40-60 cm). The two sites from the state of Acre (AC:SM and AC:XP) had higher extractability particularly for the topsoil (0-20 cm) with average extractability of 10.3% and 10.6%, respectively. On the other hand, the sites AM:AR and RO have a lower extractability (between 1 and 2%) for the three layers studied.

The average percentages of the available Se fraction (exchangeable + soluble) relative to the total Se content in soils were as follow considering the 3 layers assessed for each site (mean ± standard deviation): RO (1.52% ± 0.45), AM:AR (1.89% ± 0.15), PA (2.49% ± 0.09), AM:AN (2.96% ± 1.21), RR (3.51% ± 1.14), AP (6.25% ± 0.50), MT (6.33% ± 0.85), AC:SM (7.65% ± 2.43), AC:XP (8.23% ± 2.5).

Soils worldwide will present different behaviors, with properties changing from tropical to temperate zones. An experiment applying parallel single extraction procedures on a British meadow clay loam soil (Rothamsted), containing 423 µg kg^{-1} of native Se, indicated that water-soluble Se (comparable with the soluble fraction) accounted for 1.4 ± 0.2% of the total soil Se, while the phosphate-soluble Se (comparable with the exchangeable fraction) accounted for 20 ± 1.0%.

An interesting inference that can be made is that Se availability will also change depending on the position of the soil in the landscape (upland or lowland soil), as the availability is determined by factors such as pH, Eh, OM, oxygen availability, etc. Such difference was demonstrated by Wang et al. (2012) studying Se fractionation in agricultural soils from China, showing that soluble Se and exchangeable Se fractions accounted for less than 1% of the total Se in the upland soil, but approximately 16.1% in the paddy soil. Hence, because organic matter-bound Se in paddy soil was significantly lower than that in upland soil, the authors inferred that the ultimate accumulation of Se in the organic matter-bound fraction would be the main process in the biogeochemical cycle of Se in the study region since it provides more Se readily available for the plants.

Since there are no studies in literature focusing on Se dynamics in Brazilian soils, we can state that the predominance of Fe and Al oxides (gibbsitic and goethitic soils) combined with low pH (acidic soils) can play a great role in Se adsorption in these highly weathered soils, controlling the soluble and exchangeable fractions particularly in deeper layers where there is low OM content. Besides that, anthropogenic activities, such as soil management for agriculture (addition of fertilizers) will make Se availability in these soils be surely influenced by P and S competing anions (Abreu et al., 2011; Lessa et al., 2016; Araujo et al., 2018; Araujo et al., 2020).

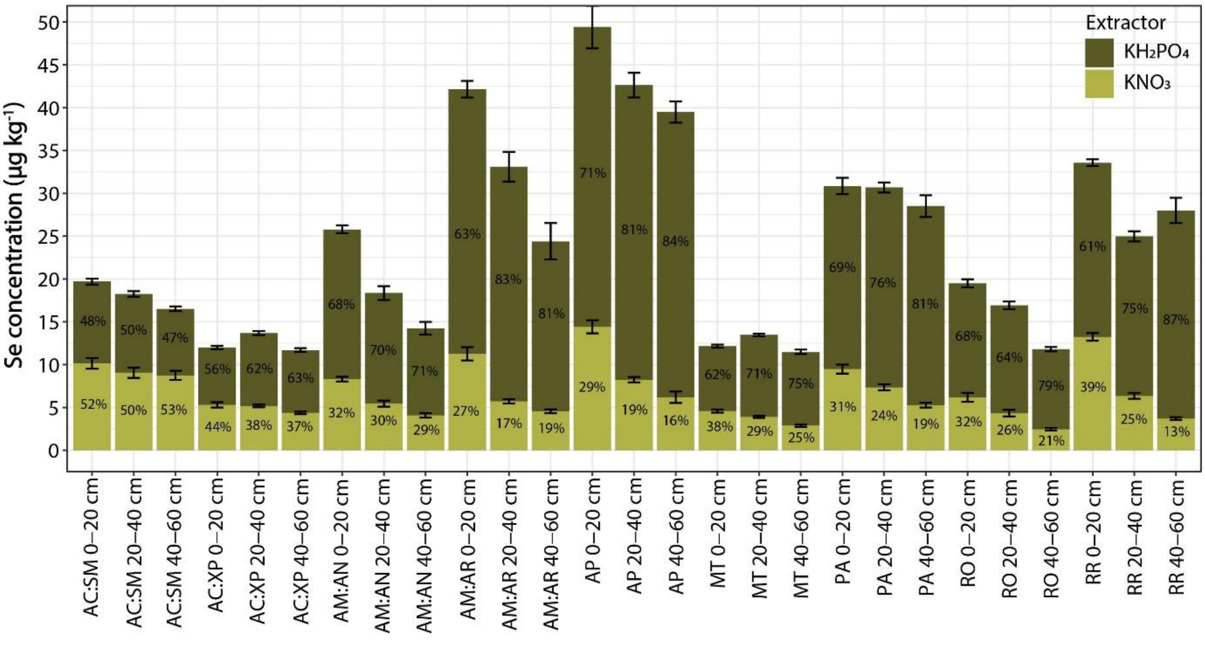

Fig. 4. Average concentration of soluble and exchangeable Se with information of relative contribution of each extractor (%) for soil samples from different sites in the Amazon region

and different layers studied (0-20, 20-40 and 40-60 cm). Each site is identified by: AC:SM (Sena Madureira, state of Acre); AC:XP (Xapuri, state of Acre); AM:AN (Anori, state of Amazonas); AM:AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); MT (Itaúba, state of Mato Grosso); PA (Santarém, state of Pará); RO (Porto Velho, state of Rondônia) and RR (Caracaraí, state of Roraima). The vertical bar plot presents the standard error of the mean for each extractor (SEM, n = 15; AM:AR, n = 18).

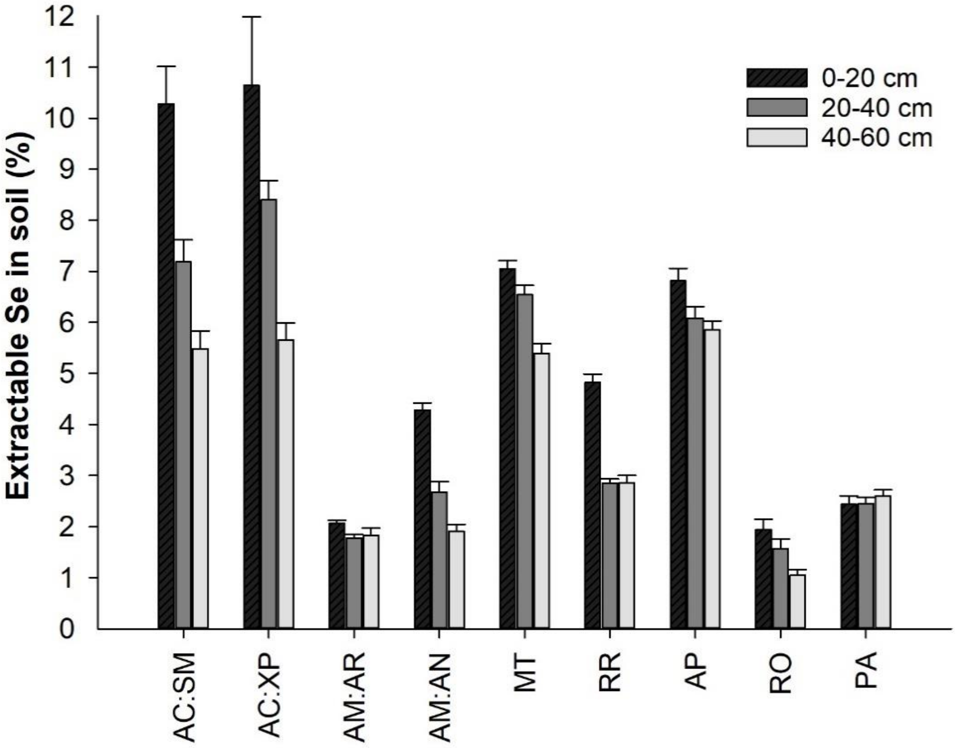

Fig. 5. Average percentage from the total of extractable/available Se in soil (soluble + exchangeable fractions) for samples from different sites in the Amazon region and different soil layers studied (0-20, 20-40, and 40-60 cm). The calculation of percentage was based on total (HF-extracted) Se in soil. Each site is identified by: AC:SM (Sena Madureira, state of Acre); AC:XP (Xapuri, state of Acre); AM:AN (Anori, state of Amazonas); AM:AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); MT (Itaúba, state of Mato Grosso); PA (Santarém, state of Pará); RO (Porto Velho, state of Rondônia) and RR

(Caracaraí, state of Roraima). The vertical bar plot presents the standard error of the mean for each layer within each site (SEM, n = 15; AM:AR, n = 18).

3.4 TMAH extractable selenium and iodine

While TMAH has been used to extract the organic-bound fraction of Se in soil samples (Ligowe et al., 2020), the same extractant has been shown to extract quantitatively the total I content from environmental samples e.g. soils, sediments, plants, and food (Watts and Mitchell, 2009). Therefore, TMAH extraction was conducted in the present study for the topsoil samples (0-20 cm).

Concentrations of Se-TMAH in soil samples studied (0-20 cm layer) ranged from 0.05 to 1.59 mg kg^{-1} (average 0.50 mg kg^{-1}). The average concentrations for each site were as follows in decreasing order: AM:AR (1.33 mg kg^{-1}) > PA (0.76 mg kg^{-1}) > RO (0.61 mg kg^{-1}) > AM:AN (0.47 mg kg^{-1}) > AP (0.45 mg kg^{-1}) > RR (0.38 mg kg^{-1}) > AC:SM (0.15 mg kg^{-1}) > MT (0.11 mg kg^{-1}) > AC:XP (0.09 mg kg^{-1}) (**Fig. 6**). The Se extractable by TMAH relative to the total Se in soil (HF-extractable Se) decreases in the following order (mean ± standard deviation): AM:AN (78.4 ± 9.89%) > AC:XP (74.3 ± 9.69%) > AC:SM (72.7 ± 8.48%) > MT (65.1 ± 4.31%) > AM:AR (65 ± 2.28%) > AP (61.8 ± 3.64%) > PA (58.4 ± 5.54%) > RO (57.3 ± 12.7%) > RR (54.5 ± 7.12%). Thus, organic-bound Se comprises in general more than 50% of the total Se in the topsoil.

Selenium extraction seeking to assess the “organic-bound” Se fraction in soils has been performed using TMAH in other recent studies (Ligowe et al., 2020; Wang et al., 2020b). Other options to quantify OM-Se fractions include the use of NaOH (Qin et al., 2012) and $K_2S_2O_8$ (Long et al., 2018). Ligowe et al. (2020) analyzed the organic fraction of Se remaining after fertilizer application in 2017 and 2018 in Malawi and the results showed that almost none of the remaining ^{77}Se was present in soluble or phosphate-extractable forms whereas virtually all of it was present in the “organic” (TMAH-extractable) fraction. In the study performed by Qin et al. (2012), OM-Se was the dominant Se fraction in Se-rich soils from China, accounting for an average 43.7% (from 31.8% to 51.3%) of total Se. Long et al. (2018) observed lower values for the organic Se fraction in the 0-20 cm layer of 4 sites in China, ranging from 30-40% from the total Se. This is below the values observed in our present study, but it still supports the significant association between Se and organic matter. Another important aspect is that Se is preferentially incorporated into the fulvic acids fraction rather than humic acids fraction (62.4 – 97.1% of OM-Se) (Qin et al., 2012).

For the TMAH extractable I in soil samples, the concentrations ranged from 0.66 to 14.2 mg kg^{-1} (0-20 cm) with an overall average of 5.43 mg kg^{-1}. The average concentration for each site decreased in the following order: AM:AR (10.9 mg kg^{-1}) > RO (8.33 mg kg^{-1}) > RR (6.36 mg kg^{-1}) > PA (6.05 mg kg^{-1}) > AP (5.68 mg kg^{-1}) > AM:AN (4.27 mg kg^{-1}) > AC:SM (2.45 mg kg^{-1}) > MT (2.28 mg kg^{-1}) > AC:XP (1.48 mg kg^{-1}) (**Fig. 6**).

Many I concentrations are of similar magnitude as those seen in iodine-deficient areas where goiter has been reported, e.g. in the UK (median 5.52 mg kg^{-1}), Sri Lanka (3.1 mg kg^{-1}), China (1.0 mg kg^{-1}), and Morroco (2.1 mg kg^{-1}) (Fordyce et al., 2000; Johnson et al., 2002; Fordyce et al., 2003; Bowley et al., 2017). However, it should be emphasized that the presence of endemic goiter is not always clearly related to the occurrence of deficient iodine in the environment (Stewart et al., 2003).

The highest Se and I concentrations were observed for the site AM:AR, where also the highest organic matter and clay content were seen. On the other hand, the lowest Se and I concentrations were observed at AC:XP, the site with the lowest available S concentration and also one of the lowest clay contents (< 25%) (**Fig. 1**), so presumably clay and OM partly determine the availability of both I and Se in the topsoil. Additionally, the correlation between

I (I-TMAH) and Se (Se-TMAH) suggests that the availability of both elements to plants may be controlled by similar factors in the soil (Bowley et al., 2017).

Fuge (2013) pointed out that the I geochemistry of soils can be summarized as being dependent on the quantity of I supplied by the parent material coupled with the soil's ability to retain it. The authors also demonstrated that sedimentary rocks show a greater range of iodine content, with clay-rich or argillaceous rocks being more enriched than the sand-rich, arenaceous rocks. The highest concentrations of iodine have been found in organic-rich shales, with concentrations as high as 44 mg kg^{-1} recorded in some bituminous shales.

Iodine in agricultural soils varied in the range of 1.3–20.9 mg kg^{-1} in La Pampa region, located in central Argentina and 0.1-10.5 mg kg^{-1} in San Juan, located in the northwest Andean region of Argentina, compared to a worldwide mean of 2.8 mg kg^{-1} (Kabata-Pendias, 2011). Roulier et al. (2019) reported a similar average (7.11 mg kg^{-1}), but a wider range of I concentrations among sites (0.39-35.6 mg kg^{-1}) in the 0-40 cm layer of French forest soils. Their results confirmed that organic matter, Fe and Al (hydr)oxides allowed higher retention of iodine in the soil. They stated that the fate of I in forest soils depends not only on soil properties but also on vegetation and climatic conditions.

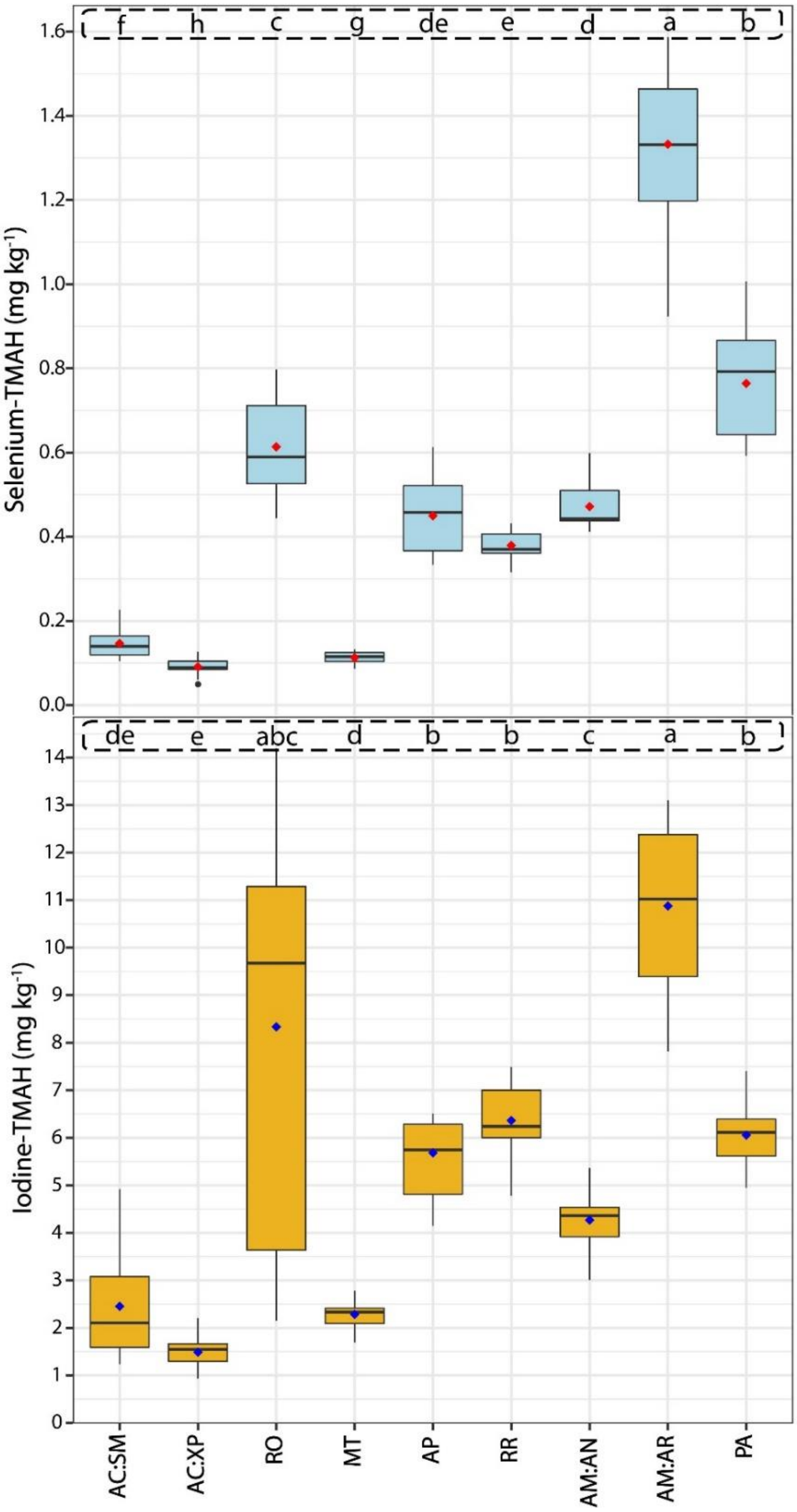

Fig. 6. Raw data for TMAH-extracted Se and I concentration in the topsoil (0-20 cm) layer on different sites of the Amazon region represented as Box-and-whisker plot (median value line within box, mean value as a rhombus). Sites are identified as follow: AC:SM = Acre-Sena Madureira; AC:XP = Acre-Xapuri; RO = Porto Velho-Rondônia; MT = Itaúba-Mato Grosso;

AP = Laranjal do Jari-Amapá; RR = Caracaraí-Roraima; AM:AN = Amazonas-Anori; AM:AR = Amazonas-Aruanã farm; PA = Santarém-Pará. Data were log-transformed before proceeding to ANOVA, subsequently applying Tukey's HSD test for mean comparison among sites.

3.5 Relationship between the studied elements (Se, I and Ba) and soil attributes

Pearson correlation and multiple regression models were employed particularly for the topsoil layer (0-20 cm) because this is the most dynamic layer in the soil, wherein information was obtained for all 6 variables of interest. The Pearson correlation matrix had a major purpose to select explanatory variables for the equations (multiple regression), built up to explain the studied response variables (total, soluble, exchangeable and organic-bound Se, total Ba, and total iodine).

Total Se was positively correlated with all three Se fractions studied (soluble, exchangeable and organic-bond Se), I, S and clay, and negatively affected by total Ba, K and sand in soil (**Table 2**). In the multiple regression, the best predictive equation (r^2 = 0.98) confirmed that the variables contributing most to the model were the organic bound-Se followed by iodine (**Table 3**). The correlation with OSe can be explained by the higher percentage of the total Se in the organic fraction when compared to the other fractions. On the other hand, the correlation with I might be explained by the shared similarities in terms of ionization and chemical bonding properties in soil. In the investigation performed by Matos et al. (2017), the Se concentration in soils from the Jequitinhonha Valley (MG), Brazil, correlated significantly and positively with Al_2O_3 (r = 0.76), Fe_2O_3 (r = 0.57), and clay (r = 0.64) and negatively with SiO_2 (r = -0.76) and sand (r = -0.54), and there was no significant correlation with organic matter, pH and silt. Therefore, clay is always an important soil attribute in the retention of Se.

Twelve variables presented significant ($p < 0.05$) correlation with total Ba in soil. To date, total Ba in soil was negatively correlated with all the main response variables studied (SeT, ESe, OSe, and I), except SSe. Other than these variables studied, OM, S and clay were also negatively correlated with total Ba (**Table 2**). In the multiple regression equation, the greatest positive contribution was given by the t value, which is the soil cation exchange capacity in its natural pH. Thus, the negative charges in the soil contribute significantly to the retention of Ba in the soil under the tropical conditions of the Amazon. The positive contribution of silt in the equation might be an indication that less weathered soils such as those from the state of Acre (AC:XP and AC:SM) tend to have higher total Ba concentrations (**Table 3**). Magalhães et al. (2011) state that Ba solubility in the soil increases under water saturation of the soil and establishment of reducing conditions. It happens because these conditions will lead to increasing the pH and reducing the Eh of the soil, consequently increasing the Ba concentration in the soil solution. Besides this, Ba demonstrates low affinity to soil organic matter, which is due to the low affinity of the Ba^{2+} cation for the binding sites of organic compounds. In summary, a low soil pH enhances the release of Ba^{2+} from the soil, while a high pH results in Ba^{2+} sorption to Al and Fe oxyhydroxide minerals, which are difficult for plants to take up (Lu et al., 2019).

The soluble and exchangeable Se in soil (SSe and ESe) presented a completely different behavior in terms of correlated variables. Despite soluble Se being dependent on the exchangeable fraction, it is more dynamic and responds faster to external influences. For example, while soluble Se was positively related to soil pH ($r = 0.48$), the exchangeable Se was not. It was remarkable to observe that in the SSe regression equation, organic-bound Se (OSe) had a negative coefficient, which can be explained by the higher retention of Se in the solid phase whenever the concentration of OM increases in the soil. The greatest contribution was given by ESe, despite that only 61% of the total variance of the original data is explained by the equation obtained. For the exchangeable Se on the other hand, the highest Pearson correlation coefficient was observed for organic-bound Se (OSe), which is confirmed in the regression model. This can be an indication that these two fractions may overlap in terms of extraction capacity in soil. In other words, both extractant solutions (ESe and OSe) can assess similar Se pool in the soil (sites in both organic and mineral colloid surfaces). Surprisingly, clay had a major negative contribution to the ESe besides exchangeable Al (**Table 2 and 3**).

It is important to mention that soil pH is an important factor determining metal availability in general, despite the present study being found significantly correlated only with soluble Se (SSe). It could not be included in the multiple regression models. Organic matter plays a key role in Se retention and partitioning between solid and liquid phases. Generally, there are more oxygenic groups in soil organic matter, such as phenolic hydroxyl and carboxyl, and these functional groups can readily complex or chelate soluble Se in soil, thereby decreasing Se availability (Coppin et al., 2006; Li et al., 2016).

The organic-bound Se was studied separately because of the particular nature of its extraction reagent used. This response variable was particularly highly correlated with total Se in soil ($r = 0.99$), which could be confirmed by inclusion as a major parameter in the multiple regression equation. The only negative coefficient in the multiple regression model was obtained for sand, which for obvious reasons has an inverse relationship with organic matter content in soils (**Table 2 and 3**).

Iodine in the 0-20 cm layer presented a remarkable positive correlation with all Se fractions (r = 0.94, 0.43, 0.78 and 0.92 for SeT, SSe, ESe and OSe and respectively). The only negative correlations were observed with BaT ($r = -0.61$), K ($r = -0.59$) and sand ($r = -0.46$) (**Table 2**). The greatest contributions to the regression equation, which were all positive, were in decrescent order: SeT > sand > S. These together could explain 98% of the variance in the I concentration in the topsoil (**Table 3**). In the literature, there is no report of correlations between Se and I in soils so far published. Thus, further research may focus on better explaining such correlation or even deny such association in soils of the region. Bowley et al. (2019) found a significant correlation between I concentrations in soils and associated vegetation. Kinetic models with pH and organic matter as dependent parameters could account for 75% of the variation in vegetation I concentration, implying also a strong relation between rainfall and I concentration in the grass.

