Lead contamination in cassava-based food and tubers (*Manihot esculenta* Crantz) in French Guiana: evidence for soil-to-plant transfer as the primary source.

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Abstract

This study investigates lead (Pb) contamination in cassava in French Guiana, focusing on its sources, accumulation processes, and. Previous studies in the area have shown that cassava-based products could be a source of lead in diets, raising concern about human exposure. The results show that cassava tubers often contain lead concentrations and 72% of the analysed samples (n=81) exceed international safety thresholds. However, processing steps such as grating (n=19), pressing (n=9), and cooking (n=30) do not increase lead levels, contrary to previous assumptions. Final products like cassava semolina (*couac*, n=17) and flatbreads (*cassaves*, n=13) reflect the median concentrations already present in raw tubers, indicating that contamination mainly originates from soils rather than processing tools and cooking utensils. The study highlights the importance of soil properties in lead uptake by these tubers. Soils rich in fine particles and low in organic matter tend to promote higher lead accumulation in cassava. Additionally, some cassava varieties, particularly yellow ones, appear to accumulate more lead than others. From a health perspective, regular consumption of cassava-based foods may pose risks, especially for children and pregnant women. However, certain products such as cassava starch (n=4) seem to contain very low lead levels and may represent safer dietary options. In conclusion, lead contamination in cassava is primarily

environmental in origin and not linked to processing practices. Further research is needed to better understand soil-to-plant transfer mechanisms and to develop strategies that reduce exposure risks for local populations.

1. Introduction

Metallic elements, such as aluminum (Al), iron (Fe), chromium (Cr) zinc (Zn), lead (Pb), cadmium (Cd) and mercury (Hg), are naturally present in Amazonian soils, but human activities can amplify their quantity and modify their biogeochemical cycle (*e.g.* Carmouze et al. [1], do Nascimento et al. [2] and Cartró-Sabaté et al. [3]). Lead contamination from human activities, such as industrial activities, mining and the use of Pb-loaded gasoline, has been an important public health issue worldwide (*e.g.* Monna et al. [4], Bollhöfer & Rosman [5-6], Eichler et al. [7] and Křίbek et al. [8]), including in Amazonia [9-11].

Depending on the degree and duration of exposure, lead can have various health effects on human beings [12-13]. Some of these effects can result in changes to the nervous and immune systems, kidney problems, cardiovascular disorders, and may affect the cognitive and behavioral developments [12-13]. In 2019, for instance, global lead exposure accounted for 5.5 million cardiovascular disease deaths and a loss of 765 million IQ points among children [13]. Preliminary studies have found elevated blood lead levels among pregnant women and children in western and southeastern French Guiana, respectively [14-15], revealing real health concerns about lead intoxication throughout the region. In addition, Santé Publique France highlighted that between 2015 and 2018, the incidence of lead poisoning (more than 50 $\mu g.dL^{-1}$ of lead in blood) was 206.9 cases among 100,000 children in French Guiana, against 4.3 in mainland France [16]. Lead isotope ratios confirm the dietary origin of lead exposure of the inhabitants, particularly in the upper Oyapock River basin [15]. Two main sources have been highlighted: wild game and cassava consumption [14-15].

Hunting is a highly valued practice in French Guiana, particularly among Indigenous peoples and Afro-descendant Maroons, and game meat is still an important part of the dietary intake [17]. However, contamination by lead ammunition is commonplace [18-21]. In addition to exposure through game meat, cassava represents another potential source dietary exposure pathway. Although cassava-related contamination is less documented in South America, it has been reported from Africa (*e.g.* Kříbek et al [8] and Udiba et al. [22]), often in relation to contaminated soils, and cassava flour. In Brazil, Barbosa et al. [23] found elevated concentrations of Pb in cassava, which increased 10-fold following transformation by roasting on large metal pans, and its consumption has been identified as an important risk factor in the Brazilian Amazon [24].