The multiple regression models were an attempt to observe the multiple factors affecting the variables in focus, but will not always be effective to capture optimal cause-effect relations for the soil phenomena studied. For some response variables, observed relations can be attributed to indirect effects. The negative correlation found between total, exchangeable and organic Se and Ba is a clear indication that these elements present an inverse relationship in the soil, and the mobility and dynamic of one is affected by the other.

The Se fractions studied seem to be mostly correlated to each other, for example, SSe x ESe and OSe x ESe, as is demonstrated by both the Spearman correlation and the multiple regression models. Such results mean that these fractions are intrinsically related and their dynamic strongly depends on each other in the soil-solution surface.

The dynamics of iodine in the soil are not completely understood yet, but indications that Se relates to its cycle in the soil environment make the study of relationships between the two elements even more interesting and valuable, especially as both are essential to humans and used in contemporary biofortification studies (Zou et al., 2019; Golob et al., 2020).

Table 2. Pearson correlation coefficients for log-normalized data between main dependent variables studied: Total Se (SeT), Total Ba (BaT), Soluble Se (SSe), Exchangeable Se (ESe), organic-bound Se (OSe) and TMAH-Iodine (I) and soil physicochemical attributes from the layer 0-20 cm, selected from the correlation matrix ($p \leq 0.05$; $|r| \geq 0.4$) (**Figure S1**).

		Response variables					
		SeT	BaT	SSe	ESe	OSe	I
Explanatory Variables	SeT	-	-0.56**	0.47**	0.83**	0.99**	0.94**
	BaT	-0.56**	-	-	-0.44**	-0.51**	-0.61**
	SSe	0.47**	-	-	0.76**	0.49**	0.43**
	ESe	0.83**	-0.44**	0.76**	-	0.85**	0.78**
	OSe	0.99**	-0.51**	0.49**	0.85**	-	0.92**
	I	0.94**	-0.61**	0.43**	0.78**	0.92**	-
	pH	-	-	0.48**	-	-	-
	K	-0.56**	0.58**	-	-0.59**	-0.58*	-0.59**
	Ca	-	-	0.45**	-	-	-
	Al	-	0.50**	-0.40**	-0.46**	-	-
	t	-	0.71**	-	-	-	-
	m	-	-	-	-0.42**	-	-
	OM	-	-0.47**	-	-	-	-
	Zn	-	0.56**	-	-	-	-
	Mn	-	-	0.40**	-	-	-
	S	0.67**	-0.47**	-	0.43**	0.63**	0.68**
	Clay	0.85**	-0.59**	-	0.52**	0.82**	0.82**
	Silt	-	0.61**	-	-	-	-
	Sand	-0.59**	-	-	-	-0.64**	-0.46**

Obs.: Data from the site PA (Santarém-Pará) were excluded from the correlation matrix because of a lack of information about texture (clay, silt, and sand). *Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

Table 3. Multiple regression equations for the relationship between the studied elements and fractions (Response variables) and other soil properties in the topsoil (0-20 cm) pre-selected via Pearson correlation (**Table 2**) and further variable selection performed via branch and bound algorithm.

Response variables	Regression equation	R^2	RSE
Total Se (logSeT)	= -1.45 + 0.11log(ESe) + 0.70log(OSe) + 0.30log(I) + 0.06log(K) + 0.17log(clay)	0.98	0.12
Total Ba (logBaT)	= 0.56log(ESe) - 0.88log(OSe) + 0.84log(t) - 0.68log(OM) + 0.26log(Zn) + 0.56log(silt)	0.99	0.51
Soluble Se (SSe)	= 4.25 + 0.35(ESe) – 2.93(OSe)	0.61	2.53
Exchangeable Se (logESe)	= 3.46 + 0.39log(SSe) + 0.58log(OSe) - 0.12log(Al) - 0.26log(clay)	0.94	0.14
Organic-bound Se(logOSe)	= -0.78 + 0.73log(SeT) + 0.24log(ESe) - 0.14log(sand)	0.98	0.13
Iodine-TMAH(logI)	= 1.18 + 0.73log(SeT) + 0.12log(S) + 0.14log(sand)	0.91	0.22

Raw data were log-transformed in order to attend parametric statistics assumptions (homoscedasticity and normality) whenever necessary. SeT = total Se (mg kg^{-1}); BaT = total Ba (mg kg^{-1}); SSe = soluble Se (µg kg^{-1}); ESe = exchangeable Se (µg kg^{-1}); OSe = organic bound-Se/Se-TMAH (mg kg^{-1}); I = Iodine-TMAH (mg kg^{-1}); K = available potassium (mg kg^{-1}); S = available sulfur (mg kg^{-1}); Al = exchangeable aluminum ($cmol_c$ kg^{-1}); Zn = available zinc (mg kg^{-1}); t = effective cation exchange capacity ($cmol_c$ kg^{-1}); OM = organic matter (%); clay (%); silt (%) and sand (%). RSE = regression standard error.

4 CONCLUSIONS

Total soil Se concentrations in the soils from different Amazon agroecosystems under study range from 0.05 to 2.48 mg kg^{-1} and therefore are within the safe range according to the Brazilian legislation (i.e. below prevention value, 5 mg kg^{-1}). Average concentrations tend to increase from topsoil to the deepest layer studied.

For Ba, the concentrations ranged from 8 to 765 mg kg^{-1}, and therefore reach levels higher than the prevention value of the Brazilian legislation (150 mg kg^{-1}), with abnormal contents in the western Amazon (sites AC:SM and AC:XP in the state of Acre). This element is distributed homogeneously as a function of soil depth on the sites studied.

Exchangeable Se content is higher than the soluble Se fraction in the soil and the extractable/available Se fraction (soluble + exchangeable fraction) accounts only for 4.45% of the total on average in the Amazon.

Selenium-TMAH extracted concentration ranged from 0.05 to 1.59 mg kg^{-1} in the topsoil and accounts for more than 50% of the total Se, which reflects the great contribution of the organic-bond fraction to Se in the surface layer. The average I-TMAH in the topsoil is 5.43 mg kg^{-1}, which is above the worldwide mean of 2.8 mg kg^{-1}.

Multiple regression models supported by Spearman correlation analyses provided the following inferences for the variables studied:

1-Selenium and Ba in soil present a clear inverse relationship in the topsoil and therefore we assume an antagonistic behavior, despite completely different dynamics in the environment;

2- Selenium fractions (soluble, exchangeable and organic), despite representing different pools, share similar factors contributing to their respective models because their dynamic is interrelated;

3-Iodine is somehow affected by Se in soil besides soil texture (sand) in topsoil (0-20 cm), although, the mechanisms that link Se and I in the soil are still unknown.

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Supplementary material

Table S1. Information about the sampling sites of soil 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
Xapuri/AC	10°50’02.02’’S	68°23’23.51’’W	262	Am
Itaúba/MT	11°06’00.32’’S	55°02’06.78’’W	387	Am
Itacoatiara (Aruanã)/AM	3°01’05.59’’S	58°49’55.60’’W	92	Af
Anori/AM	3°50’10.01’’S	61°36’11.64’’W	29	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

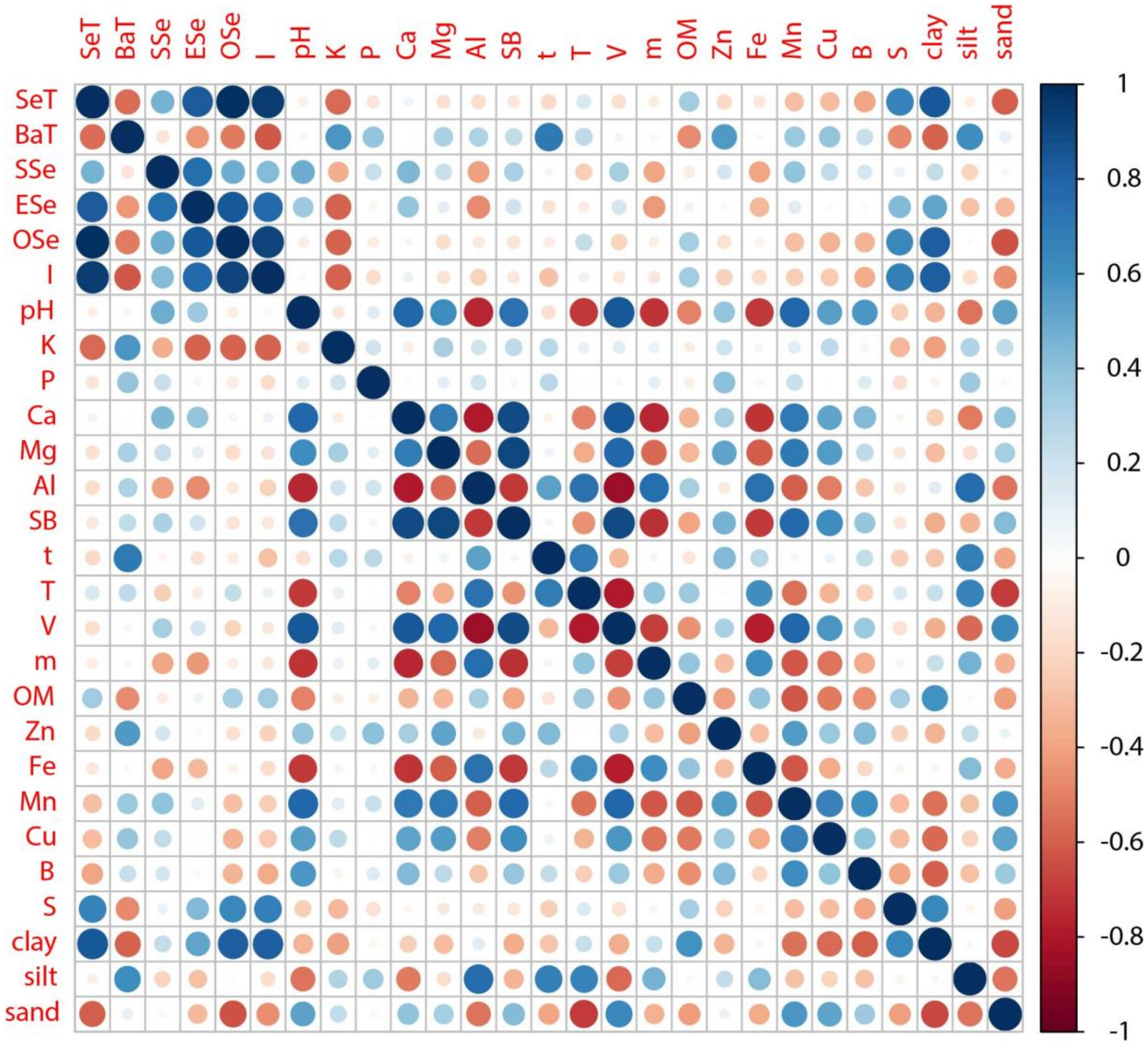

Fig. S1. Pearson correlation matrix correlation performed previously to the selection of explanatory variables used in the linear multiple regression. Data were log-transformed before proceeding to correlation analysis.

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ARTICLE 3 - Selenium and barium concentration and distribution in Brazil nuts (*Bertholletia excelsa)* seeds using μ-XRF

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Highlights

1 – Se and Ba levels in Brazil nuts can reach 356 and 7177 mg kg^{-1}, respectively;

2 – There is an inverse relationship between the levels of Se and Ba in Brazil nuts;

3 – Se accumulates mainly in the outer parenchyma tissue forming a ring shape in the nut;

4 – Ba showed hot spots mainly around the epidermal tissue of the nut;

5 – Possible association between Se-Ba and S-Ba suggest formation of $BaSeO_4$ and $BaSO_4$.

Abstract

Brazil nuts (*Bertholletia excelsa)* is native from the Amazon rainforest. They are consumed worldwide and known as the richest food source of selenium (Se) but also reported to present high barium (Ba) concentrations. This study presents the first detailed assessment of Se and Ba concentration, correlation, and localization using complementary μ-XRF techniques in Brazil nuts samples from 6 different sites in the Amazon region. Selenium concentrations ranged from 0.46 to 356 mg kg^{-1} and Ba from 12.5 to 7177 mg kg^{-1}. Spatial distribution in the seed tissues was mainly dependent on the element's concentration. Selenium accumulates mainly in the outer parenchyma tissue of Brazil nuts seeds forming a ring shape. Barium tends to accumulate in a ring more externally located in the epidermal tissue. The location and known affinity between Se-Ba and S-Ba suggest the formation of less soluble composts such as $BaSeO_4$ and $BaSO_4$ in the nut.

Keywords: Synchrotron, micro-XRF, selenium, barium, *Bertholletia excelsa.*

1 INTRODUCTION

The Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.), a member of the *Lecythidaceae* family 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 Guyana (Mori & 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 sources of income for many Amazonian indigenous and riverine communities (Ortiz, 2002; 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) and are usually found in groves of between 50 and 100 individuals, known as "castanhais" (Mori & Prance, 1990). The fruit takes around 15 months to develop and after maturing (fall during the rainy season in the Amazon), it measures 11-15 cm in diameter and weighs up to 2 kg, being enclosed by a woody capsule that contains 10-25 seeds (Mori & Prance, 1990). The seed of Brazil nuts is composed mainly by parenchyma tissues delimited by a ring of meristematic tissue, surrounded by an epidermal layer and a thin external lignified layer (Camargo et al., 2000). Corner (1976) observes that 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 by hypocotyl (Prance & Mori, 1978). A more recent description of the Brazil nuts seeds is provided by Scussel et al. (2014), who states that the edible part of the nut, which is the storage tissue, shows several different tissue/cell layers starting from the epidermis (double/triple cells sequence of round and palisade shapes) layer–the endosperm tissue. The parenchymal tissues show cells of irregular shape with small and larger sizes distributed in regular and random layers, respectively, separated by a short meristem tissue layer. The cortex cells increase in size as they approach the cotyledons junction.

Brazil nuts are rich in essential elements such as calcium, copper, magnesium, potassium, manganese, and phosphorus, containing also fiber, folate, and antioxidant compounds (Brito et al., 2019). Brazil nuts are also frequently reported to have high Se content, making them the richest known food source of Se (Chang et al., 1995; Dumont et al., 2006). A general recommendation to include one Brazil nut per day in the diet would avoid the need for fortification of food or supplements to improve the Se status (Stockler-Pinto et al., 2014), although recent studies demonstrate that Se concentrations in Brazil nuts vary geographically depending on the soil where they are grown as well as on the population provenance (Silva Junior et al., 2017).

Selenium is a required element for members of all six kingdoms and three domains of life (Dolgova et al., 2018). In humans, the adequate intake of Se has a narrow range between dietary deficiency (< 40 µg per day) and toxic levels (> 400 µg per day) (Rayman, 2012; Winkel et al., 2012). 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 several cases of overt toxicity and mortality have been reported following acute intoxication (Skalny et al., 2019; Hadrup & 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 diet. Selenium concentration in crops varies widely, not only due to species differences in terms of accumulation capacity but also due to natural variation in soil Se concentration, which is determined by geological processes (Reis et al., 2017). It has been demonstrated that there is a significant relationship between environmental and food Se levels. Besides, physicochemical aspects of the soil influence local Se speciation and availability (Lessa et al., 2016; Araujo et al., 2018; Araujo et al., 2020; Silva Junior et al., 2017; Reis et al., 2017; Skalny et al., 2019).

Besides Se accumulation, Brazil nuts are also known for presenting high Ba concentration (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 (Chang et al., 1995), in which Ba occurs in a mobile form and is easily available for plants (Kabata-Pendias & Pendias, 2001). Another important feature is the species (*Bertholletia excelsa* Humb. & Bonpl.) 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; Oskarsson & Reeves, 2007).

The recommended daily intake of Se is frequently cited as being 60 and 70 µg day^{-1} for adult women and men, respectively (Kipp et al., 2015). 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 adults' population (ATSDR, 2013; IRIS, 2005).

Micro X-ray fluorescence (μ-XRF) techniques provide a direct and versatile means of investigating trace elements *in situ* 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 biofortification of crops (Sarret et al., 2013; Lessa et al., 2019). μ-XRF is a valuable and sensitive tool for the analysis of the distribution of elements in different composts of a plant (Reis et al., 2020). 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 such as arsenic (As) and cadmium (Cd) in plant tissue (Vijayan et al., 2015). In recent years, the technique has been used in combination with or has often replaced conventional approaches (Capobianco et al., 2018).

This study presents a comprehensive characterization of Brazil nuts seeds using both benchtop and synchrotron-based microprobe X-ray fluorescence spectroscopy (μ-XRF) to set up 2D maps and investigate the distribution of Se and Ba in Brazil nuts originated from different sites in the Amazon region. Therefore, we hope not only to provide information on the role of Brazil nuts as a source of dietary Se to humans, and the precaution with Ba intoxication, but also to better understand mechanisms underlying the unique properties of Se and Ba accumulation in nuts from different Amazon agroecosystems.

2 MATERIALS AND METHODS

2.1 Sampling sites

Fruit samples of Brazil nuts were collected in different areas from 6 sites located in 6 states of the Brazilian Amazon that are important for the production of Brazil nuts (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 the years 2014 and 2016, from January to February. Information concerning geographic coordinates, sampling points and climatic conditions is provided in **Table S1**.

The number of Brazil nut trees sampled on each site was: Sena Madureira-Acre: 14, Aruanã (Itacoatiara)-Amazonas: 16, Laranjal do Jari-Amapá: 14, Caracaraí-Roraima: 14, Porto Velho-Rondônia: 7, and Santarém-Pará: 7. The Brazil nuts samples collected from the site Itacoatiara-Amazonas originated from the Aruanã farm, which is a big plantation of clones brought from the State of Pará. Brazil nuts were sampled from below the tree canopy, collecting ten ripe fruits, which naturally fall from the trees after maturation. To collect the Brazil nuts, these fruits were opened and a total of 50 randomly selected seeds (shelled nuts) from each plant were put in a plastic bag. The method for sampling 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, 3 seeds were randomly chosen and dried in an oven (Hamco Laboratory oven, Semi-Automatic, India) at 60°C until reaching constant weight (after about 72 hours). Initial and final weights were recorded for all samples. After peeling, they were grinded 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 by 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 (Atomic Absorption Spectrometry with Graphite furnace; AAnalyst™800 AAS, Perkin Elmer). The analytical determination of Ba was done using 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 mg kg^{-1}.

For quality assurance and control in Se and Ba measurements, 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 quality control. Additionally, 3 blank samples were included in each batch of digestion to calculate limits of detection and quantification.

The mean recovery value obtained for the standard materials confirmed the reliability of the data and were 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 8 blank extracts and the values were calculated with three and ten times the standard deviation (LOD and LOQ, respectively) of the 8 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 in the benchtop system, Brazil nuts (unshelled) from each sampling site were gently cut in longitudinal and transversal sections with ~ 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 a 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 in 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 matrix of 32 x 25 pixels (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 (OriginPro, 2016).

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

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

where BG$_{\mathrm{(average)}}$(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-Synchrotron analyses

2D mapping via synchrotron XRF analysis was performed in the bottom extremity of the shelled Brazil nut cut in a longitudinal section with ~ 3 mm thick, in order 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 image plane precision of the sample was 0.5 μm with three axes (X, Y, and Z) controlled by stepper motors. The samples were excited using a white beam with 30 μm of vertical extension and 30 μm horizontal, obtained by a KB micro-focus system. The pitch size was 30 μm in both directions and the acquisition time was 500 ms per pixel. The energy of the electron in the storage ring was 3.5 GeV with a current range of 200 to 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 in addition to 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 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 based on the relationship between the count rate and mass concentration for each spectral line.

2.4 Statistical analyses

A descriptive analysis of the concentrations of Se and Ba was initially performed using box plots for illustration of data distribution using the software R 3.6.2 (R Development

Core Team, 2020). Correlation analyses between the levels of Se and Ba in Brazil nuts were performed using a linear regression model. To attend the statistical assumptions previous to correlation analyses (i.e., normality, linearity, and homoscedasticity), data for Se and Ba were log-transformed before fitting the curve. Also, scatter plots showing correlation among Se, Ba, and S using µ-XRF intensities were performed using the PyMca image correlation tool (PyMca 5.2.2) (Solé et al., 2007), and are presented in **Fig. S3.**

3 RESULTS AND DISCUSSION

3.1 Se and Ba concentration in Brazil nuts

Selenium concentrations in Brazil nuts ranged from 0.46 (Sena Madureira-Acre) to 356 mg kg^{-1} (Santarém-Pará) with an average concentration of 52.2 mg kg^{-1}. The nuts sampled from States of Pará and Amazonas (clones originated from Pará grown in the Aruanã farm in Itacoatiara) presented the highest Se concentrations (median of 249 and 68.2 mg Se kg^{-1}, respectively) and the samples from Acre and Rondônia the lowest Se concentrations (median of 2.52 and 8.83 mg Se kg^{-1}, respectively).

Such variation in concentrations of Se in different Brazil nuts from different sites are believed to reflect mainly the available soil Se concentrations, and therefore the geographical position of each Brazil nut population becomes the most important variation factor when compared with plant-related characteristics, such as genotype, age, etc. The results for Se concentrations in the present study showed higher values compared with a previous study with Brazil nuts samples performed by Silva junior et al. (2017) with concentrations ranging from <0.5 to 147 mg kg^{-1} and a more recent study published by Lima et al. (2019) with Se levels ranging from 10 to 79 mg kg^{-1}, although the wide variation trends among different samples/sites are similar.

Barium concentrations varied from 12.5 (Santarém-Pará) to 7177 mg kg^{-1} (Sena Madureira-Acre). The highest median concentrations are from Sena Madureira-Acre and Caracaraí-Roraima (2533 and 650 mg kg^{-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 mg kg^{-1}, respectively) (**Fig. 1**).

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. This particular case may be explained because the clones grown in the site Itacoatiara-Amazonas (Aruanã farm) are originated from the state of Pará, i.e., the genotype of these two groups (populations) of plants share closer characteristics than when compared to the other sites studied. 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 has a great influence on the amount of Se and Ba accumulated by the plants.

An early study observed the unique capacity of Brazil nuts to accumulate Ba (Smith, 1971), with reported concentrations using Neutron Activation Analysis (190-5890 mg kg^{-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. The authors assumed that Ba moved more readily than Ca from root to shoot tissues, given that the ratio Ba/Ca in the endosperm was 67-112, and one possible explanation is the formation of organic complexes, which increase the mobility of the heavier alkaline earth ions preferentially compared with calcium, both in the upward movement of ions from the roots to the leaves and in their redistribution during fruit development.

Comparatively, with data from more recent studies, 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 mg kg^{-1} in nuts from Brazil, Bolivia, Peru, and Northern South America, and also greater than those reported in another study by Gonçalves et al. (2009), who referred to concentrations ranging from 860-2084 mg kg^{-1} in nuts from several Brazilian samples of unknown specific locations.

A 99-fold difference between the median Se concentration from the site Sena Madureira-Acre (lowest Se concentration reported) and Santarém-Pará (highest Se concentration reported) was observed. 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. Interestingly, **Fig. 1** shows that the site with the highest Se concentrations (Santarém-Pará) has the lowest Ba, and the highest Ba concentration (found in Sena Madureira-Acre) has the lowest Se, i.e., these elements are inversely correlated in the nuts. We also noticed that the sites with highest concentrations for both Se (Santarém-Pará, northern portion of the Brazilian Amazon) and Ba (Sena Madureira-Acre, 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).

The linear regression model (**Fig. 2**) with the studied variables (Se and Ba) log-transformed demonstrated a significant ($p < 0.01$) and negative ($r = -0.54$) relationship between the levels of Se and Ba in Brazil nuts. The results demonstrate that when Se concentration in Brazil nuts is high, the levels of Ba tend to decrease, being this trend confirmed in both graphs (**Figures 1 and 2**). This relationship 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, depending on the availability of these elements in soil or even to the plant metabolism itself.

There is no recent study showing association between Se and Ba in Brazil nuts or soils from the Amazon, but in turn, barium selenate showed positive effects as a slow-release fertilizer applied to subterranean clover pasture in order to provide Se for sheeps with no adverse effects from Ba to the pasture (Whelan, 1993). Also, $BaSeO_4$ injected into ewes during the breeding season elevated the Se level in milk during lactation and increased the Se concentration and GSH-Px activity in blood composts of lambs in the period when they are at the greatest risk of Se-deficiency disorders (Zachara et al., 1993). Thus, it seems that barium selenate possesses a long-lasting effect in terms of providing Se to soil, plant, and animals.

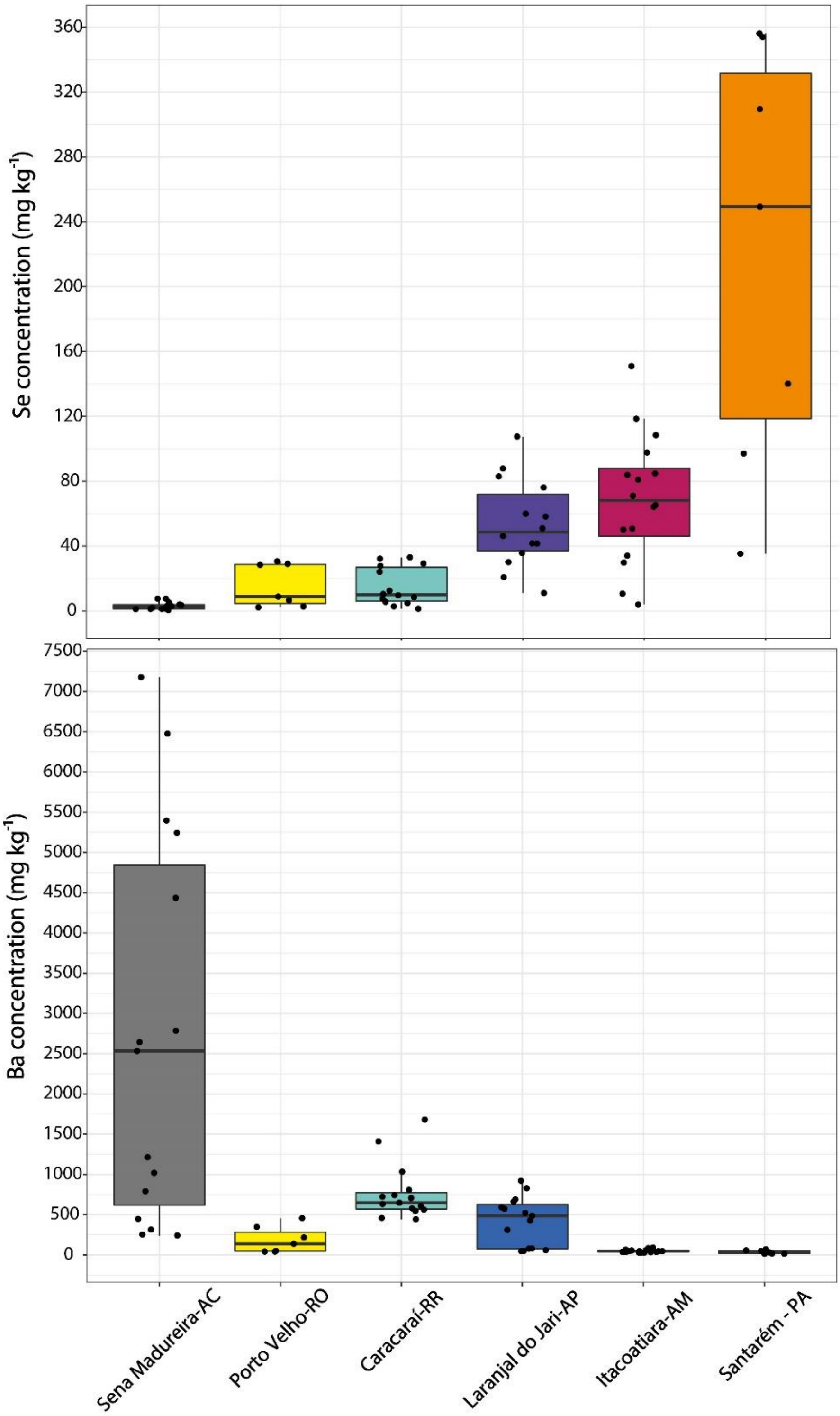

Fig. 1. Box plot for total Se and Ba concentrations in Brazil nuts samples from the respective sites: Sena Madureira-Acre (n = 14), Porto velho-Rondônia (n = 7), Caracaraí-Roraima (n = 14), Laranjal do Jari-Amapá (n = 14), Itacoatiara-Amazonas (n = 16), and Santarém-Pará (n = 7). Obs.: Data for Se in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

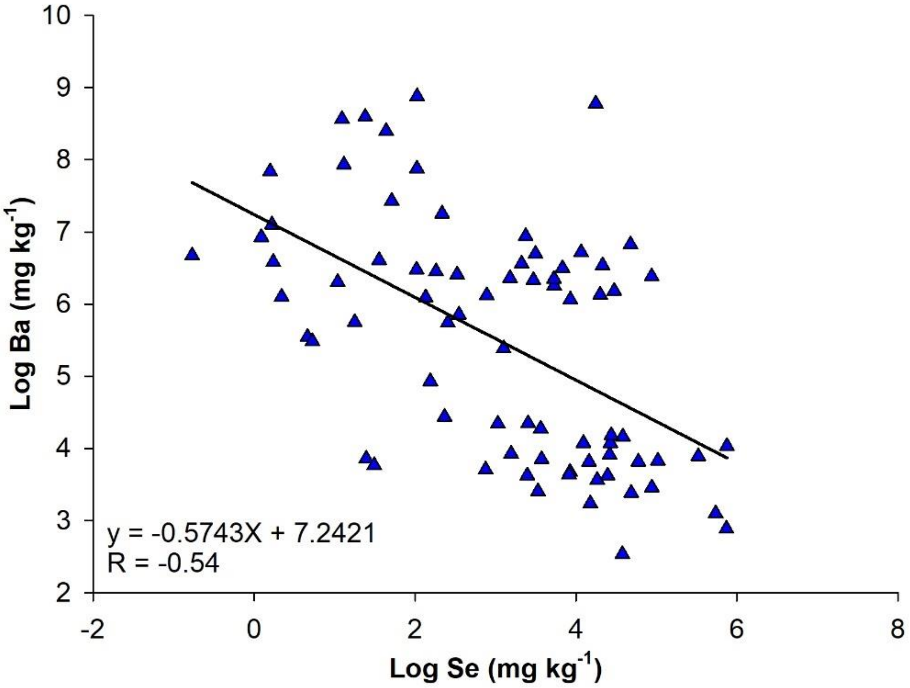

Fig. 2. Pearson correlation between total Se and Ba concentration in Brazil nuts from the 6 sites studied. Data were log-transformed before applying the linear regression model. The information expressed in the graphic represents the equation with correlation coefficient (*r*) and a significant correlation ($p < 0.05$) was obtained.

3.2 XRF 2D mapping with benchtop system

Although Se and Ba concentrations in Brazil nuts are quite high depending on the geographical location where the sample was collected, the µ-XRF 2D maps showed in the present study represent samples collected in natural conditions, mostly in native forest, in soils with no fertilizers or nutrients addition. The investigation of longitudinal and transversal sections of shelled Brazil nuts seeds by µ-XRF showed wide variation in terms of 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**.

Selenium and Ba distribution in Brazil nuts seeds presented different behavior according to the origin (site studied), which reflects in the concentration of the element in the seed. The variations in elemental distribution follow 4 different scenarios according to the concentration:

1) For the site Sena Madureira-Acre (with low Se, < 10 mg kg^{-1}; and high Ba, > 1000 mg kg^{-1}) Se is distributed randomly with a few scattered hot spots filling the seed and presenting similar distribution in the transversal section. Barium, on the other hand, for both longitudinal and transversal sections, presents notable higher intensity at the border of the seed, 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**).

2) For the site Laranjal do Jari-Amapá (with high Se, > 50 mg kg^{-1}; and medium Ba, 100-1000 mg kg^{-1}) Se tends to be more concentrated in the bottom, which happens to be the seed's root apex (radicle), perfectly visible in the longitudinal section. Curiously, the root pole (seed base), from which the primary root originates when the seed germinates, could be identified because it is larger than the stem pole, which originates the aerial part of the plant (Simone & Gurgel, 2006). The reasons why Se is more concentrated in such an important position in this meristematic tissue needs further explanation. 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 procambium ring of the seed. 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 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 mg kg^{-1}; and low Ba, < 50 mg kg^{-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. In the transversal section, the ring shape formed by Se is not regular because of the unusual anatomy of the seed sampled, but still forms a layer in the parenchyma region that is even thicker than the observed in other samples. Barium was not detected by the µ-XRF scanning in the sample from the site Itacoatiara-Amazonas because of the too low concentration for the technique (**Fig. 3**).

4) For the site Caracaraí-Roraima (with medium Se, 10-50 mg kg^{-1}; and medium Ba (100-1000 mg kg^{-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 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 mg kg^{-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 mg kg^{-1}, respectively) was more concentrated in the peripheral region of the seed, forming an almost continuous line in the border of the seed along with the epidermal tissue. In the transversal sections, the pattern of distribution 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, whereas Ba, on the other hand, 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 to 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 serving 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 to plants from herbivores, pathogens, or even toxic substances (Mehdawi & Pilon-Smits, 2012; Huang et al., 2017).

The maps with elemental distribution of S and P are presented in **Fig. S2**. From the observed samples from the sites Laranjal do Jari in longitudinal and transversal sections and Itacoatiara-Amazonas 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 and Sena Madureira-Acre, most maps presented intensities below the calculated threshold (equation 1), and therefore are below the limit of detection of this technique.

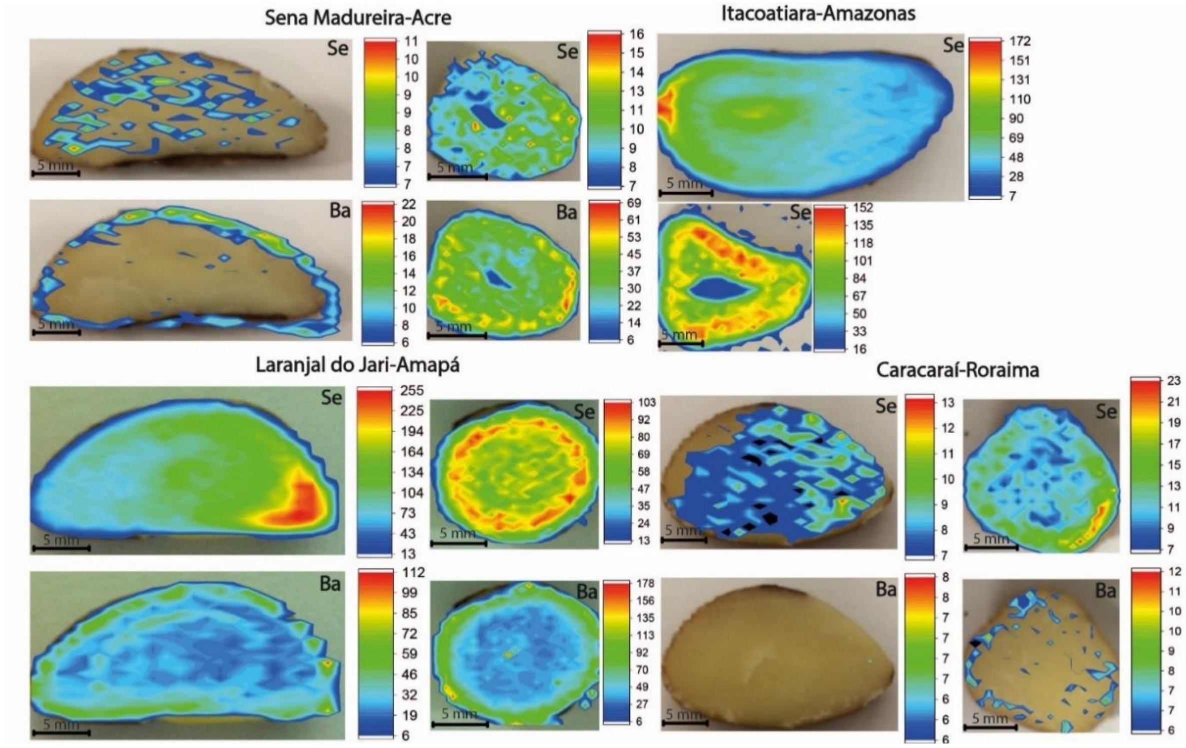

Fig. 3. Selenium and Ba distribution in Brazil nuts seeds samples from the sites Sena Madureira-Acre, Itacoatiara-Amazonas, Laranjal do Jari-Amapá and Caracaraí-Roraima, represented in longitudinal and transversal sections. Selenium and Ba average concentrations are respectively for each site: Sena Madureira-Acre: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

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 4 sites studied, as can be observed by the prominent peaks of Br visible in the mean spectrum of the µ-XRF analyses (**Fig. S1**). The maps show a different distribution behavior for each of the samples assessed and therefore it was not possible to identify a clear pattern of spatial distribution in the samples studied. What could be observed in general 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. 4**). A high Br concentration was reported in Brazil nuts by Furr et al. (1979) with 87 mg kg^{-1} dry weight, besides high levels of Se (11 mg kg^{-1}) and Ba (1764 mg kg^{-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.

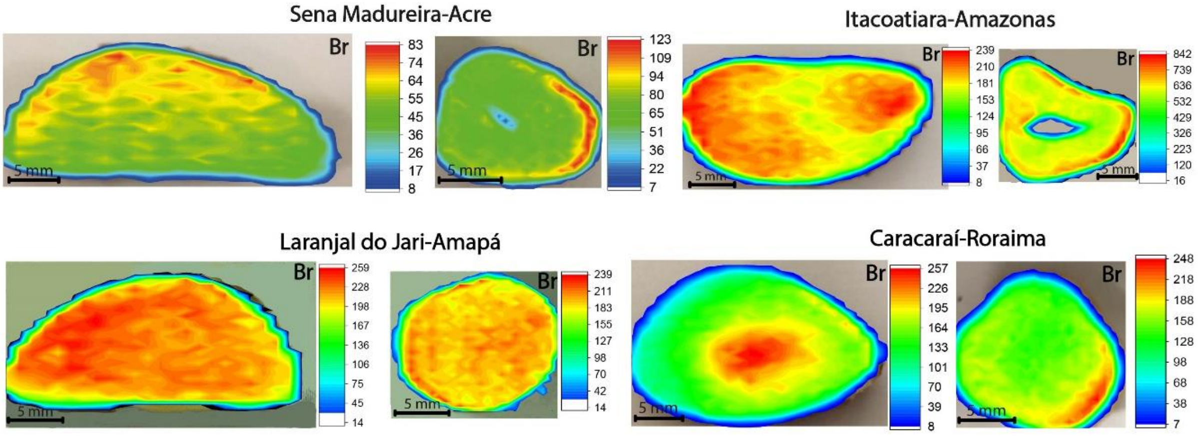

Fig. 4. Bromine (Br) distribution in Brazil nuts seeds samples from the sites Sena Madureira-Acre, Itacoatiara-Amazonas, Laranjal do Jari-Amapá and Caracaraí-Roraima, represented in longitudinal and transversal sections. The temperature bars indicate Br-Kα Net counts obtained by the µ-XRF Orbis PC EDAX.

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, which presented three elements per map and in a bigger scale, visualizing the whole seed. These RGB maps are in a smaller scale aiming 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 would provide important meristematic functions for the differentiation of the plant when it germinates.

Using the same criteria to classify the samples according to their respective Se concentrations, we have them divided also according to the sub-region in which the samples 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 in terms of the colors corresponding to the intensities of S, Ba, and Se from the perisperm layer where S is more concentrated in the procambium and epidermal layers (higher Ba intensity) and outer parenchyma (Se) in the area mapped of 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. 5**). 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 clearer, 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. Interestingly to note that 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 seed (**Fig. 5)**. These findings agree with the results observed by the images of the whole seed (benchtop equipment) (**Fig. 3**).

There is a relationship between Se and Ba in these tissues as the colors are visibly mixed and the transition from one layer to the other is diffuse, particularly in the samples with lower Se concentrations (**Figures 5A** and **5B**), which implies the formation of a less soluble compost such as barium selenate ($BaSeO_4$) at some degree. Similarly, sulfur (thinner green layer), which is in the outer layer of the seed with around 0.2 mm thick, in a tissue constituted by perisperm (lignin and mostly non-living cells), above the layer of Ba, is present forming a darker color, suggesting that there is a certain combination between Ba and S representing an association forming barium sulfate ($BaSO_4$) (**Figures 5A** and **5C**).

Since Ba does not exist in nature in its elemental form but occurs as a divalent cation in combination with other elements, the hypothesis that Ba in Brazil nuts seeds is associated particularly with S and Se is consistent. Based on the concentration of these elements present in the seeds, there is a chance that it would be partially forming barium sulfate and barium selenate, because there is no report confirming cases of toxicity by Ba due to ingestion of Brazil nuts seeds. This statement agrees with the study conducted by Gonçalves et al. (2009) and is made considering that these chemical species formed present low solubility in water, which implies a low Ba bioavailability for human consumption. Thus, it can be inferred that Se and S may play an important role in amending Ba toxicity in Brazil nuts.

For Brazil nuts seed it is still not possible to confirm if Se, which is believed to be accumulated in the parenchyma tissue cells (most of the endosperm), will be more associated with the proteins itself 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 (Moore et al., 2010; Lessa et al., 2019; Rodrigues et al., 2020).

Scatter plots showing correlation among Se, Ba, and S intensities were performed using the PyMca image correlation tool and are presented in **Fig. S3.** This supporting information reveals that Se has a positive correlation with Ba when the first is present in lower intensities, whereas when Se is increased, there is a negative correlation. In other words, it seems that Se has a limit of concentration in which it can be associated with Ba (presumably forming $BaSeO_4$), and after this limit of concentration, increasing Se in the soil-plant system, and therefore, in the nuts, makes total Ba concentrations to decrease. The association between Ba and S in nut seeds demonstrates a better affinity, and the graphs show a linear positive association between the two elements, which supports the hypothesis of the formation of $BaSO_4$, which might be making Ba partially insoluble following the consumption of the nuts. For the relationship between Se and S, the behavior is similar to Se and Ba, with the difference that the affinity between these two elements is even lower than the latter. It was observed that when Se is present in lower concentrations, S will predominate, but in a narrow range limit, and after that, the increase of Se promotes a little decrease in S concentration. Therefore, it seems that the competition between the two elements is only remarkable in a narrow range of low concentration and does not affect their metabolic functions in the Brazil nuts seeds since Se can substitute S in many amino acids such as methionine and cysteine (Trippe & Pilon-Smits, 2021).

In summary, barium selenate ($BaSeO_4$) and barium sulfate ($BaSO_4$) composts formation assumptions in the Brazil nuts studied here are supported by the graphic with inverse concentration (Se x Ba) observed in the boxplots (**Fig. 1**), the negative correlation between Se and Ba in Brazil nuts (**Fig. 2**) and also the graphics containing scatter plots with relationship Ba vs Se vs S in the supplementary material (**Fig. S3**). Theoretically, the combination Se-Ba and S-Ba is partial and based on each particular affinity and location in the seed tissue, therefore still part of Se and Ba will be linked to other elements and composts forming chemical species still soluble/bioavailable.

The germination process of Brazil nuts seeds is particularly very slow and takes 12-18 months on total to occur naturally. Such delay is due to endogenous seed dormancy, which may be attributed to the high levels of Se and Ba contained in the Brazil nuts seed embryo. To prove if this dormancy mechanism exist 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).

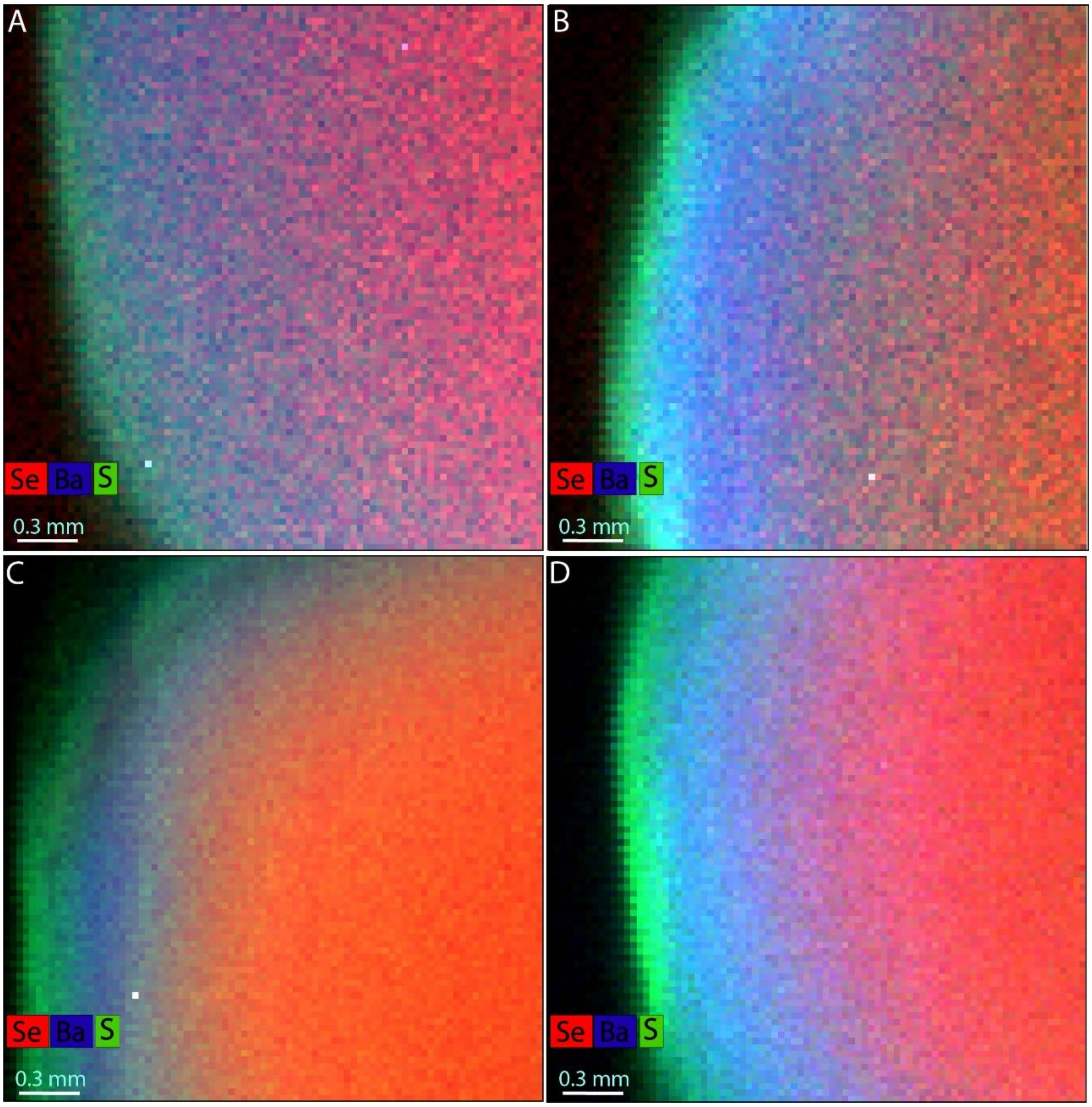

Fig. 5. XRF-Synchrotron analyses. RGB images for selenium, barium and sulfur in the basal region of the Brazil nut seed (longitudinal section). The legends for the sites, Se, Ba and S concentrations are respectively: **A** = Sena-Madureira-Acre (AC9, Se = 1.26, Ba = 2914, S = 533 mg kg^{-1}); **B** = Porto velho-Rondônia (RO14, Se = 6.0, Ba = 511, S = 1268 mg kg^{-1}); **C** = Laranjal do Jari-Amapá (AP13, Se = 314, Ba = 646, S = 1700 mg kg^{-1}); **D** = Santarém-Pará (PA1702, Se = 67.5, Ba = 24.9, S = 1293 mg kg^{-1}).