The diverse population of French Guiana, which includes Creole, Maroon and Indigenous peoples [25], still heavily relies on cassava (*Manihot esculenta* Crantz; Euphorbiaceae) as their main staple crop. Its tuberous root is the basis of several foods that are locally known as *cassave* (cassava flatbread), *couac* (torrefied cassava semolina), tapioca (cassava starch), *tucupi* or *kwabio* (boiled juice) or *cachiri* (cassava beer); the plant is a cultural keystone species [26-27] regarding not only food, but also culture and social organization for many generations [28]. Cassava is cultivated in what is known in French Guiana as *abattis*, or fields that form part of swidden and fallow agriculture [29-30], which hosts several functions: nutritional, economic, and socio-cultural. Cassava (essentially bitter varieties) is produced for subsistence, for direct and daily consumption, but also for commercial purposes [27]. Most members of the community take part in this agriculture. The plant is grown in *mayouri*, a kind of collective work where a family invites their relatives to work, providing food and cassava beer, and working in other families' swidden reciprocally [31]. This organization is necessary to cultivate large plots of land and to process large quantities of cassava, although the final products vary regionally. For example, the Pahikwene, located around Saint-Georges-de-l'Oyapock and Trois-Palétuviers (not included in the study) villages on the lower Oyapock River, prepare large quantities of cassava semolina, while the Wayãpi, located around Camopi and Trois-Sauts villages on the middle and upper Oyapock River, rather produce cassava flatbread and cassava beer.

Everywhere, cassava processing is still artisanal, and the tools used in the more remote localities (Trois-Palétuviers or Trois-Sauts) are often handmade. Traditionally, all the tools needed to process cassava tubers into food and beverages were made by men in the community, particularly basketry implements woven from plant fibers, such as the cassava press and the sieve [32]. To grate cassava, manual graters inlaid with stone and then metal elements have now been replaced by motorized graters [33-34]. To cook cassava into semolina and flatbreads, clay pans have now been replaced by large roasting pans frequently made from crafted cast-iron sourced in recycled materials, bought in Brazilian convenient stores selling hardware [35]. Today, although some wickerwork tools are still in use, particularly in Trois-Sauts, the manual cassava press has increasingly been replaced by a mechanical press made from wood [33-35], and large used tins often showing tracks of rust. Our field observations reveal that large troughs originally made of wood for fermenting cassava are now being replaced by old refrigerators or freezers.

The use of these metal tools was highly regarded as the possible source of lead in cassava products in French Guiana, a hypothesis supported by evidence in Brazil showing increased Pb levels following processing with metal equipment [23]. These concerns prompted the Regional Health Agency of French Guiana (*Agence Régionale de Santé Guyane*; ARS) to fund this study with the goal to better understand the complex dynamics of lead accumulation cassava, from the tuberous roots (although cassava produces tuberous roots, the term "tubers" is used in this paper for simplicity) to the final products consumed by the population.

Our objectives were thus to decipher i) the determinants of lead accumulation during cassava processing from the tuber to the (liquid and solid) final products, ii) at which levels it was present in the raw tuber, iii) with variations among varieties and locations, soil types, iv) at which step the lead possibly accumulates, and v) which step eventually detoxifies or concentrates it. We thus set up an interdisciplinary method combining ethnography and analytical chemistry. Ethnographic studies were carried out within the two Indigenous communities concerned by high lead exposure: the Pahikwene at Saint-Georges-de-l'Oyapock (SG) and the Wayãpi at Trois-Sauts (TS), to assess cassava tuber processing practices and identify specific stages or operations that are prone to the introduction of lead. Soil and cassava samples collected at various stages of the processes involved in transforming raw cassava tubers into different food products were analyzed to determine their lead content among other variables.

2. Materials and Methods

2.1. Study area

Fieldwork took place from April and June 2022 (rainy season) in French Guiana, in Saint-Georges-de-l'Oyapock and between July and August 2022 (dry season) in Trois-Sauts, upstream (Fig. 1). The former is mostly inhabited by Pahikwene (commonly referred to as Palikur in French and English, Pahikwene and Parikweneh are their self-designated endonyms) Indigenous people from the Arawakan linguistic family [36], while the latter is inhabited by Wayãpi Indigenous People from the Tupi-Guarani linguistic family [37]. Sites were selected because cassava cultivation is extensively practiced today, and is an important part of cultural habits, as well as being the main staple food and pillar of local Indigenous food systems [27].