The XRF images showed in **Fig. 6** 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 that is mostly composed by lignin and dead cells, where those elements coincide with their hotspots. The micronutrients (Mn, Fe, Cu, and Zn) on the other hand, are more homogeneously distributed throughout the bottom of Brazil nut seed mapped. The estimated concentrations via XRF technique are respectively P: 1801 mg kg^{-1}, S: 1270 mg kg^{-1}, K: 3404 mg kg^{-1}, Ca: 2021 mg kg^{-1}, Mn: 4.42 mg kg^{-1}, Fe: 23.7 mg kg^{-1}, Cu: 18.7 mg kg^{-1}, and Zn: 72.8 mg kg^{-1}. Detailed information of the sample RR7 elemental mapping is presented in **Table S2**.

Quantification of major and microelements in Brazil nuts was performed previously by Brito et al. (2019), who reported ranges for K (337 to 2981 mg kg^{-1}), Ca (142 to 3436 mg kg^{-1}), P (64.9 to 6708 mg kg^{-1}), Fe (5.7 to 36.8 mg kg^{-1}), Mn (0.1 to 11.4 mg kg^{-1}), and Zn (3.1 to 48.9 mg kg^{-1}). Lima et al. (2019) measured also S (1.6 to 4.8 g kg^{-1}) and Cu (11.2 to 56 mg kg^{-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 content of these elements may vary according to climate and soil characteristics, which influence nutrients uptake levels by the plant (Cardoso et al., 2017).

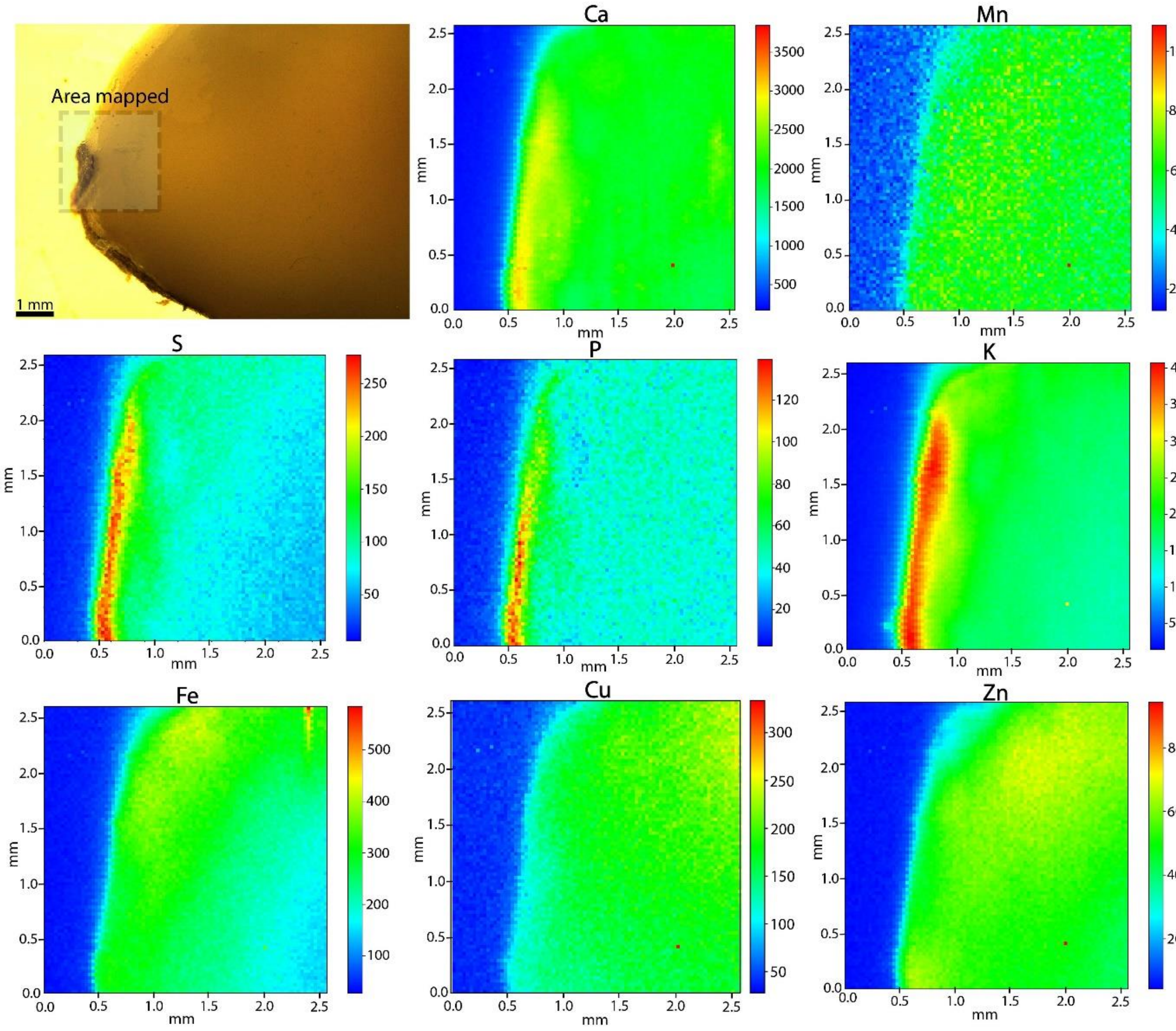

Fig. 6. Images of the mapped region of a Brazil nut sample from Caracaraí-Roraima (RR7) in a small scale (root pole in the bottom extremity of the seed), in longitudinal section with the spatial distribution of chemical elements. The temperature bars indicate net counts of each element obtained by Synchrotron µ-XRF.

4 CONCLUSIONS

Selenium and Ba total concentrations in Brazil nuts can be considerably high depending on the origin site (population), and their spatial distribution in Brazil nuts seeds varies considerably depending on the site where the samples are obtained. The results suggest that concentration is the main factor influencing the behavior of elemental spatial distribution in the seed tissues.

Selenium was found to be accumulated mainly in the outer parenchyma tissue of Brazil nuts seeds forming a "ring shape" surrounding the seed, confirmed by longitudinal and transversal section visualizations in samples with high concentrations. Another accumulation pattern observed was that Se intensity for those high-Se samples presented a hot spot in the bottom extremity, where the root apex is located. 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 spatial location of the elements and known affinity between Se-Ba and S-Ba suggest the formation of $BaSeO_4$ in a lower degree and $BaSO_4$ in a higher degree, supporting the results and hypothesis so far reported by the literature on the formation of these composts of low solubility and resulting presumably in low bioavailability of Ba for human consumption.

The μ-XRF technique in both benchtop and synchrotron approaches agree in terms of results obtained and showed to be successful techniques to mapping the distribution of Se, Ba, and other 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. As highlighted by the present study, the anatomy of *Bertholletia excelsa* seeds needs more comprehensive studies to confirm the patterns of distribution found in the present study and to investigate the possible association between Se, Ba, and S contents in Brazil nuts seeds and the bioaccessibility of these elements in the nuts.

Acknowledgments

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Supplementary material

Table S1. 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

Köppen classification source: Climate-data.org (2020); * Samples collected from clones originated from the State of Pará.

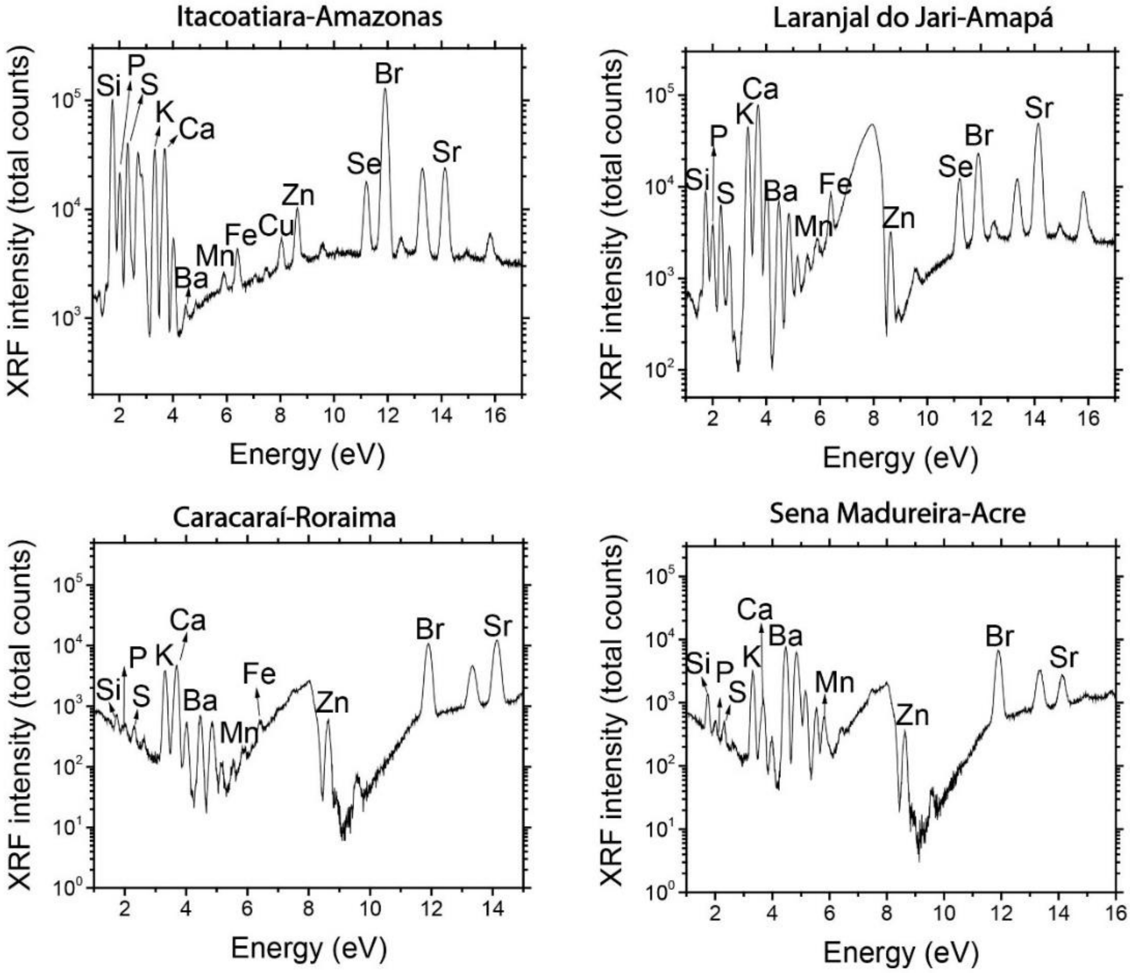

Fig. S1. Mean spectrum obtained by the measurements in the μ-XRF Orbis PC EDAX in each set of Brazil nuts samples from each site showing the fluorescence intensities of major and trace elements.

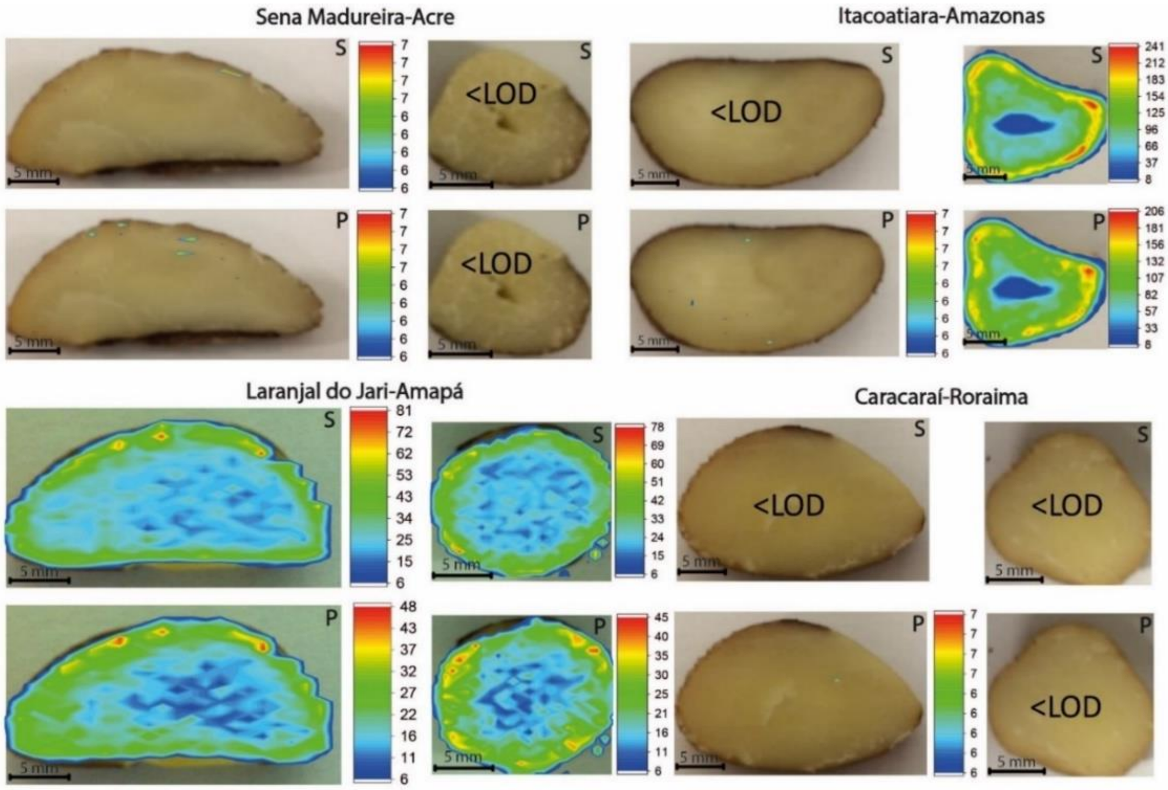

Fig. S2. Sulfur and P distribution in Brazil nuts samples from Caracaraí-Roraima in transversal and longitudinal sections. The temperature bars indicate net counts of each element obtained by μ-XRF Orbis PC EDAX.

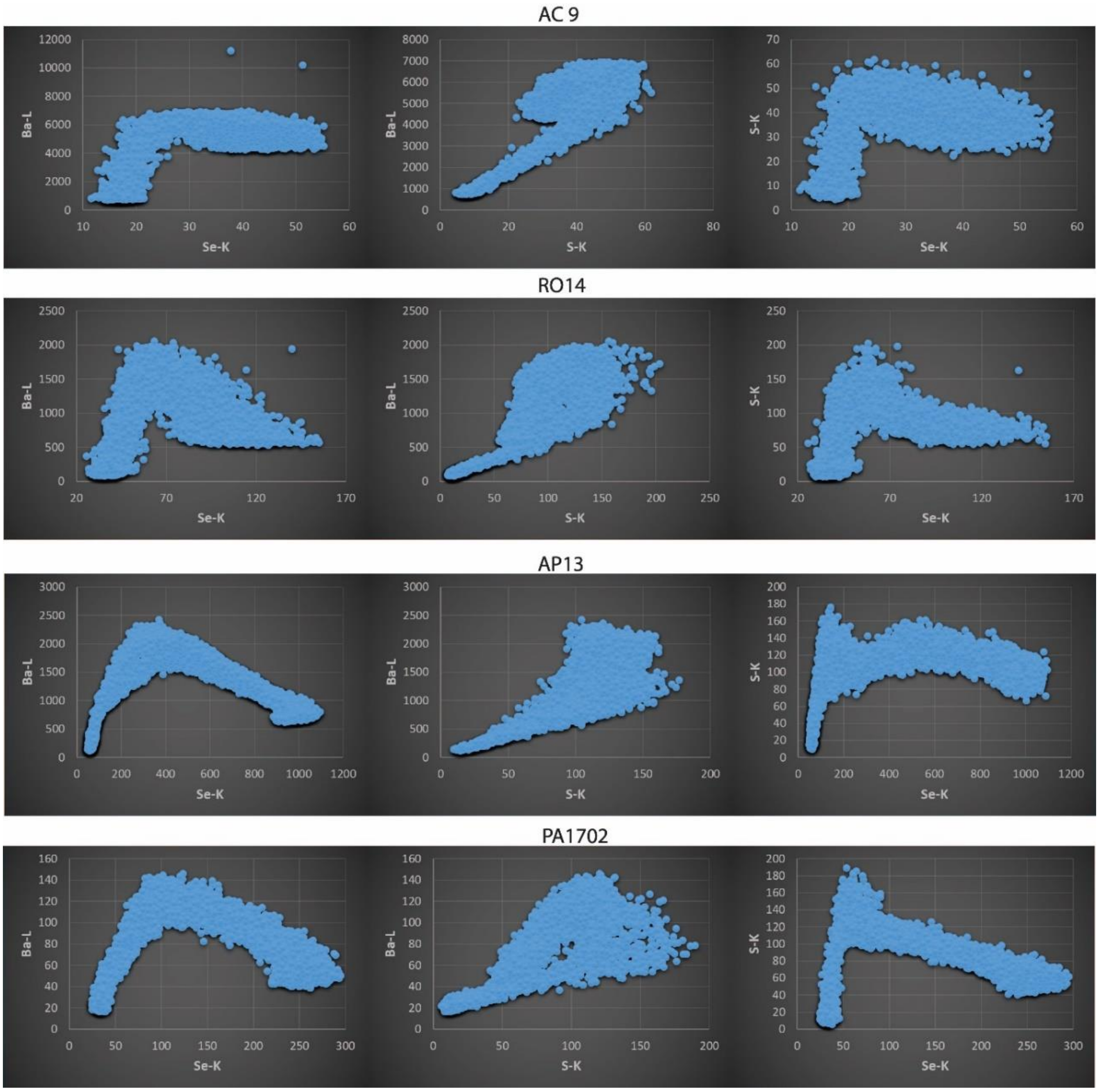

Fig. S3. Scatter plots for Se-K x Ba-L, S-K x Ba-L, and Se-K x S-K for the selected samples collected from sites in Sena Madureira-Acre (AC9), Porto Velho-Rondônia (RO14), Laranjal do Jari-Amapá (AP13), and Santarém-Pará (PA1702). The graphics are related to samples in figure 4. K and L are the shells from the element atom (Se, B, or S) in which the specific X-ray fluorescence intensity was detected after exciting the electrons.

Table S2. Elemental concentration of the sample RR7 mapped area, related to 2D Synchrotron µ-XRF images in figure 5.

Element	Group	Fit area	Sigma area	Concentration (mg kg^{-1})
P	K	2.574227e+01	8.59e+00	1,801
S	K	6.273790e+01	1.07e+01	1,270

K	K	1.846142e+03	5.38e+01	3,404
Ca	Ka	1.759474e+03	4.75e+01	2,021
Mn	K	4.677562e+01	1.07e+01	4.42
Fe	K	2.152966e+02	1.86e+01	23.7
Cu	K	1.161969e+02	1.46e+01	18.7
Zn	K	4.423073e+02	2.54e+01	72.8

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ARTICLE 4 - Occurrence and bioaccessibility of essential and toxic trace elements in Brazil nuts from Amazonian agroecosystems

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Highlights

- Concentrations of As, Cd, Cr, Hg, and Pb in Brazil nuts are below detection limit;
- Average Se and Ba bioaccessibility in Brazil nuts are 50.3% and 3.31% respectively;
- There is wide variation of Se and Ba bioaccessibility among the samples studied;
- Recommendation of 1-3 nuts per day is safe, considering Se and Ba uptake limits.

Abstract

Brazil nuts is the richest food source of selenium (Se) but also known to accumulate high barium (Ba) contents. However, not all Se and Ba are released from the Brazil nut matrix and become available for absorption into the bloodstream upon digestion in the gastrointestinal tract. Therefore, oral bioaccessibility of Se and Ba in Brazil nuts from 8 different sites in the Amazon region was assessed *in vitro*, followed by measurement of total Se, Ba, As, Cd, Cr, Hg, Ni, Fe and Pb concentrations. Results showed that total concentration of the toxic trace elements assessed are under safe levels. The average bioaccessibility in the gastric phase (2 h) and small intestine (4 h) were for Se 18.1% and 50.5% and Ba 54.8% and 3.3%, respectively. Based on the bioaccessible contents, the recommendation of 1-3 nuts per day is safe, considering the uptake limits for Se and Ba.

Keywords: Brazil nuts; selenium; barium; Amazon agroecosystems; oral bioaccessibility.

1 INTRODUCTION

Selenium (Se) is an important micronutrient to health in humans as well as animals. Selenium is incorporated into selenoproteins that have a wide range of pleiotropic effects, ranging from antioxidant and anti-inflammatory effects to the production of active thyroid hormone (Rayman, 2012). In nature, Se is found in soil, air, and water. Typical concentrations of Se in soil range from 0.01 to 2 mg kg^{-1}, with a global average of 0.40 mg kg^{-1} (Fordyce, 2007). In the environment, however, there are concerns about both toxicity and its deficiency, because Se intake is variable across the world, owing to several factors, such as Se content in the soil where crops are grown, Se speciation, soil pH, organic matter content, and the presence of ions that can compete with Se (Winkel et al., 2012). Both deficiency and excess have been associated with adverse health effects that have often been characterized by a Ushaped relationship. Therefore, increased mortality is associated with both low and high Se status (Rayman, 2019).

Barium (Ba) on the other hand, is an omnipresent element, and its reported average soil concentration ranges globally from 362 to 580 mg kg^{-1} (Kabata-Pendias, 2011). When available at high levels in soils, it can cause toxicity to plants and invertebrates (Coscione & Berton, 2009). The toxicity of Ba compounds in the human body depends on their solubility. Acute or chronic exposure to barium salts may result in many disorders, including renal intoxication, hypertension, and cardiac malfunction (Oskarsson & Reeves, 2007).

The trace elements considered contaminants, arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb) are harmful to humans at high concentrations. To date, As is commonly recognized for adversity in human (carcinogenic) and marine animals. Most environmental arsenic contamination originates from anthropogenic activities (Alka et al., 2021). Cadmium is a group IIB element and is characterized as heavy metal. Environmental Cd contamination can be the result of anthropogenic activities or natural changes. Exposure to Cd can be associated with various toxic effects on kidneys, liver, lungs, bones, etc. (Zhang & Reynolds, 2019; Đukić-Ćosić et al., 2020). Chromium (Cr) is a catalytic metal capable of inducing oxidant generation and biomolecular oxidative damage such as lipid oxidation. Chromium exists mostly in two valence states: trivalent and hexavalent, namely Cr(III) and Cr(VI), respectively. Indeed, Cr(III) is an essential trace element for humans and plays a relevant role in glucose and fat metabolism. On the other hand, Cr(VI) species are of great environmental and biological concern due to their recognized toxicological and carcinogenic properties (Jomova & Valko, 2011). Mercury (Hg) is a naturally occurring element that is toxic and bioaccumulative in its elemental and organic forms (i.e. methylmercury). Acute exposures to Hg and its compounds from natural and anthropogenic sources are associated with serious health conditions including adverse renal and cardiovascular impacts, and developmental neurotoxicity (Mohan et al., 2019). Lead (Pb) possesses many useful physical and chemical properties and was among the first metals used by humans. Elemental lead is virtually indestructible. Once in the environment, it is cyclically reintroduced, a process driven by natural and human factors. Lead is also associated with negative health outcomes across most animal species, including humans (Levin et al., 2020).

The trace elements Nickel (Ni) and Iron (Fe) are considered essential and required in low concentrations in the human organism. Although, they have the potential to become toxic at high levels. Nickel is present in the Earth's crust at the level of about 0.009%, existing both in soluble and insoluble compounds in soils, fumes, and waters. Nickel is present in most foods, such as green beans, broccoli, peas, canned vegetables and spaghetti, canned fruit, dried fruit, nuts, cocoa, and chocolate. Despite its poisoning potential, Ni plays fundamental biological roles for plants, bacteria, archaea, and unicellular eukaryotes, making it a double-faced element for life (Zambelli et al., 2016). Iron is a metal highly abundant in the earth's crust and essential for human life. By the end of the nineteenth century, it was acknowledged as a micronutrient needed for recovery from anemia. Quantitatively, the main biological function of Fe is oxygen transport, but small quantities of Fe participate in > 200 enzymatic systems that are essential for cellular functions (Blanco-rojo & Vaquero, 2019).

Bertholletia excelsa, popularly known as Brazil nut, is a tree species widely distributed in upland forests in the Brazilian Amazon rainforest. It occurs in different countries throughout the region, including Brazil, Bolivia, Peru, Colombia, Venezuela, and Guyana (Baldoni et al., 2020). It is an extremely valuable forest commodity that critically supports local communities' livelihoods and regional economies through the thriving market for exportation (Bongiolo et al., 2020). Brazil nuts fruits are capsular, possessing a thick, woody pericarp and containing around 10-25 edible seeds inside, each of around 5x2 cm size with a distinct triangular shape (Mori & Prance, 1990). The species is found in areas with average annual rainfall between 1400 and 2800 mm, a mean annual temperature between 24 °C and 27 °C and relative humidity between 79% and 86% (Diniz & Bastos, 1974).

Brazil nuts are consumed worldwide, being known for their nutritional composition, health benefits and as an effective way to improve Se status (Cominetti et al., 2012; Stockler-Pinto et al., 2015; Cardoso et al., 2017) since it is considered as the richest food source of Se. In the literature, concentrations of up to 512 mg Se kg^{-1} have been reported (Chang et al., 1995; Dumont et al., 2006, Silva Junior et al., 2017). Surprisingly, the highest levels of Ba in foods so far reported are also found in Brazil nuts seeds, with values ranging from 190 to 5890 mg kg^{-1}, which depends on the growth site (Smith, 1971; Kabata-Pendias & Mukherjee, 2007). Particularly in the Amazon region, profuse amounts of a psilomelane (manganese oxides) group of minerals called hollandite ($Ba_2Mn_8O_{16}$), found in the natural environment of the Brazil nut trees (Amazon rainforest) and containing around 130 mg g^{-1} of Ba, are probably the source of Ba that comes from the soil to the edible nuts (Mazokopakis & Liontiris, 2018).

It is known from the literature that there is a wide variation in the contents of Se and Ba in Brazil nuts, depending on the place where they were grown, and there are even variations among nuts coming from the same place, presumably reflecting the concentrations of Se and Ba in regional soils and the ability of each Brazil nut genotype to accumulate this element in the nuts. Such variations were reported by Chang et al. (1995), Dumont et al. (2006) and Silva Júnior et al. (2017) for Se, by Gonçalves et al. (2009) for Ba, and Parekh et al. (2008) reported a wide variability for both elements.

It is known that the total level of a nutrient or contaminant in a portion of food that is eaten may be quite different from the level effectively absorbed by humans. The bioaccessible fraction of an element can be defined as the part of the ingested nutrient or contaminant that is released from the food matrix in the lumen of the intestine, and that can thereby possibly be absorbed through the intestinal mucosa (Thiry et al., 2013; Sun et al., 2017). For such assessment, there are two main methodological approaches for assessing oral bioaccessibility: *in vitro* and *in vivo* studies. The *in vivo* approach is very time demanding, requires a painstaking planning and specific resources for adequate experimental control, and has some analytical and ethical constraints. On the other hand, the *in vitro* methods in their huge variety offer a wider experimental scope, which may be useful in providing deeper insight into the phenomena underlying bioaccessibility (Cardoso et al., 2015).

The results obtained by bioaccessibility estimation provide an accurate assessment of the risks and benefits associated with food consumption in a new era in the field of human nutrition. This simplification can be used as a worst-case scenario for contaminants since it maximizes the number of absorbed contaminants. Also, the measurement of bioaccessibility provides valuable information to select the appropriate dosage and source of food matrices to ensure the nutritional efficacy of food products (Fernández-garcía et al., 2009).

So far, there are no studies on the bioaccessibility of Se and Ba in Brazil nuts in a wide scale covering different sites in a regional campaign. Therefore, many uncertainties regarding how much these elements can be assimilated by the human gastrointestinal system and the possible factors that determine their higher or lower accessibility for absorption by the human body remains uncertain. In this context, information about the oral bioaccessibility *in vitro* for Se and Ba becomes essential, as a first step before *in vivo* approaches studying bioavailability since the fractions and their potential beneficial or harmful effect on the human organism are not yet known.

The objectives of the present study were: a) Measure the total concentration of a series of relevant essential and toxic trace elements in Brazil nuts; b) Correlate Se and Ba concentration in Brazil nuts with relevant soil physicochemical attributes; c) Evaluate the oral bioaccessible fraction of Se and Ba in Brazil nut samples from different plant populations in the Amazon region through simulated gastrointestinal *in vitro* digestion and d) Estimate the average amount of nuts that can be ingested daily per adult person for optimal supplementation with Se and having safe ingestion below the uptake limit for both Se and Ba.

2 MATERIALS AND METHODS

2.1 Characterization of the study area and Brazil nuts sampling

This study involved a major survey collecting Brazil nuts samples from different plant populations in native areas and a local cultivated farm in the Amazon region. Eight target sites were selected to better understand the natural variation in concentrations of Se and Ba as well as the bioaccessible fractions of these elements.

The chosen target sites for this study were the same ones as those used by Silva Junior et al. (2019) in their survey assessing the natural variation of arsenic fractions in soils of the Brazilian Amazon with exception of the site from the state of Mato Grosso (MT) because samples collected on that site were already deteriorated and discarded. Each site corresponds to a Brazil nut stand (forested area or plantation), identified by: AC:SM (Sena Madureira, state of Acre); AC:XP (Xapuri, state of Acre); AM:AN (Anori, state of Amazonas); AM:AR (Aruanã farm-Itacoatiara, state of Amazonas); AP (Laranjal do Jari, state of Amapá); PA (Santarém, state of Pará); RO (Porto Velho, state of Rondônia) and RR (Caracaraí, state of Roraima). Aiming to better understand the position of each site in the Amazon region, they were grouped taking the Amazon river mainstream as a reference, being: Northern Amazon: RR and AP; Southern Amazon: RO; Western Amazon: AC:SM and AC:XP; and Central Amazon: AM:AN, AM:AR and PA.

On each site, the original sampling campaign consisted of 15 collection points that were sampled in the native Amazon rainforest, except for the site AM:AR (Itacoatiara), where samples of 18 points were collected in the Aruanã farm, a big Brazil nut plantation. Samples were collected between the harvest seasons of 2014 and 2017, from January to February.

Information concerning geographic coordinates, sampling points, and climatic conditions are provided in **Table S1**.

Brazil nuts were sampled from below the tree canopy, collecting ten ripe fruits from each plant, which naturally fall from the trees after maturation. To collect the Brazil nuts seeds, these fruits were opened and a total of 50 seeds (unshelled nuts) were randomly selected from each plant and then were put in a plastic bag. The method for sampling below the trees in the sites is represented schematically by Silva Junior et al. (2017).

2.2 Study design

The study involved 2 main phases: **a**) General analysis of total concentrations of relevant trace elements, i.e. Se, Ba, As, Cd, Cr, Fe, Hg, Ni, and Pb in Brazil nuts selected from the 8 sites and 45 different plant materials, with analysis being conducted in duplicate for each material (total of 90 samples for digestion and ICP-MS analyses). The number of samples selected for each site were as follows: AC:SM (4), AC:XP (3), AM:AR (4), AM:AN (3), RR (12), AP (4), RO (7), PA (8). **b**) After measuring total concentrations in the samples, materials were selected for the *in vitro* assessment of Se and Ba oral bioaccessibility based on the following criteria: For each site, materials with lowest, highest and median total Se concentration were selected, therefore having 3 materials for each site (total = 24). For the samples from sites AC:SM, AC:XP, AM:AR, AM:AN and AP there was a screening of previously published data (Silva Junior et al., 2017).

2.3 Total concentration for multielement analysis

From the 50 nuts collected from each plant, 3 nuts were randomly chosen and dried in an oven at 60 °C until reaching constant weight (after 72 hours). Initial and final weights were recorded for all samples to keep drying until constant weight. After peeling, the samples' weights were recorded again in order to calculate the average weight for samples and sites, then the samples were ground using a pestle and mortar.

For the digestion procedure, first 0.2 g of the previously dried and grinded Brazil nuts seeds were weighted in duplicate. The material was put in Teflon tubes and 10 mL of Pico-Pure 65% HNO_3 (Chem-Lab, Belgium) were added to each tube. The tubes were left with the extracts resting for 30 min. Afterward, the tubes were placed in a sonicator (Sonorex RK103H, Germany) with distilled water for 30 min at 50 °C to guarantee a complete mixing. Then, the samples went for the digestion run in the microwave oven (Mars 6, CEM, US) that takes ~ 1 h. After digestion is finished the extracts were put in falcon tubes (50 mL) and diluted/filled with Milli-Q water. HNO_3 2% and Milli-Q water were used to rinse the digestion tubes into the falcon tubes up to 50 mL. From the falcon tubes, the extracts are ready to be analyzed by ICP-MS. Depending on the concentration expected, it was necessary to dilute with 13% HNO_3. Both standard addition and external calibration were used during ICP-MS measurement.

For quality assurance and control, a selection of standard reference materials was analyzed, including materials from the Institute for Reference Materials and Measurements (White Clover - BCR 402, IRMM, Geel, Belgium), from the National Institute of Standards and Technology (Peach Leaves - SRM 1547, NIST), Rye Grass (ERM-CD281) and also Sea Lettuce (BCR-279). The mean recovery rate value obtained for the standard materials was satisfactory which confirmed the reliability, except for Hg that despite an acceptable average recovery, presented a high standard deviation. The recovery rates for the CRM’s are presented for each element in **Table S2** of the supplementary material.

The limit of detection (LOD) and method detection limit (MDL) were determined according to Equations (1) and (2) in which S_{blank} was determined as the standard deviation of 10 blank measurements and *a* the sensitivity from calibration:

$$\mathrm{LOD} = 3 \times (S_{\mathrm{blank}} \div a) \qquad (1)$$

$$\mathrm{MDL} = 3 \times (S_{\mathrm{blank}} \div a) \times \text{final volume} \div 0.2 \qquad (2)$$

The results for the LOD and MDL for each element are as follows (µg kg^{-1}): Se (0.100, 25), Ba (0.250, 62.5), As (0.063, 15.8), Cd (0.007, 1.8), Cr (0.200, 50), Fe (1.42, 354), Hg (0.046, 11.5), Ni (0.120, 30), Pb (0.032, 8).

2.4 *In vitro* bioaccessibility experiment

In the present study, the biochemical reactivity found in the human gastrointestinal tract (oral cavity, gastric environment, and intestinal lumen) was sequentially simulated, with sampling performed in the gastric and small intestine phases. The bioaccessibility experiment was performed based on a standardized static *in vitro* method suitable for food, which is an international consensus method developed by Minekus et al. (2014).

Previous to the incubation day, the main solutions (inorganic salts) were prepared in advance and autoclaved, cooled down and stored at room temperature until use for incubation in the next morning. Composition of the simulated saliva fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) are presented in **Table S3**.

For the incubation, each material that was previously grinded (according to section 2.3) and weighted (~5 g) into falcon tubes of 50 mL in triplicate. Then, the saliva medium (SSF) was shaken very well and amylase + $CaCl_2$ were added freshly. The pH of the solution was adjusted to 7 with 1 M HCl and the solution was prewarmed at 37 °C. Then, 10 mL of the prepared SSF solution was added to the falcon tubes and mixed with the 5 g sample (food/saliva ratio 1:2). The mixture was agitated manually a little after finishing the preparation.

In the meantime, the stomach fluid (SGF) was prepared: pepsin + calcium chloride was added and the pH was adjusted to 2 with 5 M HCl. Stomach fluid was added in a 1:1

ratio, i.e. 10 mL saliva mixture + 10 mL stomach fluid. Then, the sample was incubated in the stomach medium for 2 h at 150 rpm at 37 °C on an orbital shaker (KS 4000 i control, IKA, Staufen, Germany). In this step, before putting samples on the shaker, pH was measured and controlled. After 1 h of shaking, the pH of the mixture was checked again, and modified if needed to be 2.0 ± 0.1 for all extracts. After finishing (2 h), 5 mL of the extract from the gastric phase was collected from each sample for analysis.

Finally, the pancreatic juice, here referred to as simulated intestinal fluid (SIF), was prepared: the pH of the original solution was adjusted to pH 7 with 1 M NaOH/5 M HCl after adding the enzymes. Then, the same sample extracts obtained from the gastric phase (GP) were mixed with 15 mL pancreatic juice (1:1 ratio). Subsequently, the pH was adjusted to 7 again, followed by incubation for 2 h while shaking at 150 rpm and 37 °C. The small intestine phase was sampled 2 times (T1 and T2), after 2 and 4 h of incubation respectively, and is referred to in the results as SI-T1 and SI-T2. After finishing each time point, 5 mL was collected from the extract and poured into a small centrifuge tube (10 mL).

After that, the extracts collected in each phase were centrifuged at 10.000 rpm for 15 minutes. To separate the soluble bioaccessible phase and the lipid fraction, two centrifugation steps were needed, since the Brazil nuts formed 3 layers of material after centrifugation: pellets in the bottom, bioaccessible fraction in the middle, and lipid phase in the top, mixed with particulate light organic material (residues) (**Fig. S2**). After the first centrifugation step, the supernatant containing the bioaccessible (solution) fraction plus lipid fraction were separated from the pellet in the bottom and poured into another centrifuge tube. Then, after the second centrifugation step the bioaccessible fraction that remains in the bottom was collected with a pipette, and for the tubes containing the SI-T2 the lipid fraction left was separated from the particulate material using a 0.45 µm syringe filter (Millex syringe driven filter unit, Millipore, Cork-Ireland).

Before analysis, all the solutions were digested in a microwave oven in closed vessels to destroy the remaining material and proceed to analysis on the ICP-MS. Each of the 3 phases is referred to as Gastric phase (GP), small intestine phase after 2 h (SI-T1), small intestine phase after 4 h (SI-T2), and the lipid fraction, which was extracted from the SI-T2 and discussed separately from the 3 gastrointestinal phases since it is a fraction that cannot be considered bioaccessible yet.

The relative bioaccessible fraction of Se and Ba was calculated for each digestion phase as:

$$\%\ \text{Bioaccessibility} = \frac{\text{Concentration in collected bioaccessible solution (mg/kg)}}{\text{Total concentration in the Brazil nut sample (mg/kg)}}\ \text{x}100$$

To obtain the concentration of Se or Ba in the collected bioaccessible solution (mg kg^{-1}) it was necessary to consider dilution by the incubation in the gastric and small intestine phases (incubation solution/sample weight), the supernatant ratio (bioaccessible solution/full extract) after centrifugation, and dilution in the microwave digestion process (final volume extract/sample weight).

For the relative Se and Ba content in the lipid fraction, a similar formula was used:

$$\%\ \text{Content} = \frac{\text{Concentration in collected lipid fraction (mg/kg)}}{\text{Total concentration in the Brazil nut sample (mg/kg)}}\ \text{x}100$$

Similarly to the previous formula, to obtain the Se/Ba content in collected lipid fraction (mg/kg) it was necessary to consider dilution by the incubation in the small intestine phase (SI-T2) (incubation solution/sample weight), the lipid ratio (lipid/full extract) after centrifugation and dilution in the microwave digestion process (final volume extract/sample weight).

2.5 Analytical determinations of Se, Ba and other trace elements

The determination analyses of Se, As, Cd, Cr, Fe, Hg, Ni and Pb in extracts for total analysis and the oral bioaccessibility assessments were performed by inductively coupled plasma-mass spectrometry (ICP- MS, NexION^{TM} 350D, Perkin Elmer, USA). For Ba, the determination analysis of the total and bioaccessible extractions was performed by Inductive coupled plasma-optical emission spectrometry (ICP-OES, VARIAN Vista MPX with SPS5).

Data for total concentrations in Brazil nuts are reported on a dry weight basis (DW). Results for Se, Ba, Fe and Ni are expressed in mg kg^{-1} and data for As, Cd, Cr, Hg, and Pb are expressed in µg kg^{-1}.

2.6 Statistical analysis

A descriptive statistical analysis for the total concentrations of the trace elements and their bioaccessible contents in the Brazil nuts selected for the bioaccessibility experiment was performed. Pearson correlation analysis was performed between Se and Ba concentration in Brazil nuts versus several other soil physicochemical attributes in the 0-20 cm layer for the sites studied. The data for Se and Ba bioaccessibility in each of the 3 simulated gastrointestinal phases (GP, SI-T1, and SI-T2) were compared after verifying the significance through ANOVA using Tukey's HSD test with the package emmeans v 1.4.4 (Lenth, 2019) in R 3.6.2 (R Development Core Team, 2020). Logarithmic transformation was performed for Ba bioaccessibility to meet statistical requirements for normality and homoscedasticity before ANOVA. The bar plots were performed with SigmaPlot version 14 (Systat Software, Inc., San Jose, CA, USA) and box plots with R 3.6.2 (R Development Core Team, 2020).

3 RESULTS AND DISCUSSION

3.1 Total trace elements concentrations in Brazil nuts

Concentrations of potentially harmful trace elements such as As, Ba, Cd, Cr, Hg and Pb and other essential elements such as Se, Fe and Ni in different Brazil nut populations of the Amazon are reported here to present the general status of these elements and compare their levels among different sites in the Amazon.

The levels of As, Cd, Cr, Hg and Pb were below the detection limit for the nuts sampled in all sites studied (**Table 1**). Despite the values for Brazil nuts were below the limit of detection, high levels of total and available As were found in soils from the sites AC:SM and AC:XP, in the Amazon (Silva Júnior et al., 2019). But similarly to the present study, Kluczkovski et al. (2020) did not detect As, Cr, Hg and Pb in Brazil nuts when assessed with ICP-MS.

The average level of Cr in Brazil nuts in the study performed by Welna et al. (2008) was 7.89 ± 1.06 mg kg^{-1}. For Cd, values were below the limit of detection just like in the present study, therefore it is reasonable to say that Brazil nuts plants are not prone to accumulate Cd in the edible nuts.

Studies also reported high levels of Hg in rivers and lake water, soils and bulk precipitation in the Amazon several years ago (Fadini & Jardim, 2001) as well as the high daily intake of Hg previously shown to result in high levels in human populations of the Brazilian Amazon (Passos et al., 2008). Yet, the presented results being below the limit of detection demonstrate that either Brazil nuts in the sites sampled are not under soils with high levels of Hg or Brazil nut trees specifically do not efficiently translocate Hg to the edible nuts. In the study performed by Welna et al. (2008), the levels reported for Hg in Brazil nuts were also below the limit of detection.

For Fe, the concentrations obtained ranged from 15.78 to 35.72 mg kg^{-1}. The lowest average concentration was observed for AM:AN and the highest was found on the site PA.

Cardoso et al. (2017) reported a much wider range for Fe in Brazil nuts (< 1.0-74 mg kg^{-1}) but a similar average concentration compared with the present investigation (25 mg kg^{-1}).

Nickel levels ranged from 0.39 to 8.89 mg kg^{-1} with the lowest average observed for the site PA and highest for AC:XP (**Table 1**). Lima et al. (2019) reported a quite similar range for Ni obtained from 26 commercially available Brazil nuts purchased from a U.S.A. website specialized in nuts and also from a big supermarket franchise, all imported from Brazil (0.9-6.2 mg kg^{-1}). Such high variation in Ni contents in Brazil nuts from Amazon may be explained by the comparatively high variation in the Ni contents in soils of the region as reported by Souza et al. (2018), in which the average concentration in sites studied was 19.6 mg kg^{-1} and the coefficient of variation was 50%. High Ni concentration in nuts is something typically observed and may be related to the fact that Ni is involved in nitrogen metabolism and as a structural compost of essential enzymes i.e. urease and hydrogenases and aminoacids as suggested by Brown et al. (1990) and Lavres et al. (2016). In Europe, for example, for the Flemish region of Belgium, the Ni content investigated in different nuts obtained from local markets, Babaahmadifooladi et al. (2020) reported values ranging from 577 to 1092 µg kg^{-1} for almonds, from 1196 to 3846 µg kg^{-1} for hazelnuts, from 406 to 1740 µg kg^{-1} for pistachios and from 721 to 4624 µg kg^{-1} (DW) for walnuts.

Since there is an overall concern about the levels of Ni present in nuts, **Table 2** presents the results for individual Ni content in Brazil nuts from selected samples in the Amazon and provides information on the contribution of nuts to dietary Ni intake. Notably, the wide variation among and within the sites studied, considering that the average weight of Brazil nuts seed also varies greatly among these sites. The average individual contents ranged from 3.30 µg (PA) to 19.6 µg (AC:XP). Taking the recommendation of 1 nut per day, these levels would be still below the lowest $BMDL_{10}$ (benchmark dose at limit 10 % extra risk) of 1.1 µg Ni kg^{-1} b.w. (77 µg considering a body weight of a person with 70 kg) (EFSA, 2015).

But the ingestion cannot exceed 23 nuts per day considering the average individual Ni content for the samples from the site PA and ~4 nuts per day for the samples from the site AC:XP.

Total Se and Ba concentration in the Brazil nuts samples selected for the bioaccessibility experiment are presented in **Fig. 1**, which are the 24 samples selected out of the 45 materials assessed previously in the first screening (**Fig. S1**) for total analysis and chosen according to criteria described in the Material and Methods section. Selenium concentrations ranged from 0.31 to 370 mg kg^{-1} (average = 51.1 mg kg^{-1}) and Ba levels ranged from 14.7 to 10394 mg kg^{-1} (average = 2153 mg kg^{-1}).

The samples with respectively lowest and highest Ba concentrations are PA1705 and XP16, and for Se, they are XP26 and PA1705 (**Fig. 1**). Remarkably, the sample with the highest Se is also the one with the lowest Ba concentration (PA1705), which confirms the inverse relationship between the two elements as demonstrated in **Fig. S3** of the supplementary material and unpublished data by Silva Junior et a. (2020) presented in the previous chapter. Briefly, we assume that if there is too much Ba available in the soil it may lead to the formation of $BaSeO_4$, which is less mobile and soluble in soil. Then, the remaining free Se anions absorbed and translocated for the edible nuts will be much less when compared with the absorption in sites and by plants in which there is less Ba available in the soil relative to Se.

A notable unusual aspect in the Brazil nuts fruits (pods) was observed visually as a substance excreted from outside the woody capsule that involves the nuts with a shape of dark brown rounded nodules and gelatine-like consistency (**Fig. S4**). Such characteristic was only observed in samples from the site AC:XP with a great frequency in the plants sampled from this local, which is also the site with samples containing the highest Ba concentration (> 10000 mg kg^{-1}). It is still too early to confirm that such atypical behavior is caused by the excess of Ba in soils and accumulated by the Brazil nuts tree, but further investigation must be done in order to prove this hypothesis.

The wide variation for both total Se and Ba concentrations in Brazil nuts from the Amazon region (3 orders of magnitude) is similarly reported in previous studies (Parekh et al., 2008; Silva Junior et al., 2017). In the present work, we are aiming at observing the relative bioaccessibility for these samples and confirm if bioaccessibility follows a similar trend.

Table 1. Descriptive statistics for multielement analysis in Brazil nuts from the 8 target sites studied in the Amazon region (average ± SD).

Site	Se (mg kg^{-1})	Ba (mg kg^{-1})	As (µg kg^{-1})	Cd (µg kg^{-1})	Cr (µg kg^{-1})	Fe (mg kg^{-1})	Hg (µg kg^{-1})	Ni (mg kg^{-1})	Pb (µg kg^{-1})
AC:SM	42.5±52	7145±1763	<15.8	<1.8	<50	24.5±3.0	<11.5	4.71±1.15	<8.0
AC:XP	9.51±11.9	6404±4158	<15.8	<1.8	<50	24.7±0.62	<11.5	6.64± 3.86	<8.0
AM:AR	136±71.6	31.7±3.75	<15.8	<1.8	<50	24.6± 4.33	<11.5	1.19±0.17	<8.0
AM:AN	21.5±19	3420±1178	<15.8	<1.8	<50	19.8± 0.3	<11.5	4.02±0.56	<8.0
RR	31.3±19	753±259	<15.8	<1.8	<50	24.7± 3.45	<11.5	3.40±0.76	<8.0
AP	54.3±11.4	289±81.3	<15.8	<1.8	<50	25.0±3.8	<11.5	4.94±1.58	<8.0
RO	15.5±7.18	185±165	<15.8	<1.8	<50	24.4±6.4	<11.5	2.11±1.02	<8.0
PA	239±131	34.2±22.2	<15.8	<1.8	<50	26.3±4.9	<11.5	1.09±0.39	<8.0

Table 2. Average individual content of nickel (Ni) in Brazil nuts from different sites in the Amazon.