Fig. 1: Sampling sites and Oyapock River basin map. In SG (zoom A), sites 3, 6, 7, 9, and 11 correspond to *Wacapou* area in this study, while other sites are for *Crique Gabaret*. Zoom B shows the different wayãpi villages and the sampled areas. Basemap: Natural Earth (public domain), MapBiomas Brazil (public domain), Parc Amazonien de Guyane (Licence Ouverte Etalab 2.0). Sampling locations, villages and rivers: this study. Maps were done with QGIS 3.40.10.

2.2. Consultations

This study was carried out after prior consultation with and oral approval from Pahikwene and Wayãpi traditional authorities. Administrative permits were granted by the Prefecture de Guyane to access the restricted access area if the municipality of Camopi (authorizations n° 2022-23 and n°2025-04).

Data were initially collected using an ethnographic approach. This involved conducting semi-structured interviews based on a list of topics to be covered, as well as informal and spontaneous interviews alongside participant observation by participating in activities related to the transformation of cassava tubers at swidden and fallow fields and observing each step of the operational sequence. Sampling occurred concomitantly with consultations. Altogether, 21 families were interviewed while working in the abattis, with various other people present during the discussions. Family or community assistance with the transformation process involved between four and fifteen people. However, the individuals leading the production were six men and five women in Saint-Georges, and two men and eight women in Trois-Sauts.

Exchanges were carried out in the interviewees' languages of preference, namely French and French Guianese Creole, as well as Parikwaki and Wayãpi, with the help of an interpreter. Participants were selected based on the criteria that they partake in cassava agriculture and were identified by snowball sampling. Names were initially provided through existing relationships between community members and three of the authors (DD, GO, and MR) who have extensive experience working with Pahikwene and Wayãpi communities [27, 35, 38-43], and by consulting with local village Chiefs (*i.e., Chefs coutumiers*).

2.3. Sampling strategy

Raw cassava tuber samples were collected in farms during several processes leading alternatively to cassava flatbread (*cassava*), semolina (*couac*) or beer (*cachiri*), which were fully documented and sampled at each step and between each step (Fig. 2). The cassava tubers transformation processes are complex and involve physical, chemical and biological processes [44]. Sweet potato (*Ipomoea batatas* (L.) Lam.; Convolvulaceae) and yam (*Dioscorea* spp.; Dioscoreaceae) tubers, which are optionally included in the production of cassava beer, were also collected. Pictures of tools used at transformation steps are gathered in figures A and B S1 text. In total 10 fields in SG and 9 in TS (Fig. 1) were sampled. Local names and descriptions of the varieties were identified with farmers, and raw samples were collected at different locations of the field to cover spatial heterogeneity. A piece of one tuber from each plant was collected. Occasionally, a composite sample was done by sampling multiple pieces of different tubers from the same plant (later homogenized). Bitter cassava tubers are broadly classified by colour (*i.e.*, yellow and white). Tubers were peeled, cleared of mud with deionized water and frozen at -18°C before being freeze-dried until all water content was removed. Fresh and dry weight were taken to calculate an average moisture content for tuber. For liquid products, the difference between fresh and dry weight was used to calculate the water content and use either a dry weight concentration (in $\mu g.g^{-1}$) or a concentration in liquid (in $\mu g.L^{-1}$). For each cassava tuber sampled, a 10 to 20 cm depth of corresponding topsoil was collected. All soil samples were dried at 40°C and sieved at 2 mm for homogenization.

Composite samples (multiple aliquots of the same batch) from transformation steps and final products (Fig. 2) were collected at every relevant step (Fig. 2), frozen at -18°C, and freeze-dried (until all water content are removed) before analysis. Solids were stored in Ziplock bags and liquids in 50 mL, 10% HCl (Trace Metal Grade) cleaned Falcon tubes. Cassava flour and sun-dried cassava flatbread were not frozen and/or freeze-dried. Additional cassava semolina samples were collected in Saint-Georges-de-l'Oyapock and Cayenne markets. Most of the freeze-drying was done at Geosciences Environnement Toulouse (GET) facilities in Toulouse (France), whereas several were done at the Ecology, Evolution, Interactions of Amazonian Systems Laboratory (LEEISA) in Cayenne (French Guiana) before being shipped out. Number of samples per field and the type of products collected are summarized in S1 and S2 tables.