Site	n	Ni content (µg) ± SD
AC:SM	4	13.2 ± 2.16
AC:XP	3	19.6 ± 11.8
AM:AR	4	3.56 ± 0.49
AM:AN	3	16.2 ± 2 .06
RR	12	11.0 ± 2.89
AP	4	14.6 ± 3.27
RO	7	8.33 ± 5.35
PA	7	3.30 ± 1.30

Values for Ni content are presented as average ± standard deviation.

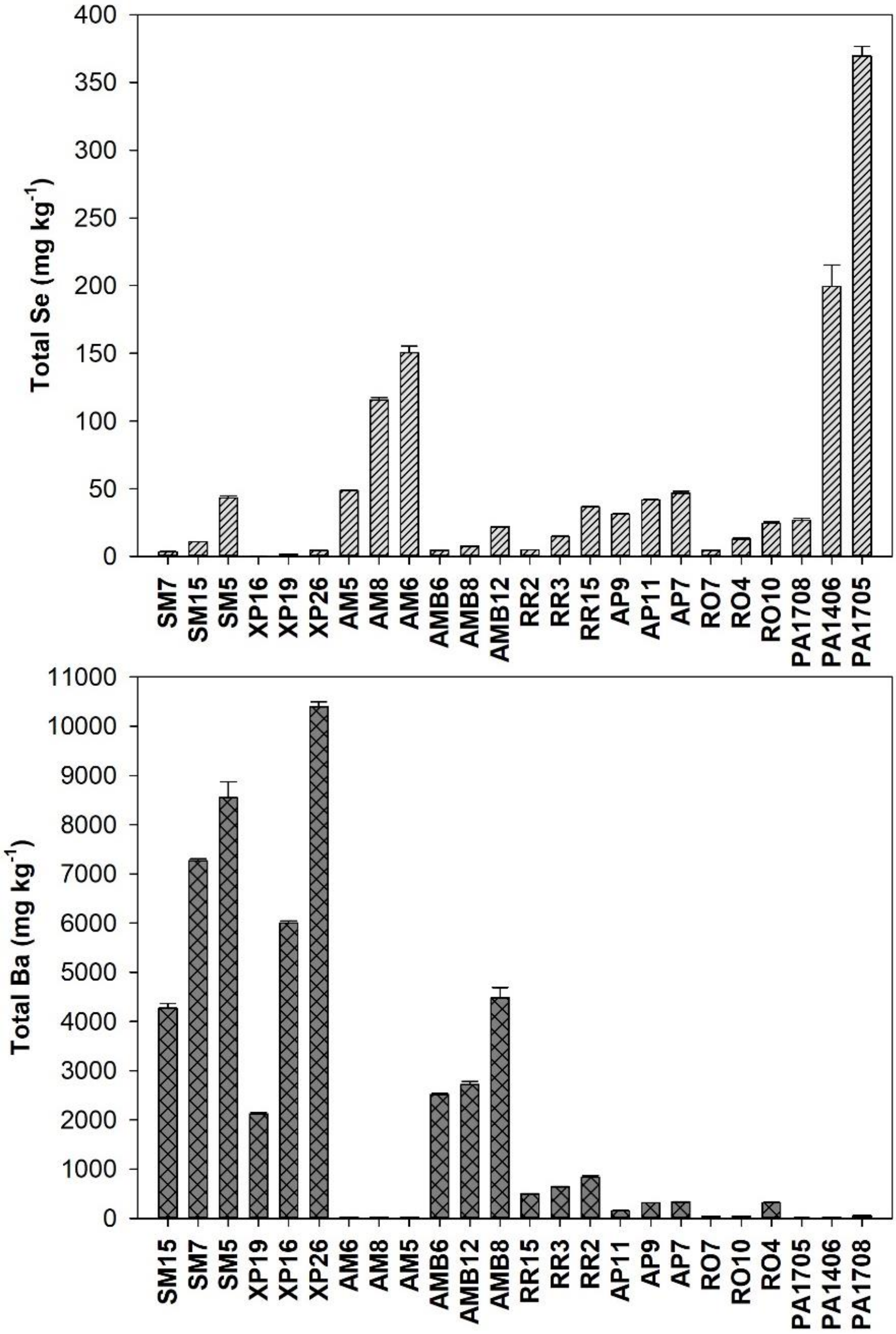

Fig. 1. Total Se and Ba concentration in Brazil nuts for the material selected (24 samples from 8 sites) for the bioaccessibility experiment. The prefix representing each site are described as: SM: Sena Madureira-Acre, XP: Xapuri-Acre, AM: Aruanã-Amazonas, AMB: Anori-Amazonas, RR: Caracaraí-Roraima, AP: Laranjal do Jari-Amapá, RO: Porto Velho-Rondônia and PA: Santarém-Pará. Error bars represent standard error of the mean for the sample replicates of the laboratory analysis (SEM, n=2).

3.2 Relationship between Se, Ba in Brazil nuts and soil physicochemical attributes

Pearson correlation analysis was performed using dataset of Se and Ba in Brazil nuts, Se and Ba in soils in the layer 0-20 cm and 21 other soil physicochemical attributes in the same soil layer. Only the significant relationship ($p < 0.05$) was selected and showed in **Table 3**. Thus, Se and Ba in Brazil nuts were considered the main variables of interest for the present discussion.

For Se in Brazil nuts, only Se in soil (0-20 cm), K and clay presented significant correlation (r = 0.44, -0.34, and 0.33 respectively). The positive correlation between Se concentration in soil and Se concentration in Brazil nuts is explained by the obvious pathway of translocation and accumulation of this element through the soil-plant system, which is also affected by several other soil attributes besides texture (as we found clay being positively correlated as well). The reason for the negative correlation between Se in Brazil nuts and K is indirectly related to the availability of Se in soil and is consistent with the findings reported by Li (2020) who explained that the reason is because K is concentrated mainly in K-feldspar or muscovite, which are characterized by poor Se sorption. Presumably, the same soils which are rich in K in the Amazon, are also not able to retain high Se concentrations and therefore less Se will be available for plant uptake in these soils (**Table 3**).

For Ba in Brazil nuts, were found a total of 11 significant correlations. It was positively correlated to Ba in soil (0-20 cm), K, P, Al, t (exchange capacity in natural pH), m (aluminum saturation) and silt (with r being 0.82, 0.55, 0.64, 0.47, 0.43, 0.37 and 0.42 respectively). The high correlation coefficient found for Ba in soil x Ba in Brazil nuts indicates that the increase of Ba concentration in Brazil nuts is strongly controlled by the total concentration of the element in soils of the Amazon. The fact that t value is also positively related to Ba concentration in Brazil nuts is explained by the higher availability of this element whenever the negative charges increase in soil and can retain more the cation Ba^{2+}. Another interesting relationship is with silt in soil, which is usually present in higher percentage in soils in a low weathering phase such as those in the state of Acre, which also coincides with the presence of 2:1 clay minerals (Bernini et al., 2013). Barium concentration in Brazil nuts was negatively correlated to Se in soil (0-20 cm), OM, S, and clay (-0.61, -0.38, -0.33, and -0.57 respectively). The negative correlation between Ba in Brazil nuts and Se in the soil might be explained by the formation of $BaSeO_4$, which is barely soluble (Fordyce, 2013), and therefore, the higher the Se content in the soil, the lower will be Ba uptake and accumulation in the edible nuts. Similarly, the negative correlation Between Ba, S and OM is explained by the formation of $BaSO_4$ and organometallic complexes respectively in the soil. For clay, on the other hand, the negative correlation might be explained by the higher retention of Ba^{2+} in soils with a higher percentage of the clay fraction, which makes the sites in soil colloids release fewer cations Ba^{2+} to the soil solution (**Table 3**).

Table 3. Significant Pearson correlation between Se and Ba concentration in Brazil nuts and soil physicochemical attributes in the 0-20 cm layer for the 8 sites studied.

Row	**Column**	***r***	***p***
[Se] in Brazil nuts	[Se] in soil	0.44	0.006
[Se] in Brazil nuts	K	-0.34	0.034
[Se] in Brazil nuts	Clay	0.33	0.037
[Ba] in Brazil nuts	[Se] in soil	-0.61	0.000
[Ba] in Brazil nuts	[Ba] in soil	0.82	0.000
[Ba] in Brazil nuts	K	0.55	0.000
[Ba] in Brazil nuts	P	0.64	0.000
[Ba] in Brazil nuts	Al	0.47	0.002

[Ba] in Brazil nuts	t	0.43	0.007
[Ba] in Brazil nuts	m	0.37	0.019
[Ba] in Brazil nuts	OM	-0.38	0.016
[Ba] in Brazil nuts	S	-0.33	0.043
[Ba] in Brazil nuts	Clay	-0.57	0.000
[Ba] in Brazil nuts	Silt	0.42	0.007

3.3 Selenium and barium relative bioaccessibility

The results of the *in vitro* bioaccessibility experiment indicate that the relative bioaccessibility of Se in Brazil nuts samples studied is higher in the small intestine phase on the two time points assessed, being SI-T1 and SI-T2 (after 2 and 4 h of incubation respectively), when compared with the gastric phase (GP). Therefore, while in the gastric phase the average bioaccessibility for Se was 18.1% (3.6-48.4%), for SI-T1 it was 48.5% (3.1-94.2%) and similarly for SI-T2 it was 50.3% (5.7-86.9%). It is interesting to observe such great variation for the Se bioaccessibility in the small intestine, which may reflect the variation in the Se speciation of the nuts for the different sites studied (**Fig. 2**). Similar to the present study, Se bioaccessibility for other food matrices (rice and maize) was higher in the intestinal digestion when compared with the gastric digestion (Jaiswal et al., 2012). Overall, the results suggest that the solubility of Se is affected by the pH of the gastrointestinal environment and its chemical form as also discussed by (Silva et al., 2020). Zhang et al. (2020) go even further and suggest that besides pH, the enzyme activity during the gastrointestinal digestion also affects the bioaccessibility of Se, and according to their results, pancreatin, which is the major enzyme component added during the intestinal phase at neutral pH, is a mixture of many enzymes and decomposes complex nutrients into simple molecules, making Se more bioaccessible.

For Ba, on the other hand, the bioaccessibility in the gastric phase (GP) is much higher than in the small intestine phase on the two time points (SI-T1 and SI-T2). The average bioaccessibility for Ba in the GP was 55% (25-92.9%), for the SI-T1 it was 3.6% (1.1-9.4%) and for the SI-T2 it was 3.3% (1.6-6.3%) (**Fig. 2**). The causes for such low Ba bioaccessibility in the small intestine are still unknown but may be related to the neutral pH (~7) of the simulated pancreatic juice, which affects the speciation of Ba in the solution and even leading to the formation of low solubility Ba compounds such as $BaSO_4$, which comes from the nuts themselves as stated elsewhere (Gonçalves et al., 2009) and also in agreement with the results observed in the previous chapter by our research group (data not published) in which the spatial location of the elements in the nuts and known affinity between Se-Ba and S-Ba suggest the formation of less soluble components such as $BaSeO_4$ and $BaSO_4$. Due to its insolubility, barium sulfate is also used as a contrast in X-ray imaging of the intestine even though Ba itself is toxic (Den et al., 2019). Another hypothesis for the lower bioaccessibility of Ba in this phase is the precipitation of $BaCO_3$ in the small intestine since it is added as compost of the simulated intestinal fluid 12.5 g/L $NaHCO_3$ (**Table S3**), but confirmation could only be made with further speciation analysis of the extracts after incubation *in vitro*.

Tukey's HSD test also demonstrated that there is no statistical difference between the two time points assessed in the small intestine phase (SI-T1 and SI-T2) for both Se and Ba bioaccessibility (**Fig. 2**). Since the variability of the data in the SI-T2 is lower for both elements, it will be used to calculate the final/actual bioaccessible content, as presented in the last section below.

The present study is so far the first to use the standardized protocol described by Minekus et al. (2014) for Brazil nuts seeds. Therefore, a comparison with other studies has to be made with the proper considerations to the methods used. Yet, it is relevant to mention that other studies reported reasonable bioaccessibility of Se and selenomethionine present in

Brazil nuts. Thomson et al. (2008) reported bioavailability of Se for humans after ingestion of Brazil nuts, in which the level of Se in plasma increased by 64.2% after consumption of 2 nuts per day during 12 weeks when compared with 7.6% in the placebo group. Silva et al. (2013) observed selenomethionine to be bioaccessible in Brazil nuts, corresponding to 74% of the total Se present in the sample after simulated *in vitro* gastrointestinal digestion. These results are in agreement with Wrobel et al. (2003), who also showed this species as being the most abundant in Brazil nuts, with concentrations around 75% of the total Se.

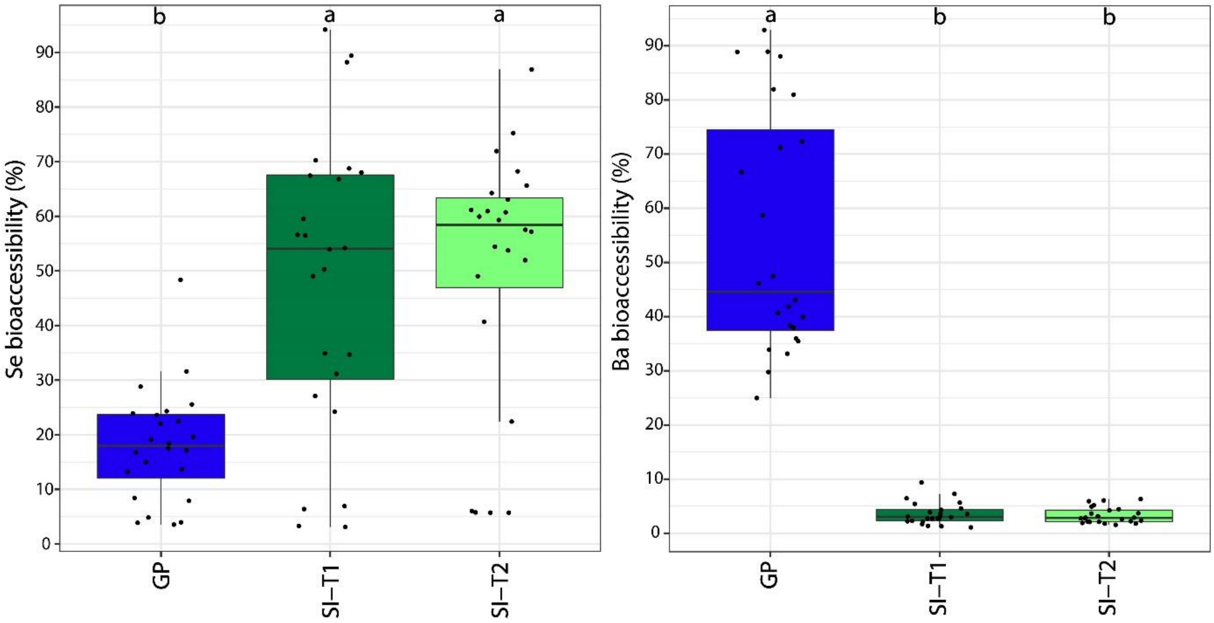

Fig. 2. Raw data and distribution of Se (left) and Ba (right) bioaccessibility of selected Brazil nuts samples (n=24) from 8 different sites in the Amazon region in 3 different simulated oral gastrointestinal phases. Random horizontal noise was added to the points (raw observations) to facilitate visualization. The letter on top of each phase indicates the result of Tukey's HSD test ($p \leq 0.05$), following a significant ANOVA ($p < 0.001$). Data for Ba bioaccessibility was log-transformed before ANOVA to attend parametric statistical assumptions.

The bioaccessibility data presented in **Fig. 3** provides a better visualization of the variation among the sites studied. Average Se bioaccessibility in the GP increases in the following order: AM:AR (12.2%) < RO (13.9%) < AC:XP (15.8%) < AC:SM (17.1%) < PA (18.9%) < AP (19.2%) < RR (20.5%) < AM:AN (26.9%). For Ba, the average bioaccessibility in the GP increases as follows: RO (31.6%) < AP (36.0%) < AC:SM (36.4%) < AC:XP (42.5%) < AM:AR (67.4%) < RR (70.6%) < PA (71.0%) < AM:AN (84.3%).

Data for Se bioaccessibility in the small intestine phase (T1 and T2) presents a wide variation within each site, noticed by the wider error bars when compared with Ba in the same phase. If the SI-T2 is taken as the standard bioaccessible phase for the small intestine, the site with the highest average is AC:SM with 69.7% (65.6-75.2%) and the site with the lowest average is PA with 23.7% (5.7-59.3%) for Se.

For Ba, the bioaccessibility in the small intestine was always below 10% and as assumed previously, the possible explanation is the formation of composts with low solubility such as $BaSO_4$ and/or $BaCO_3$ in this gastrointestinal phase. Moreover, it is noticeable that the variation within each site is small. The site with the highest bioaccessibility is AM:AR with 5.5% (4.5-6.1%) and the one with the lowest bioaccessibility is AC:XP with a bioaccessibility of 2.24% (1.92-2.6%) (**Fig. 3**).

The sample with the highest total Ba concentration, XP26 (10200 mg kg^{-1}) originates from the site with the lowest average relative bioaccessibility (AC:XP). But for such high total concentration present in this sample means that the total amount being bioaccessible would be still high. This association might be explained by the formation of insoluble compounds such as barium sulfate ($BaSO_4$) regardless of the Brazil nut population from which the material originates, which allows avoiding excess and possible intoxication by this element in the small intestine. To confirm this hypothesis, Gonçalves et al. (2009) performed fractionation of Ba in Brazil nuts and found that Ba is mainly in the form of $BaSO_4$, which is unavailable to the human organism. However as stated previously, we can still expect that precipitation with carbonate ($BaCO_3$) to play a role in the precipitation of Ba during the small intestine phase.

Lisk et al. (1988) found that, differently from the present study, after the consumption of a single 92-g portion of Brazil nuts by humans, only about 10% of the barium dose was excreted in urine and feces in the first six days, which means that a major part of the Ba ingested would be absorbed by the gastrointestinal system. However, the authors mentioned that Ba apparently has a low order of toxicity by the oral route. But sometimes there are contradictions since accidental poisoning from soluble barium salts has been reported in the literature (Ananda et al., 2013). This is a clear indication that Ba toxicity is more related to the chemical form and speciation than its concentration in food, soil and environment in general.

Selenium speciation analysis was initially planned in the present work, but unfortunately could not be performed and therefore, no statement can be done regarding the main chemical Se species present in the simulated gastric and small intestine phases. It is recommended for future investigations to perform Se and Ba speciation in the Brazil nuts, as not all compounds present the same level of bioaccessibility.

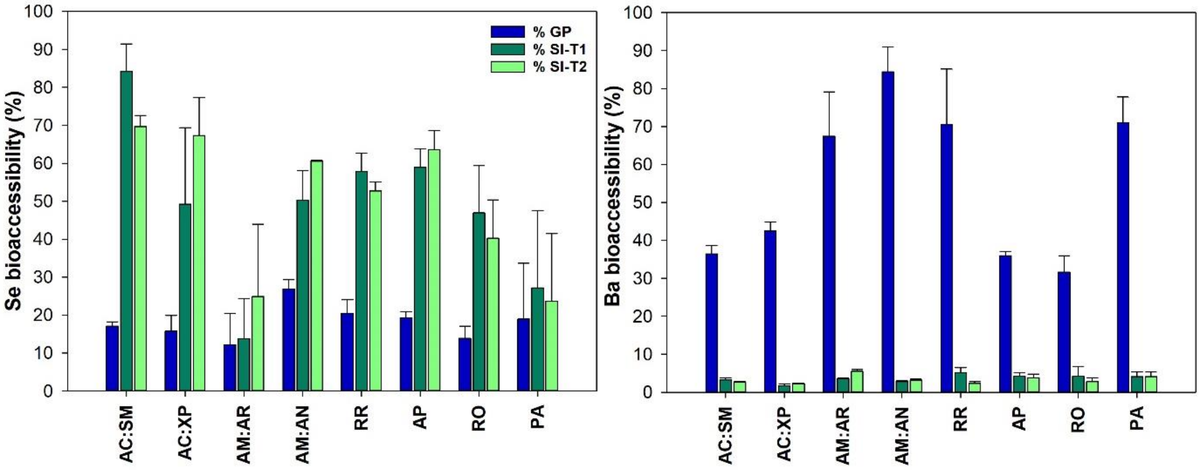

Fig. 3. Average relative Se (left) and Ba (right) bioaccessibility in Brazil nuts in 3 different gastrointestinal phases for the 8 sites from the Amazon region studied. Error bars are standard errors of the mean for each phase within the sites (SEM, n = 3).

3.4 Selenium and barium in the lipid fraction

The matrix of Brazil nuts is quite complex (66–67% fat, 14% proteins, and 13% of carbohydrates) (Dumont et al., 2006), so in the bioaccessibility experiment, the behavior presented by this material was atypical when compared to most other foodstuffs tested using a similar setup. This is mainly due to the high lipid content. Instead of having divided the material into two fractions (pellet and supernatant), it was divided into 3 fractions (pellet, bioaccessible fraction, and lipid with particulate material) after centrifugation.

The discussion concerning Se and Ba concentration in the lipid fraction was separated from that of the bioaccessible fraction because despite this fraction affects directly the bioaccessibility, it cannot be considered fully (100%) bioaccessible and therefore available in the gastrointestinal tract for the food matrix studied. Therefore, we can only state that the Se and Ba content in the lipid fraction of Brazil nuts might become soluble and available for absorption in the lumen only if this fraction gets disperse into micelles (Thakur et al., 2020).

Since lipid is a fundamental part of the food matrix, it affects the bioaccessibility of trace elements (Alava et al., 2015). So far there is no research in the literature showing the effect of the lipids in the bioaccessibility of Se and Ba in food specifically, but comparatively, a previous study revealed that fat has a major role in modulating As bioaccessibility presumably by their interaction with bile salt micelles. The results showed that the presence of fat resulted in an increased bioaccessibility of both inorganic and organic arsenicals in the presence of bile salts (Alava et al., 2013).

It is stated that intact cell walls play an important role in regulating the bioaccessibility of intracellular lipid and other nutrients in almond seeds (Mandalari et al., 2008). Novotny et al., (2012) estimated that only 76% of the energy contained within almonds (based on the Atwater factors) is actually metabolized in the human body, and that explains why their consumption does not result in increased body weight.

A wide variation among and within the sites is observed for Se and Ba concentrations in the lipid fraction. The overall average of the relative occurrence of Se in the lipid fraction was 26.1%, with the highest average for Se observed for sample AMB12 (50.2%) and the lowest for sample PA1406 (2.25%). For Ba, the average relative occurrence in the lipid fraction was 21.4%, with the highest percentage observed for sample AMB8 (39.3%) and the lowest for sample AC26 (9.28%) (**Fig. 4**).

Welna et al. (2008) investigated two extraction procedures to obtain the lipid fraction of Brazil nuts, which provided contents of 68.0 ± 0.6% and 68.2 ± 1.2%, using petroleum ether (PE) and chloroform: methanol 2:1 mixture (C + M), respectively. Among the elements studied, they found that Ba was predominantly concentrated in the defatted fraction, which is in line with the low percentage of Ba in the lipid fraction observed in the present study.