Fig. 2: The various transformation steps of raw bitter cassava. Red arrows correspond to sampled steps. Dashed lines departing from sweet potatoes and yam tubers represent optional pathways in the operational sequence leading to cassava beer.

2.4. Soil physico-chemical parameters measurements

Cation Exchange Capacity (CEC) and pH were measured at the GET laboratory, while grain size composition and analyses of total and organic C, H, N and S were performed at the Biodiversity and Environment Research Center (CRBE) in Toulouse (France). For pH measurements, 5g of sieved soil samples were dissolved in 25mL of ultrapure water (milli-Q®, Merck) and agitated for 1h. After centrifugation, the pH of the supernatant liquid was measured with a Mettler Toledo FiveEasy Plus®. CEC was determined with cobaltihexamine [45], 2g of sample was suspended in 30mL of a cobaltihexamine solution at 20.8 mmol.L^{-1} and agitated for 1h. Samples were then centrifuged and filtered, and the supernatant was analyzed by spectrophotometry (Cary-50, Agilent®) at 475 nm. Grain-size distributions of the <2mm fraction of sediment were measured in ultrapure water by light scattering using a LA-960V2 Laser Particle Size Analyzer from Horiba® according to NF ISO-13320 standards. Each sample was measured in duplicate. C, H, N, S and organic carbon (all in %) were measured with an elemental analyzer Flash 200 Thermo Scientific® after decarbonation (organic carbon only) according to NF ISO-10694/ NF ISO-13878 standards.

2.5. Determination of total and phyto-available Pb concentrations

All sample preparations and analyses were done in GET. Soil samples were digested on hot plates in a white room for total Pb concentrations. 50 mg of sample was digested for 12h at 120°C with reverse aqua regia (1 dose of bi-distilled HCl and 3 doses of bi-distilled HNO_3). After evaporation at 70°C, the residue was digested with a mixture of 3mL of bi-distilled HNO_3 and 1mL of HF (Trace Metal Grade) for 12h at 80°C. After total evaporation, the residue was dissolved in HNO_3 2% and diluted prior to analysis. San Joaquim soil (SRM 2709a, NIST©) was used to check the recovery of the digestion. Average lead concentration measured for San Joaquim soil was 17.7 ± 2.1 µg.g^{-1} (n=12), which represents a recovery of 102.5 ± 11.7 % compared to the certified value (17.3 ± 0.1 µg.g^{-1}). No Pb was detected in digestion blanks. In addition to total Pb concentrations, soil phyto-available fraction from soil samples was obtained following the Mehlich III protocol [46]. Briefly, 2g of dry soil was mixed with 20 mL of Mehlich solution, and agitated for 1h.

Samples were then centrifuged, filtered, and acidified to reach HNO_3 2% (v/v) solutions. Prior to ICP-MS analysis, they were diluted 100 times.

Cassava tuber and cassava transformed products were micro-wave digested (CEM® Mars 6 with iPrep vessels). Briefly, 100mg of sample was digested with 12mL of bi-distilled HNO_3 using the program "plant tissue", consisting of 30 min ramp to 200°C, followed by a holding time of 10 min before cooling. After total evaporation, the residue was dissolved in HNO_3 2% and diluted prior to analysis. Apple leaves certified sample (SRM 1515, NIST©) was used to check the recovery of the digestion. Average lead concentration measured for SRM 1515 was 0.430 ± 0.067 $\mu g.g^{-1}$ (n=15), which represents a recovery of 91.6 ± 14.2 % compared to the certified value (0.470 ± 0.024 $\mu g.g^{-1}$). No Pb was detected in digestion blanks.

For digestions and Mehlich III protocols, every 10 samples triplicates were done. Otherwise, only one digestion was performed by sample. For triplicates, average and standard deviation of the three measured concentrations were reported as concentration uncertainties while propagation of uncertainties from Pb concentration measurements and solution dilutions were used for other samples.