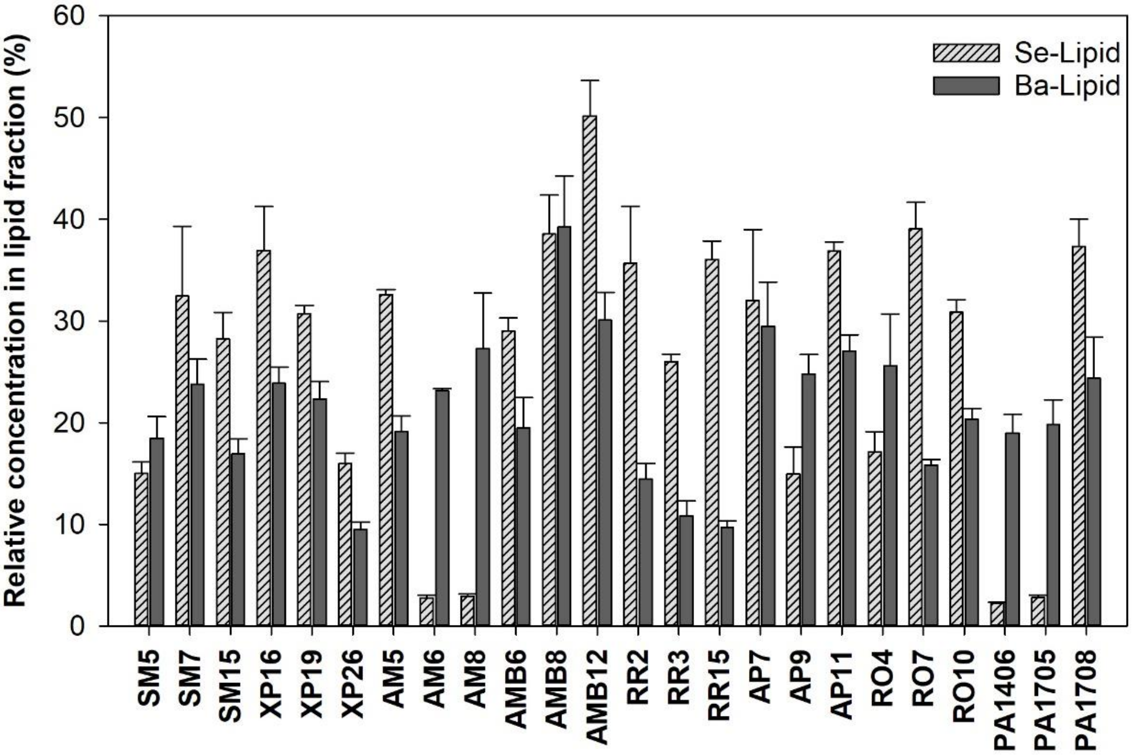

Fig. 4. Average relative concentration (%) of Se and Ba in the lipid fraction of Brazil nuts from the 8 sites studied. Error bars are standard error of the mean (SEM, n = 3). The prefix representing each site are described as: SM: Sena Madureira-Acre, XP: Xapuri-Acre, AM: Aruanã-Amazonas, AMB: Anori-Amazonas, RR: Caracaraí-Roraima, AP: Laranjal do Jari-Amapá, RO: Porto Velho-Rondônia and PA: Santarém-Pará.

3.5 Selenium and barium average intake per nut consumed

Brazil nuts are ingested by local populations and also throughout the world in many different ways. Perhaps the most common is the ingestion of the raw nuts that usually are processed, being unshelled and dried after the collection of the fruits in the forests around the Amazon region. These nuts are available in markets and food stores. The packages available in the local markets rarely present nutritional information for Se and never for Ba, therefore just a small part of the population is aware of their Ba and Se content. Only populations in the big cities such as São Paulo and Rio de Janeiro and those who are already eating Brazil nuts as a Se supplement are in fact demanding this information. Yet, it is valuable to present nutritional information on Se and Ba intake towards consumers of Brazil nuts and for the elaboration of public health policies.

In **Table 4** we crossed information about the individual content of Se and Ba in Brazil nuts, which is based on the total concentration multiplied by the average weight (g) of individual Brazil nuts (**Table S4**). Subsequently, we took the information of the relative bioaccessibility in the small intestine phase T2 (4 h after incubation) as the effective/actual relative bioaccessibility of Se and Ba, multiplied by the individual Se and Ba content to obtain the bioaccessible content (BAC), which is the effective amount of Se or Ba being potentially available for absorption in the lumen when ingesting a certain number of nuts.

In this sense, as the bioaccessibility is highly variable, using the standard recommendation of ingestion of 1 or 2 nuts per day in the diet, that is normally used by people who want to avoid the need for Se fortification (Thomson et al., 2008), seems to be a not so straightforward concept.

As we could expect, there is a wide variation in individual contents and bioaccessible contents (BAC) of both Se and Ba in nuts of the sites studied. While the individual contents of Se ranged from 1.01 to 1346 µg per nut (average = 163 µg), those for Ba ranged from 53.4 to 28278 µg (average = 7125 µg). For the bioaccessible contents, the values for Se ranged from 0.88 (XP16) to 105 µg (AP7) (average = 37.1 µg) and for Ba, the bioaccessible contents ranged from 1.96 (PA1505) to 758 µg (SM7) (average = 183 µg) (**Table 4**).

For Se bioaccessibility in the small intestine T2 (after 4 h incubation), we can observe material with considerably low bioaccessibility (~ 6%) as wells as material with reasonably high bioaccessibility (> 50%) on the same site, as is exemplified in the sites AM:AR and PA (**Table 4**). For Ba on the other hand, despite the great variation in the individual contents, the bioaccessibility is below 7% for all samples and variation is low within the sites studied. Therefore, the common factor that keeps Ba bioaccessibility low in Brazil nuts, regardless of the origin, composition and the wide variation in the total concentrations of the sample seems to be the addition of $NaHCO_3$ (12.5 g/L) in the small intestine phase, which might be causing the precipitation of Ba in the form of $BaCO_3$.

For Se supplementation purposes, if we use the recommended ingestion of 1 nut per day, the samples with adequate Se amount would be SM5, AM5, AP7, and PA1705 being originated from the sites AC:SM, AM:AR, AP and PA respectively, considering the optimal daily intake of 60 and 70 µg of Se for women and men, respectively (Kipp et al., 2015).

On the other hand, if we consider the uptake limits stipulated for both Se (400 µg day^{-1}) (Prabhu & Lei, 2016) and Ba (14000 µg day^{-1}, a person weighing 70 kg) according to IRIS (2005), the ingestion of 1 nut per day would still be safe, although this limit cannot exceed 3 nuts if we take the sample with highest bioaccessible Se content and 18 nuts if we take the sample with highest bioaccessible Ba content. Yet, the toxic effects caused by the excess of both elements should only be an issue if the exposure to such high concentrations extends for a long time. This is probably the reason why intoxication in humans by ingesting Brazil nuts is not commonly reported in the literature.

Table 4. Detailed information about individual Se and Ba contents in Brazil nuts and bioaccessible Se and Ba contents in Brazil nuts (BAC) which was calculated from the relative bioaccessibility obtained in the small intestine phase T2 (SI-T2, 4 h incubation).

Site	ID	Se content (μg)	Ba content (μg)	Se (SIT2)(%)	Ba (SIT2)(%)	Se BAC (μg)	Ba BAC (μg)
AC:SM	SM5	121.6±4.92	23943±1267	75.2±5.08	2.11±0.12	91.5±6.18	505±27.9
	SM7	13.1±0.19	27255±212	68.2±1.20	2.78±0.07	8.96±0.16	758±19.4
	SM15	33.7±0.35	13252±402	65.6±4.59	2.78±0.41	22.1±1.55	368±54.2
AC:XP	XP16	1.01±0.10	19296±183	86.9±9.48	2.60±0.34	0.88±0.10	501±66.4
	XP19	4.63±0.70	6078±134	61.1±3.48	2.21±0.08	2.83±0.16	135±4.85
	XP26	11.7±0.00	28278±382	53.7±3.42	1.92±0.11	6.30±0.40	544±32.4
AM:AR	AM5	146±2.45	90.2±0.98	63.1±5.92	6.07±0.24	91.7±8.62	5.47±0.21
	AM6	422±19.5	69.6±3.42	5.73±0.30	4.45±0.23	24.2±1.27	3.10±0.16
	AM8	308±6.06	69.8±0.99	5.76±0.33	5.91±0.33	17.8±1.03	4.13±0.23
AM:AN	AMB6	14.5±0.10	8024±127	60±0.52	3.66±0.51	8.68±0.08	294±41.0
	AMB8	37.3±1.63	21881±1444	60.9±3.44	2.90±0.30	22.7±1.28	634±66.1
	AMB12	91.2±1.14	11439±392	60.7±4.23	2.89±0.15	55.4±3.86	331±16.7
RR	RR2	23.7±0.33	4269±230	49.0±2.76	1.78±0.21	11.6±0.65	75.8±9.07

	RR3	39.7±1.80	1734±29.4	52.0±2.31	3.16±0.18	20.6±0.92	54.8±3.12
	RR15	90.3±1.63	1229±28.1	57.2±2.88	2.12±0.25	51.6±2.60	26.1±3.07
AP	AP7	163±7.48	1158±42.1	64.3±1.96	4.23±0.39	105±3.20	49.0±4.50
	AP9	92.2±2.30	931±11.5	71.9±0.45	5.16±0.43	66.3±0.42	48.0±4.03
	AP11	100±0.96	371±12.0	54.4±2.37	2.17±0.25	54.4±2.36	8.06±0.94
RO	RO4	17.6±0.39	970±14.2	22.4±1.19	1.79±0.21	3.95±0.21	17.4±2.01
	RO7	19.7±0.94	143±2.12	57.5±2.07	1.55±0.13	11.3±0.41	2.21±0.19
	RO10	98.8±1.05	207±7.10	40.7±4.03	4.95±0.40	40.2±3.98	10.3±0.82
PA	PA1406	616±68.8	61.8±2.69	5.72±0.44	6.33±0.84	35.2±2.72	3.92±0.52
	PA1705	1346±36.8	53.4±3.80	6.03±0.46	3.68±0.14	81.2±6.13	1.96±0.07
	PA1708	92.5±6.64	202±0.19	59.3±2.36	2.33±0.25	54.9±2.18	4.72±0.50

Values are average ± standard deviation, being Se/Ba content (n = 2); Se/Ba (SI-T2) (n = 3) and Se/Ba BAC (n = 3). Se/Ba content = individual content of Se or Ba in Brazil nuts in micrograms; Se/Ba (SI-T2) = relative bioaccessibility (%) of Se or Ba in the small intestine phase after 4 h incubation (T2); Se/Ba BAC = Se or Ba bioaccessible content based on the relative bioaccessibility in SI-T2 multiplied by the individual content.

4 CONCLUSIONS

The main objective of this investigation was to provide novel information regarding the bioaccessibility of Se and Ba in Brazil nuts from different sites in the Amazon using a standardized method for oral bioaccessibility assessment *in vitro*, which was successfully attained.

Despite the current concerns on levels of As, Cd, Cr, Hg and Pb in the Amazonian environment (soil, food and water resources) the levels found in Brazil nuts of the sampled sites are all below the detection limit.

Pearson correlation showed that Se concentration in Brazil nuts is mainly related to Se in soil (0-20 cm) and clay (positive correlation). Barium concentration in Brazil nuts, on the other hand, is positively and highly correlated with Ba in soil (0-20 cm) and negatively correlated with Se in soil (0-20 cm).

The overall data showed that Se bioaccessibility in the simulated gastric phase (GP) is lower than in the small intestine phase (SI-T1 and SI-T2), while for Ba the opposite occurs.

The average relative content of Se and Ba in the lipid fraction upon *in vitro* digestion extracted from the small intestine phase (SI-T2, 4 h incubation) is 26.1% and 21.4%, respectively. Further studies are needed to confirm the bioaccessibility of Se and Ba in this fraction.

Bioaccessible contents of Se and Ba in individual Brazil nuts vary widely among and within the sites studied, but the recommendation of ingestion of 1-3 nuts per day can be considered safe, considering the current uptake limits stipulated for Se and Ba in the literature.

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Supplementary Material

Table S1. Information about the sampling sites of Brazil nuts 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
Xapuri/AC	10°50’02.02’’S	68°23’23.51’’W	262	Am
Itacoatiara/AM	3°01’05.59’’S	58°49’55.60’’W	92	Af
Anori/AM	3°50’10.01’’S	61°36’11.64’’W	29	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

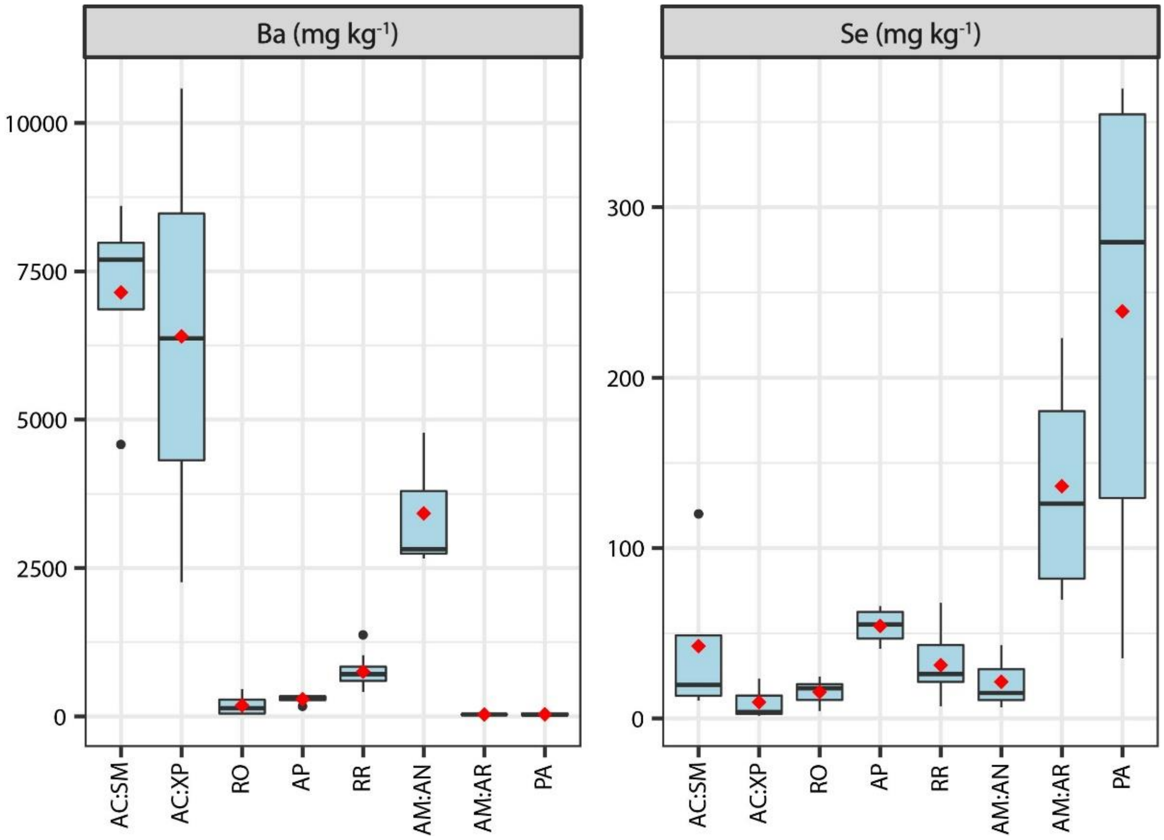

Fig. S1. Total Ba and Se concentration in Brazil nuts from different sites of the Amazon region represented as Box-and-whisker plot (median value line within box, mean value as a rhombus). Sites are identified as follow: (AC:SM = Acre-Sena Madureira; AC:XP = Acre-Xapuri; RO = Porto Velho-Rondônia; AP = Laranjal do Jari-Amapá; RR = Caracaraí-Roraima; AM:AN = Amazonas-Anori; AM:AR = Amazonas-Aruanã farm; PA = Santarém-Pará.

Table S2. Average recovery rate (%) for the standard reference material used for the quality control/assurance during the multielement analysis of Brazil nuts. Values are average ± SD.

Element	White clover (BCR-402)	Peach leaves (NIST-1547)	Ryegrass (ERM-CD281)	Sea lettuce (BCR-279)
Se	107±3.3% (n=4)	-	-	-
Ba	-	99.8±0.8% (n=2)	-	-

As	-	-	-	94.7±5.8% (n=2)
Cd	-	-	89.9±2.9% (n=2)	98.4±16% (n=2)
Cr	96±1.9% (n=4)	-	104±0.3% (n=2)	-
Fe	-	90.9±1.0% (n=2)	-	-
Hg	-	-	107±34% (n=2)	-
Ni	-	-	99.7±0.4% (n=2)	-
Pb	-	85.8±0.9% (n=2)	-	89±0.3% (n=2)

Table S3. Composition of simulated gastrointestinal fluids and enzymes for incubation *in vitro*.

Solution	Product	Amount (g L^{-1})
SSF (pH = 7)	KCl	1.1264
	KH_2PO_4	0.5032
	$NaHCO_3$	1.1424
	$MgCl_2(H_2O)_6$	0.0305
	$(NH_4)_2CO_3$	0.00576
	Amylase 500 U/mg protein*	0.150
	$CaCl_2.2H_2O$ (147.0146 g mol^{-1})*	2.5002
SGF (pH = 2)	KCl	0.51474
	KH_2PO_4	0.1224
	$NaHCO_3$	2.1
	NaCl	2.7612
	$MgCl_2(H_2O)_6$	0.0244
	$(NH_4)_2CO_3$	0.048
	Pepsin 666 U/mg protein*	3.003
	$CaCl_2.2H_2O$ (147.0146 g mol^{-1})*	0.25
SIF (pH = 7)	KCl	0.5073
	KH_2PO_4	0.1088
	$NaHCO_3$	12.5
	NaCl	2.2464
	$MgCl_2(H_2O)_6$	0.0671
	Pancreatine	0.9
	Fresh dehydrated bile (DIFCO oxgall)*	6
	$CaCl_2.2H_2O$ (147.0146 g mol^{-1})*	1

SSF = Simulated Saliva fluid; SGF = Simulated Gastric fluid; SIF = Simulated Intestinal Fluid. *Enzymes components and $CaCl_2$ were added freshly right before pre-warming the solution previous to *in vitro* incubation procedure.

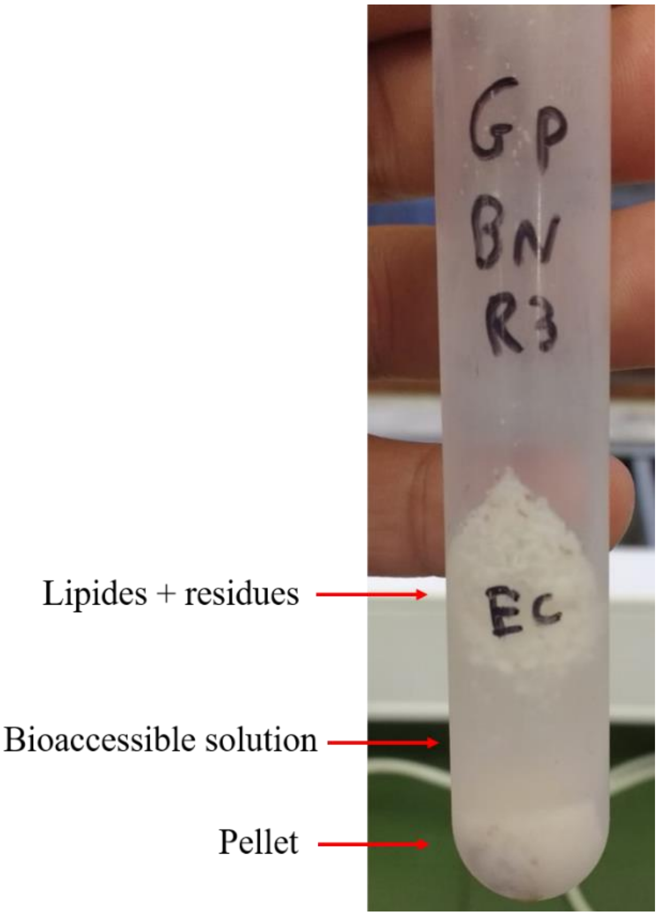

Fig. S2. Fractions separated after centrifugation step of the bioaccessibility extracts collected during simulated *in vitro* gastrointestinal experiment with Brazil nuts sample.

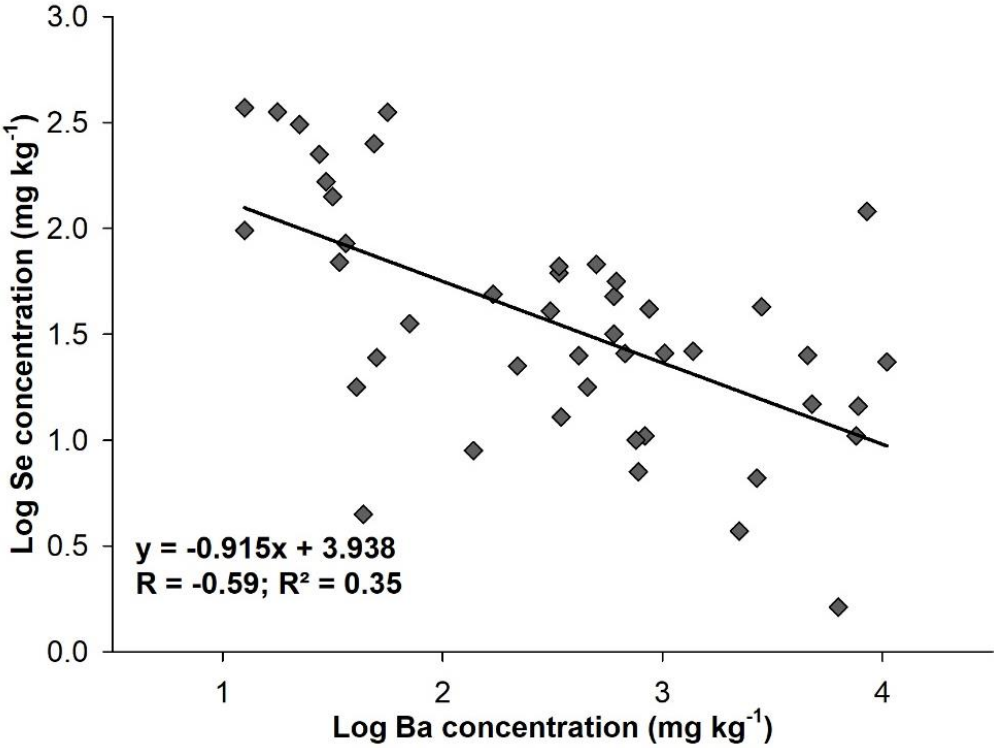

Fig. S3. Scatter plot for total Se x Ba concentration in Brazil nuts from 8 different sites in the Amazon. Data was log-transformed to attend parametric statistical assumptions for linear regression.

Table S4. Average weight of individual Brazilian nuts (n=3) from each plant and average weight for each respective site studied.

Site	Plant ID	Weight (g)	Average weight ± SD (g)
	SM5	2.80	3.22±0.49
AC:SM	SM7	3.75	
	SM15	3.11	
	XP16	3.22	2.94±0.26
AC:XP	XP19	2.87	
	XP26	2.72	
AM:AR	AM5	3.0	2.82±0.17

	AM6	2.81	
	AM8	2.66	
AM:AN	AMB6	3.19	4.09±0.85
	AMB8	4.88	
	AMB12	4.21	
RR	RR2	5.08	3.42±1.45
	RR3	2.70	
	RR15	2.46	
AP	AP7	3.49	2.95±0.55
	AP9	2.96	
	AP11	2.40	
RO	RO4	3.02	3.73±0.83
	RO7	3.53	
	RO10	4.63	
PA	PA1406	3.09	3.39±0.28
	PA1705	3.64	
	PA1708	3.46	

Fig. S4. Dark brown nodules found in Brazil nuts fruits ("ouriços") from the site AC:XP (Xapuri, state of Acre, Brazil).

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4 GENERAL DISCUSSION AND CONCLUSION

This chapter highlights the major results and contributions of this Ph.D. thesis and provides a broader view on the research outcomes presented in the 4 research chapters. It also points out the limitations of the conducted research and identifies the main research gaps that remain and need further investigation in the future.

4.1 Expanding knowledge on Se, Ba and other relevant trace elements in Brazil nuts

Research on Se and Ba in Brazil nuts (*Bertholletia excelsa*, Lecythidaceae) in the Brazilian Amazon was initiated only quite recently. Important pioneer studies provided a necessary pathway for research on the soil-plant relationship and gave a first notion on the wide variation in the concentrations of these elements in the nuts (SMITH, 1971; CHANG et al., 1995). Advances in analytical techniques for Se and *in vitro* models for bioaccessibility assessment made it possible to study bioaccessibility and speciation of Se in Brazil nuts, which are proven to be essential factors for improvements in human micronutrient status.