Lead concentrations were measured with a triple quad inductively coupled mass spectrometer (QQQ-ICP-MS) ICAP TQ from Thermo Scientific®. Indium and Rhenium were used as internal standards. Calibrations were controlled with SLRS-6 certified river samples (NRC, Canada). Average measured (± SD) concentration for SLRS-6 was 168.0 ± 19.0 $ng.L^{-1}$ (n=14) for a certified value at 170.0 ± 26.0 $ng.L^{-1}$ (NRC, Canada). Limits of Detection (LOD) and Quantification (LOQ) were respectively 0.008 and 0.026 $ng.L^{-1}$ in HNO_3 2% (v/v) and 0.014 and 0.048 $ng.L^{-1}$ in diluted Mehlich solution. All concentrations reported here (in solids or liquid samples) are over quantification limits for each matrix and have been corrected for the dilution factors and digested masses. Samples indicated as not detected mean that regardless of the dilution factor tested for analysis, concentrations were below reported LOD.

2.6. Data analysis

Bio-accumulation factor (BF) and soil phyto-available lead fraction ($\%Pb_{Phyto\text{-}av}$ of total Pb) were calculated to assess the proportion of total Pb by soil transfer to cassava tubers and the percentage of phyto-available Pb, respectively. BF is calculated as in equation 1, while $\%Pb_{Phyto\text{-}av}$ in equation 2 (Eq. 2):

$$BF = \frac{[Pb]_{tuber}}{[Pb]_{soil}} \quad (1)$$

$[Pb]_{tuber}$ is the lead concentration in tuber and $[Pb]_{soil}$ is the lead concentration in topsoil (10-20 cm), both in $\mu g.g^{-1}$ and dry weight.

$$\%Pb_{Phyto-av} = \frac{[Pb]_{Phyto-av}}{[Pb]_{soil}} \times 100 \quad (2)$$

$[Pb]_{Phyto\text{-}av}$ is the phyto-available lead concentration estimated with Mehlich III protocol and $[Pb]_{soil}$ is the lead concentration in the topsoil, both in $\mu g.g^{-1}$ and dry weight.218

2.7. Statistical analysis

Statistical analysis was done with RStudio® (Version 2024.09.1+394) using R-4.4.2 and standard packages [47]. R packages "FactoMineR" [48] and "factoextra" [49], cluster [50] and rstatix [51] were used.

Shapiro- wilk tests were used to verify normality of the data. skewed data were not transformed. Lead concentrations gathered by transformation steps (raw tuber to final products) showed skewed distribution except for cassava semolina in SG, burnt flatbread in TS and cassava beer in TS which are normally distributed. However, considering the low numbers of observations for those groups ($n<10$), only Kruskal-Wallis tests were done for group comparisons. When the test was significant, a Dunn test with Holm's correction was used to determine the groups that were significantly different. Paired comparison tests were done with Mann-Whitney tests. Median comparisons to threshold were done with one-sample Wilcoxon signed-rank tests. Lead concentrations in tuber gathered by vernacular names showed normal distribution for some groups and skewed ones for others. Given the low numbers of observations for those groups, paired comparison with Mann-Whitney tests only were performed. In all cases, a confidence level of 0.95 was chosen. Medians were significantly different when the p-value was less than $\alpha = 0.05$. Effect sizes, epsilon-quared (ε^2) for Kruskal Wallis were calculated. During statistical analysis, for not detected data (some transformed products, see tables S1 and S2), the retained value was 0.001 $\mu g.g^{-1}$ which was the lowest concentration observed in this study.

To assess relationships between the different variables (granulometry, total lead concentrations in tubers and soils, % of phyto-avalaible lead in soils, pH, CEC and soil chemistry), a principal component analysis (PCA, with Spearman coefficient) was performed on a correlation matrix calculated using the Spearman coefficient as most of the data showed a skewed distribution. Individuals were clustered using k-mean clustering on the first five principal component scores using 100 random initializations. The optimal number of clusters was determined using the gap statistics (1 to 10 clusters with 50 Monte Carlo bootstrap reference datasets) and then selected according to the *firstmax* criterion. Significance of clusters was calculated using $R^2_{clustering}$ as follows in equation 3:

$$R^2_{\mathrm{Clustering}} = \frac{\mathrm{SSB}}{\mathrm{TSS}} \quad (3)$$

where SSB is the Sum of Square Between, corresponding to the intra-cluster variance (which must be low for well representative clusters), and TSS is the Total Square Sum, corresponding to the total variance, *i.e.,* the sum of SSB and the inter-cluster variance, or Sum of Square Within (SSW). In addition, the silhouette index was calculated to check cluster separation.

3. Results

3.1. Lead concentrations in tubers (bitter cassava and others)

Measured median lead concentration in bitter cassava tubers is 0.357 $\mu g.g^{-1}$ (dry weight, dw), while average concentration is 0.616 $\mu g.g^{-1}$ (dw) and standard deviation is 0.709 $\mu g.g^{-1}$ (dw). Maximum measured lead concentration is 3.543 $\mu g.g^{-1}$ (dw) and minimum is 0.002 $\mu g.g^{-1}$ (dw). All values are summarized in S1 and S2 tables, whereas general statistics are in Table A S1 text. Lead concentrations in bitter cassava tubers are slightly, but not significantly (p-value = $6.65.10^{-2}$), higher in TS compared to SG based on median values, 0.543 and 0.282 $\mu g.g^{-1}$ (dw) respectively (Fig. 3).

Fig. 3: Box plots f lead concentrations distributions in cassava-based products during transformation steps. A) for all dry products from all locations (including 8 cassava semolina from local markets); B) for liquid products from all locations. Cassava beers made with boiled tubers are made with sweet cassava tubers; C) ~~is~~ for dry products from Saint-Georges-de-l'Oyapock; D) for dry products from Trois-Sauts. Lead consumption limit for tubers and dry products corresponds to 0.19 $\mu g.g^{-1}$ (dw), based on ANVISA, Resolução - RDC n°42 [53], CXS 193-1995 [54] and UE regulation 2023/915 [52]. Limits for mineral water, juice and nectar are from CXS 193-1995 [54] and UE regulation 2023/915 [52]. Statistics are summarized in table A

S1 text and in table 1 for cassava semolina. Letters (a, b, c) indicate distinct statistical classes (p-value < 0.05) between categories.

The European Commission from the European Union (Commission Regulation 2023/915 [52]), the Ministry of Agriculture and Livestock of Brazil (ANVISA, Resolução - RDC n°42 [53]) and the Food and Agriculture Organization of the United Nations (CXS 193-1995 [54]) recommend a threshold value at 0.1 $\mu g.g^{-1}$ (fresh weight, fw) for tuber ready for human consumption [52-54]. Considering all results are dry weight, the latest threshold (fw) is converted into dry weight, using an average tuber water content of 46%, based on our sampling and measurements. Consequently, the retained threshold is 0.19 $\mu g.g^{-1}$ (dw). Median concentration for bitter cassava tubers is significantly higher than this international limit value for edible tubers entering in the human diet (p-value = 6.19 $.10^{-7}$, fig. 3A), which indicates that 72% (58 over 81) of all analyzed tubers concentrations exceed this threshold. In SG, median concentrations are significantly higher than 0.19 $\mu g.g^{-1}$ (dw, p-value = 7.55 $.10^{-4}$, Fig. 3C) as well as in TS (p-value = 2.75 $.10^{-5}$, Fig. 3D) with 70% of (32 over 45) and 75% (27 over 36) exceeding the threshold value, respectively.

No clear spatial patterns can be observed in TS, however, sub-localities present different concentrations in SG, where two important areas were cropped: one called *Wacapou* and the other called *Crique Gabaret* (Fig. 1). Concentrations in bitter tubers are significantly higher in *Wacapou* (n=23) compared to *Crique Gabaret* (n=22), with median concentrations being 0.349 and 0.207 $\mu g.g^{-1}$ (dw, p-value = 0.039), respectively (Table A S1 text). At the plot scale, in both TS and SG, lead concentrations in bitter cassava tubers are widespread and show a wide range of concentrations (Fig. 3A, 3C and 3D).

Other tubers (yams and sweet potatoes) have significantly lower lead concentrations than overall bitter cassava tubers from all locations, with a median concentration equal to 0.058 $\mu g.g^{-1}$ (dw) compared to 0.357 $\mu g.g^{-1}$ (dw, p-value = 7.63 $.10^{-3}$) (Fig. 3A). The same trend is observed in TS, where other tubers were sampled, with bitter cassava tubers having significantly higher lead concentrations with 0.058 $\mu g.g^{-1}$ (dw) and 0.543 $\mu g.g^{-1}$ (dw, p-value = 4.78 $.10^{-3}$) respectively (Fig. 3D). Despite low median concentrations, yams and sweet potatoes are not significantly different than 0.19 $\mu g.g^{-1}$ (dw, p-value = 0.44), however, only six samples of this category were collected during this study, and more samples are needed to confirm the trend.