One of the main contributions of this thesis is the information provided on the levels of Se and Ba that one can expect when consuming Brazil nuts originated from different sites in the Brazilian Amazon. This is a continuation of a previous study on Se in Brazil nuts and soils of the Amazon, conducted by Silva Junior et al. (2017). Other previous research only provided information on the trace elements level that could be expected in a certain lot or package of Brazilian nuts obtained in a local market or food store somewhere else in the world (USA, Europe, or Southeast Brazil), wherein the specific local origin of the nuts can often not be identified. In the current study, geographical coordinates of the specific plants and stand of plants (called “Castanhal”) from where the samples were collected could be provided for the 9 sites being studied. This achievement was only possible due to collaboration with researchers from EMBRAPA, a Brazilian research company focused on agriculture and livestock production, in different states of the region (Acre, Rondônia, Mato Grosso, Amazonas, Amapá, Roraima, Pará). Most sites used for sample collection in the present study were areas already being investigated and involved in a research project called “MapCast”, which is a research initiative to improve the management of Brazil nuts in the native forests of the region by performing a socio-environmental and economic characterization of Brazil nut production systems in the Amazon.

Given the 9 sites studied, it was also possible to confirm the great variation in the Se and Ba concentrations not only among these sites but also within each site studied when comparing the 15-18 plants from each site. This variation has been reported in the literature, but for a better comparison among the Brazil nuts populations studied, it was necessary to select sites in each of the 7 Amazonian states (Acre, Rondônia, Mato Grosso, Amazonas, Roraima, Pará, and Amapá). This allowed to include at least one site in each of these states and therefore also the assessment of variability "among states" of the Amazon region, i.e., at larger spatial scales. Brazil nut plants were selected inside the native forest/farm and the nuts (10 fruits) were collected below the tree canopy. The minimum necessary distance from one Brazil nut tree to the other was 50 m to not mix samples from different plants, and the geographic coordinate of each tree was recorded with GPS. This approach provided new evidence on how Se and Ba concentrations in Brazil nuts vary between sites, plants, and states in the Amazon. The thesis also demonstrated, according to results obtained in the last three chapters, an inverse relationship (negative correlation) between Se and Ba in both soil and Brazil nuts samples, which indicates that their fate in the global environment is mutually affected.

Another important contribution from the thesis is the identification of genotypic variation as an important factor affecting Se and Ba accumulation in Brazil nuts, despite not being included in the scope of the research. A good example of how Se and Ba accumulation in Brazil nuts are strongly influenced by the genotypic variation is taken from the studied case of similar concentrations found for both elements (Se and Ba) in Itacoatiara-Amazonas (Aruanã farm) - a Brazil nut plantation represented by clones originated from a population of the State of Pará -, and Santarém (Pará), which are plants grown in their natural environment. The similarities observed in terms of Se and Ba accumulation capacity when comparing different Brazil nut plant populations can be an indication of the genotypic effect on the ability of these plants to absorb and translocate such elements from the soil to the edible nuts. Therefore, it is possible that even for plants geographically distant to share genetic characteristics as exemplified by the clones originated from the state of Pará grown in the state of Amazonas and other cases of propagation of Brazil nut plants by humans. For the current genetic structure of Brazil nut populations, some studies have considered the critical role of humans in the distribution of Brazil nut trees throughout the Amazon, interpreting the population distribution as orchards created by pre-Colombian Indians (more than 500 years ago). The anomalies in Brazil nut‘s modern distribution in the forest also support this hypothesis, since only human preferences can explain the absence of the species in some areas (CLEMENT et al., 2010; SHEPARD; RAMIREZ 2011). Sujii et al. (2015) does not exclude the possible role of pre-Colombian Indians as ‘‘seed dispersers’’, as previously mentioned, but his dataset suggests that local differentiation has taken place, which can include demographic and adaptation processes. Therefore the relative contribution of landscape, adaptation, gene flow, and genetic drift for this pattern merits further investigation. The authors found an excess of heterozygotes in most populations of Brazil nuts studied, with a significant negative inbreeding coefficient as a consequence of self-incompatibility within populations. These assumptions are in line with and confirm the presence of great genetic diversity within populations of Brazil nuts, which could explain the differences in capabilities of plants from the same site for accumulating Se and Ba in the edible nuts.

Concentrations of As, Cd, Cr, Hg, and Pb in Brazil nuts are reported to be below the detection limit in the present study. This is relevant information considering that all these contaminants represent a great risk for the consumption of Brazil nuts and yet they are found frequently in reasonable amounts in soil or water samples in studies conducted within the region. For example, toxic levels of As were reported in water resources and other environments in all of the 20 Latin American countries (BUNDSCHUH et al., 2020). Mercury being found below the detection limit can be explained by the low levels of Hg present in the soil. In the same context, a study performed by Zhang et al. (2012) showed Se levels inversely related to levels of IHg (inorganic mercury) and MeHg (methylmercury) in grains of rice. Besides, a consistent reduction in translocation of both IHg and MeHg in the aerial shoots (i.e., the stem, leaf, husk, and grain) with increasing Se levels in the soils was observed. The same inverse relationship can be expected to occur in the soil-plant relationship for Hg-Se in the present study with Brazil nuts, indicating that the increase of Se in soil reduces the translocation of Hg to plant shoots and may avoid the accumulation in edible nuts.

Adding to this idea, it has been proven that while high Se or Hg exposures can each individually induce toxicity, the co-occurrence of moderately high concentrations of these elements does not produce additive effects but, rather, antagonistic effects. Therefore, their co-occurrence can mutually reduce the toxic effects of each element (PETERSON et al., 2009). This assumption is supported by research showing that Se potentially reduced the bioaccumulation of Hg in the higher trophic levels of food webs in the Amazon basin, showing an antagonistic effect in part of the food chain (LINO et al., 2020). Another study showed significant inverse relationships between Se and Hg in perch and walleye tissue, suggesting a strong antagonistic effect of Se on Hg assimilation by these fish species (CHEN et al., 2001). According to some authors, the Se protection mechanism, concerning Hg, occurs due to a high molecular weight complex being formed between the two elements. Selenite (SeO_3^{-2}) is reduced into selenide (Se^{-2}) that binds to MeHg, generating the complex selenide-methylmercury $(CH_3Hg)_2Se$, which, thus neutralizes toxic effects of Hg (NAGANUMA;

IMURA, 1980; FAIAL et al., 2015; LINO et al., 2018). This is good evidence that the consumption of Brazil nuts can serve also to counteract possible toxic effects of Hg contained in food and water in the region.

The local population is rarely aware of the levels of Se and Ba present in the Brazil nuts that they eat very frequently, even though it is reported that dietary Se intake in specific studied populations in the region is higher than for populations in the southeast region of the country (MAIHARA et al., 2004). The main cause for such elevated levels of Se in the typical diet of Amazonian riverside populations, which leads to blood Se levels up to 1500 µg/L, is the regular consumption of Brazil nuts among other products containing high Se concentrations such as domestic chicken and game meat (LEMIRE et al., 2010). Yet, there is no evidence of selenosis from a selenium-rich diet in the Brazilian Amazon (LEMIRE et al., 2012). So, it would be important to start providing information concerning levels of Se contained in the food people eat and set intake limitations related to these diets for the communities that have Brazil nuts as a means of livelihood in the Amazon. On the other hand, the economic value of Brazil nuts can be increased if the exploratory and production sectors are aware that certain populations and sites wherein Brazil nuts are grown present high levels of Se.

4.2 Identification of baseline levels of Se, As, Ba, and I in soils of the Amazon

Information regarding Se in soils of the Amazon region is still rare, with only a few published investigations so far in the literature. Yet, studies on As and Ba in soils of the region are even more scarce. For iodine, this Ph.D. thesis is the first study ever reporting concentrations of this element in the topsoil of the Amazon rainforest. Therefore, the thesis delivers important contributions and valuable data on contaminants and essential trace elements.

There are equally few reports on contamination of food, water, and other environmental resources with these elements, reaching harmful levels in humans. The high levels of particular elements such as As and Ba are restricted to gold mining sites and related anthropic activities in the region, and normally do not affect populations nearby (BUNDSCHUH et al., 2020; PEREIRA et al., 2020). Other reasons for this lack of research could be explained by the economic status of the region, which is less developed than the central and southeast Brazilian regions, and on this account, lower financial resources are assigned to studies in the region, as a result of a lower political concern on local environmental and human health issues.

The main consequence of a lack of research on Se, As, Ba, and I conducted so far on soils of the Amazon is the absence of Quality reference values (QRVs) that properly reflect the status of soils of the region in the national regulation. Nowadays the available QRVs can barely be used for the Amazon because the levels do not take into account its pedological and geochemical specificities, which occur as a result of a completely different climate, parent material, and vegetation. For example, the levels of total As reported for some sites in the present study (notably in the western Amazon, including the states of Acre and Rondônia) reach up to 41.7 mg kg^{-1} which are above the thresholds reported by Conama (CONAMA 2013) for prevention level (15 mg kg^{-1}). Yet the results obtained for As can be considered as baseline levels for the studied sites and used to derive reference values for the national legislation because there is no evidence of contamination and harmful effects to plants, animals, humans, or other organisms originated from these soils. The same underestimation of soil reference values is found for Ba in the national regulations. The levels of Ba in the western and central Amazon (including states of Acre and Amazonas) in the topsoil (0-20 cm) ranged from 100 to 765 mg kg^{-1}, being quite above the prevention level established by the Brazilian legislation (150 mg kg^{-1}). In Brazil we have 3 Quality Reference Values: they work as the traffic lights: 1-Reference value (green light), characterizes the background level for the soil and all soil environmental functions are working well; 2- Prevention value (yellow light), lies above the expected normal concentrations and can affect the normal quality of the soil concerning its main functions in the environment; 3- Investigation values (red light), represent potential direct or indirect risks to human health and the environment and are used to define control actions for soil decontamination in three different exposure scenarios: agricultural, residential, and industrial. When the concentrations of a certain contaminant are above the prevention level as frequently pointed in the discussion, it represents a warning for a more comprehensive assessment of the soil quality to check if the contaminant is compromising the normal quality and main soil functions. This warning and the subsequent evaluation are important in order to avoid taking more serious control measures, e.g., soil cleanup actions, which might happen when the values are above the “red light” (investigation values). This also highlights the importance of having accurate quality reference values in the legislation for each state to avoid under or overestimation of the real soil status. Therefore, the present Ph.D. thesis dataset provides a completer and more reliable dataset for As and Ba that could be used for policymakers and the overall community, delivering enough evidence for a reformulation of the current Brazilian regulations for reference and prevention values.

Another important contribution of the present Ph.D. thesis is the information provided for the total I in topsoil (0-20 cm) and the diverse Se fractions (total, soluble, exchangeable, and organic-bound). This is relevant, firstly because the information about the concentration of Se and I in topsoil can be used for establishing a reference value for the region in the case of I, and updating the old value in the case of Se. This information is essential to support future studies and public policies focused on the soil as the main resource of livelihood. Furthermore, biofortification strategies strongly depend on the element's behavior in soils and its phytoavailability. The knowledge built up can, therefore, help to find efficient application strategies for Se or I in these soils (i.e., correct approach, dose, fertilizer, and time of application) to provide a better nutritional quality of the food crops grown in these soils. This investigation can help to support studies on I biofortification that already started in the country, e.g., a trial evaluating the application of I through soil and foliar fertilizers on rice plants (CAKMAK et al., 2017). This is of critical importance in Brazil since the country still depends upon iodized table salt as a basic I supplementation for the entire population. The problem of using salt as the sole basic source of I for the population is that increases in the consumption of salt also increase the rate of hypertension issues in the population (CAMPOS et al., 2015). Therefore, salt addition should be avoided and it would be much healthier to include I in staple crops to increase the intake and status of the population.

4.3 Unraveling spatial distribution of trace elements in Brazil nuts seeds

Until not so long ago, scientific research measuring trace elements in biological samples could only provide information on the total concentration, and this was the only kind of information available for Brazil nuts seeds as well. For this approach, it is necessary to completely destruct the structure of the seed (i.e., grinding) without accounting for important information related to the unique anatomy of the seeds and mechanisms involved. With the advance of technology and new scientific methods such as the use of XRF (X-ray fluorescence), it became possible to investigate the spatial distribution of elemental contents. In the present study, the use of XRF-based techniques (benchtop equipment and synchrotron light) allowed us to uncover important information regarding the spatial distribution of toxic trace elements besides Se and Ba in the seed internal tissues.

Selenium was found to be accumulated mainly in the outer parenchyma tissue of the seed, forming a "ring shape". The Se distribution in Brazil nuts with high concentrations tends to be located in hotspots on the bottom extremity of the seed (root apex). This is remarkable because Se is located in an important meristematic tissue of the seed and may have an important role in the seed physiology during germination or even offering protection against biotic (herbivores and pathogens) and abiotic (cold, drought, high light, water, salinity, and heavy metals) stresses as suggested by previous research (MEHDAWI; PILON-SMITS, 2012; FENG et al., 2013). The spatial distribution of other trace elements studied such as Ba, Br, S, P, K, Ca, Mn, Fe, Cu, and Zn in Brazil nuts seems to be all dependent on the concentration and tissue location in the Brazil nuts seed sample. Information on the spatial distribution of these trace and major elements can help elucidate the role of different elements in seed development and enhance seeds' values in human diets.

Furthermore, our study could confirm that:

1) Barium is associated with Se and S in Brazil nuts seeds tissues, probably due to the formation of less soluble composts ($BaSeO_4$ and $BaSO_4$), which may lead to the unavailability of this element in the gastrointestinal system.

2) The bioaccessibility of Ba in the small intestine was confirmed to be very low (3.31% on average). This is novel information and will support future research. This outcome is relevant because there was a certain concern that such high Ba concentrations found in Brazil nuts in sites of the western Amazon (sometimes higher than 10000 mg kg^{-1}) would be toxic to humans after absorption by the human gastrointestinal system. Our study thus explains why there is no report or evidence of Ba intoxication in humans when eating Brazil nuts from the Amazon, although the very high total concentrations were reported previously in the literature.

4.4 New insights on Se and Ba bioaccessibility in Brazil nuts

The insightful observation made by Paracelsus (1493–1541) "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy", is very relevant today, and applies very well for the present study. With this statement, Paracelsus defined a basic law of toxicology: An increase in the amount or concentration of compounds causes increasing negative biological effects, which may lead to inhibition of biological functions and, eventually, to death (SELINUS et al., 2013). However, despite the harmful effects of some elements (such as Ba in the present study), others are essential for life (such as Se in the present study). For Se particularly, deleterious biological effects can result from either increasing or decreasing its concentrations. That being the case, defining a safe amount of Brazil nuts that can be ingested is of paramount importance in this research field.

In our study, the prediction of how much of a nutrient or contaminant reaches the bloodstream of humans started with an *in vitro* simulation of the gastrointestinal digestion. The use of this approach for Brazil nuts samples aiming to predict Se and Ba bioaccessibility provided essential and practical information for the consumption of Brazil nuts. The results showed a wide variation in the bioaccessibility values observed, due to the diverse general composition and concentrations in the nuts, depending on the geographic location where samples are collected. Average bioaccessibility was 50.3% and 3.31% for Se and Ba, respectively, in the small intestine phase, indicating that: 1) Brazil nuts, on one hand, are yet a good source of Se potentially bioavailable for supplementation to increase Se status in the population: and, 2) the low average bioaccessibility for Ba and certainly the low exposure upon oral intake is the key for the absence of harmful effects of this element upon Brazil nuts ingestion by humans. It is important to clarify such information for society, confirming that Brazil nuts represent a reliable source of Se, whereas Ba contained in these nuts is not harmful if only the number of nuts necessary to supply the daily Se needs is consumed, which would not be more than 3 nuts per day. Taking into account the average amount of bioaccessible Se found in the present study (37 µg/individual nut, n = 24), an optimal supplementation can be reached by eating ~2 nuts per day to reach the daily demand of 60-70 µg/day for an adult.

Another important contribution during the *in vitro* bioaccessibility assessment was the separation of the lipid fraction after the centrifugation of the extracts and the quantification of Se and Ba contained in this fraction. It is known from the literature that lipids in different food types are not 100% bioaccessible, which depends on several factors such as particle size of the food, the predominant type of fat, and lipid classes in the food studied (GRASSBY et al., 2017; GARCIA et al., 2019). Therefore, it would be necessary to develop a proper approach to quantify the bioaccessibility of Se in the lipid fraction present in the Brazil nuts.

Our study on Se in Brazil nuts may play a role in demonstrating towards the population the feasibility of using Brazil nuts as a good Se supplementation source, avoiding the need for the fortification of foods or for expensive supplements to improve Se status (THOMSON et al., 2008). An Internet search on the supplements available on the market in Brazil illustrates that prices of supplements range from 2 to over 10 US dollars, for a dose sufficient for 60 days (considering daily demands of 60 µg of Se). Comparatively, using data from our present study (average weight and bioaccessible Se content of Brazil nuts) and using the average price of Brazil nuts available in the country (n = 3) found on the internet, it would be possible to provide the same Se supplementation (60 days with 60 µg per day) with 3.87 US dollars. Therefore, in most cases, it is cheaper to use Brazil nuts as a dietary Se supplement. However, a comparison of the prices of Brazil nuts in the north of the country versus the south/southeast reveals that this food is more easily accessible for people in the north and the Amazon in general. Therefore people from other regions of the country still rely on alternative sources of selenium if they want to supplement using bioavailable organic Se forms.

Furthermore, Se in supplements is mostly present as inorganic Se (i.e., selenate or selenite), with a lower overall Se bioavailability compared with food supplements containing organic forms of selenium (mostly selenomethionine), as is the case of Brazil nuts (THIRY et al., 2013).

4.5 Limitations of the thesis and recommendations for future research

The methods and results presented in this thesis have limitations due to the obstacles in the design of the optimal sampling coverage and also the limitation of technology available during the investigation. The main limitations and opportunities for future studies are discussed below.

Study of Se and Ba concentration in the soil subsurface

Perhaps the biggest limitation was the soil sampling equipment available allowing the collection of samples down to a depth of 60 cm. However, it is reasonable to imagine that such a big tree as the Brazil nuts tree, reaching heights of 50 m, has extensive roots going down below 1 or 2 meters deep in the soil profile at least. Because of this limitation, it was not possible to study accurately the correlations between the soil contents of Se and Ba, as well as other physicochemical soil attributes and the levels of Se and Ba accumulated in Brazil nuts, in addition to making further inferences on the accumulation capacity of several elements by different plants and populations of Brazil nuts in the Amazon. The ideal solution would be to have a bigger auger drill for collecting soil samples in the subsurface (below 1 m deep). This would allow reaching soil layers that potentially match the depth where the Brazil nuts roots are absorbing most nutrients, water, and trace elements.

Mapping Se and Ba distribution in soils of the Amazon

The geographic coverage limitation involving the number and distribution of samples in the region needs further investigation. Despite the reasonable good number of sites (9) and plants/points sampled (138) and different layers (3) used for the analysis of soil and Brazil nuts samples, a great weakness is yet the representativeness of each site within each state and also the number of samples from each site (15-18) distributed in a small area inside the forest/farm. Therefore, generalizations about the concentrations of Se and Ba in Brazil nuts and about these and other trace elements and soil attributes cannot be made for an entire state or region where the sites are located, and therefore, this can only give us an idea on what to expect in terms of variation for a section of a Brazil nut population and for the region studied. For better representativeness in each state studied, it is necessary to study sites more or less homogeneously distributed in the entire state with a greater number of samples in each site. Consequently, it is still not possible to build a map showing a regional variation of Se and Ba in soils of the Brazilian Amazon using the dataset obtained in the present Ph.D. thesis. Overcoming this constraint of geographic distribution is, however, not an easy task, taking into account the huge dimensions of the Amazon Region in Brazil as well as the difficulty for reaching most sites in this region.

Genotypic variation in the accumulation of Se and Ba in Brazil nuts

There is yet a limitation in the knowledge about genotypic variation and plant mechanisms of trace elements absorption and translocation in Brazil nuts trees. With the present Ph.D. research, we could only account for the natural distribution and variation of Se and Ba concentrations in different plants from selected populations of Brazil nuts trees. The soil-plant relationship was previously studied by Silva Junior et al. (2017), but because of the limitations concerning the depth of soil layers evaluated, as mentioned previously, such correlation was still not ideal for reliable inferences on the soil factors leading to higher or lower accumulation of Se and Ba in the edible nuts. Therefore, so far, no inferences could be made on the specific ability of each plant and population to accumulate these elements. Factors that were not involved in the present study such as plant age, size, diameter among others could also account for the variation in accumulation of trace elements. Another factor important to mention is the genetics of Brazil nuts trees' populations. This is a topic studied more recently and the overall consensus is that there is great genetic divergence within and among populations of Brazil nut trees since this species is alogam (reproduce by cross-fertilization) (SUJII et al., 2015; COELHO et al., 2017; VIEIRA et al., 2019; BALDONI et al., 2020). This is perhaps the key information that would explain such wide variation in the concentrations of Se and Ba accumulated in Brazil nuts among and within the sites studied.

Regarding the wide variation in the total and bioaccessible concentrations of Se and Ba in Brazil nuts throughout the Amazon region, it would be essential to have in the future a larger dataset with more samples from each site and more sites studied to have a better prediction on what to expect in terms of concentration covering each state of the Amazon region. This is because each state represents specific environmental conditions in terms of soil, weather, and Brazil nut population. Such information could also help to keep a sustainable exploration of the native trees in the forest and to preserve these sites from deforestation.

Application of Caco-2 cell bioassay as an effective approach to predicting Se and Ba bioavailability of Brazil nuts in humans

The present thesis performed a bioaccessibility assessment test, with an *in vitro* simulation of the gastric and small intestine phase, which is the first step before doing *in vivo* experiments. Yet, it is ideal to perform a more comprehensive assessment *in vitro* using Caco-2 cells aiming to better predict the bioavailability of Se and Ba. Research has demonstrated that the Caco-2 cell bioassay is a fast and cost-effective approach for screening a set of samples before the assessment *in vivo* (TAKO et al., 2016). Therefore, for future studies, this would be the preferred approach for research aiming to refine the results on Se and Ba in Brazil nuts obtained so far.

A wider and standardized measurement of radium in Brazil nuts from Amazon

In the literature, there are a couple of studies measuring radium (Ra) in Brazil nuts (SMITH, 1971; PAREKH et al., 2008; MARTINS et al., 2012; KLUCZKOVSKI et al., 2020). However, for most of them, there is no control over the geographical origin of where the samples are collected and the number and distribution of samples are not enough for a real notion on the levels expected in different Brazil nut populations in the Amazon region. Therefore, it would be recommended for future research topics to advance in this field and use a modern and standardize methodology to measure the levels of Ra in a larger number of Brazil nuts samples.

FINAL CONSIDERATIONS

Studying natural resources in Amazonian agroecosystems is an honorable duty, considering that the livelihoods of many people can be improved with such scientific knowledge. It is anticipated that new insights generated in the doctorate thesis will support many public policies in the region. The natural resources described in the present study, i.e., soil and Brazil nuts, were proven to have very heterogeneous intrinsic characteristics and a diverse composition throughout the Amazon basin, which reflects the environmental (climate, relief, parent material, and organisms) particularity of each state and site in the Brazilian Amazon. Therefore it is emphasized that generalizations about the whole Amazon region cannot be made concerning the occurrence of essential and toxic trace elements in soils and Brazil nuts and each site should be dealt taking into account its environmental specificity.

The contributions to the scientific knowledge on Amazonian resources provided by the present study are also meant to reach the widest possible public and not be retained among the scientific community. The information should be spread into the local communities that work directly with the Brazil nuts in their daily life and use it as a source of income. Therefore, the main current challenge is to make all this information available for society, which is only possible if we can handle to bring together our scientific knowledge with the practical know-how obtained by the local communities during the next years. This can be done by giving incentives to researchers, professors, and professionals of forestry and agronomy to be better informed on the current research and pass this knowledge by providing courses and field lessons to update these communities.

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