3.2. Lead concentrations in processed products

Lead concentrations (dry weight) at different stages of cassava tuber transformation present significant differences when all types of samples (excluding liquids) from all locations are considered and most of them exceed the international threshold for Pb in tubers (Fig. 3A). This includes samples collected from cassava semolina purchased in different markets of French Guiana (Fig. 3A and table 1). Kruskal-Wallis tests showed significant differences between groups (p-value = $1.36\ .10^{-3}$, moderate size effect as ε^2=0.110). with starch and roasted starch being significantly lower than the rest (Fig. 3A and 3C). Post hoc pairwise comparisons using Dunn's test with Holm adjustment showed that only starch (Pb concentrations between 0.001 and 0.042 $\mu g.g^{-1}$, dw, see S1 and S2 tables) differed significantly from fresh cassava tubers (adjusted p = 0.011), whereas no other pairwise comparisons were statistically significant (adjusted p > 0.05). No significant differences were also observed when transformation steps are compared step by step using Mann-Whitney tests (all p-value > 0.05, Fig. 3A) and all lead concentrations were similar or lower than median lead concentrations in tubers (Fig. 3A and table A S1 text). Finally, the evolution of mean and median lead concentrations during transformation steps showed slopes at or close to 0 (Fig. C S1 text), which highlights the absence of variation in lead concentrations during cassava transformation for all locations.

Lead concentrations in liquids (cassava juices and cassava beers) are expressed in $\mu g.L^{-1}$ (Fig. 3B). For statistics, all types of cassava beers are combined. All, except one cassava juice, are below the limit of 30 $\mu g.L^{-1}$ recommended for juices and nectars by CDX 193-1995 [54] and UE regulation 2023/915 [52] (Fig. 3B) and both cassava juices and cassava beers are significantly lower (p-values = 0.039 and 0.031, respectively). On the contrary, some samples are higher than the threshold for mineral water (10 $\mu g.L^{-1}$, [52, 54]) even though most of them are close or below. This is particularly true with cassava beers made with boiled sweet (opposite to bitter) tubers (Fig. 3B). However, no significant differences from this threshold are found between 10 $\mu g.L^{-1}$ and both cassava juices and beers (p-values = 0.82 and 0.16, respectively).

In SG, Kruskal-Wallis test showed ~~no~~ significant differences between transformation steps (p-value = $2.7\ .10^{-3}$, with large size effect as ε^2=0.178), except for starch as already mentioned and which could explain the large size effect in the test. Indeed, post hoc pairwise comparisons showed that starch contained significantly lower lead concentrations than fresh tubers (adjusted p = 0.010), grated cassava (adjusted p = 0.031), and pressed cassava (adjusted p = 0.050). No significant differences were detected among the other processing stages (adjusted $p > 0.05$). Pb concentrations in final products (flatbread and semolina) are quite homogenous with median concentrations varying between 0.099 and 0.254 µg.g^{-1} (dw, Fig. 3C and table A S1 text). During transformation steps, lead concentrations are significantly different (all p-values < 0.05) from the 0.19 µg.g^{-1} (dw) limit. However, only semolina as a final product, is significantly higher (p-value = 0.016), whereas starch is significantly lower (Fig 3C). Flatbreads (with different proportions of starch) are not significantly different and close to the limit of 0.19 µg.g^{-1} (dw). Furthermore, no significant differences were observed between the different production sites in SG (p-value = 0.36), *Wacapou* and *Crique Gabaret* (table 1), whereas lead tuber concentrations are significantly higher in the first (see section 3.1). Finally, no significant differences (p-value = 0.73) were observed between cassava semolina purchased from markets in French Guiana and those produced by participants of this study (table 1). In TS, the same results as in SG were observed and no significant difference is reported between processing steps (Fig. 3D). Kruskal-Wallis test was not significant (p-value = 0.16 with small size effect as ε^2=0.045). The variability is also visible at all stages of processing, as emphasized by the quartiles of each transformation in figure 3D. None of the steps presents lead concentrations significantly different from 0.19 µg.g^{-1} (dw) but ready-to-eat cassava flatbread highlight variable concentrations, from 0.098 to 0.990 µg.g^{-1} (dw).

Table 1: Cassava semolina lead concentrations in µg.g^{-1} (dry weight) from different locations gathered in this study. N/A = Non-Applicable.

	n=	Average µg.g^{-1} dw	SD	Median	Min	Max
Cassava semolina from SG	8	0.293	0.124	0.254	0.191	0.583
Cassava semolina from SG – Wacapou area	4	0.248	0.058	0.238	0.191	0.326
Cassava semolina from SG – Crique Gabaret area	3	0.256	0.020	0.252	0.239	0.279
Cassava semolina from TS	1	0.189*	N/A	N/A	N/A	N/A
All	9	0.244	0.047	0.246	0.189	0.583

*measured value

3.3. Lead concentrations in soils, BF and phyto-available fractions

Individual data are gathered in S1 and S2 tables-while statistics are in table A S1 text. Median Pb concentrations in soils in TS is 11.14 µg.g^{-1} (n=31), while it reaches 19.68 µg.g^{-1} (n=48) in SG lower than the median measured in French soils of 27.8 µg.g^{-1} calculated on several thousand soil samples (n= 2114, incl. French Guiana and overseas territories [55]). Lead concentrations are significantly higher in SG soils compared to TS (p-value = 2.53 $.10^{-5}$). Maximum and minimum Pb concentrations are respectively 72.84 and 8.53 $\mu g.g^{-1}$ for SG and 37.06 and 5.45 $\mu g.g^{-1}$ for TS. In SG, Pb concentrations in soil in *Wacapou* are significantly higher than in *Crique Gabaret* (p-value = 6.81 $.10^{-3}$). Median Pb in soils are respectively 22.40 (max = 72.84, min = 8.53) and 17.79 (max = 37.88, min = 8.80) $\mu g.g^{-1}$. Despite higher lead content in SG, BF is significantly higher in TS compared to SG (p-value = 4.42 $.10^{-3}$) with median values being 0.038 (max = 0.231, min = 0.003) and 0.015 (max = 0.123, min = 0.001) respectively. In SG, BF in *Wacapou* and *Crique Gabaret* are not significantly different (p=0.212), even if median BF is higher in *Wapacou* than in *Crique Gabaret* (0.022 and 0.012, respectively.

$\%Pb_{Phyto\text{-}av}$ is significantly higher in TS (med = 7.6%, max = 39.1%, min = 3.6%) than in SG (p-value = 2.43 $.10^{-10}$) with a median value at 2.9% (max = 26.3%, min = 0.7%). Besides, $\%Pb_{Phyto\text{-}av}$ is significantly higher in *Wacapou* compared to *Crique Gabaret* (p-value = 0.031), with median values being 2.7 and 3.1%, respectively.

3.4. PCA/Clustering to assess Pb uptake from soil to tuber

PCA analysis was run without distinction of territories to assess relationships between Pb in cassava, Pb in soils, physico-chemical and chemical compositions of soils (Fig. 4). The aim is to understand which parameters control Pb uptake from soil to tuber. First two axes explain 60.2% of the total variation, while BF was used as supplementary axis (Fig. 4). Hence, first and second principal components explain 43.2% and 17.0% of the variation (Fig. 4).

Fig. 4: Principal component analysis biplot for 14 variables (black arrows). Bio-accumulation factor (BF, bold dotted arrow) is a supplementary variable (no influence on axis). 73 individual samples are represented (those with too many missing variables were removed). Clusters were determined by k-means clustering (k=3). $R^2_{clustering}$ is equal to 0.58 and average silhouette is 0.35. % Clay, % Fine Silts, % Sand, % C_{org}, % C_{inorg}, % H, % S, % N, CEC, pH, C/N and % $Pb_{phtyo\text{-}av}$ are in soils.

Proximity of Pb concentrations in soils with fine silts (% Fine Silts) and clay (% Clay) fractions suggest that they are highly correlated (Fig. 4), meaning that Pb concentrations are higher when fine particle contents increase. This is also confirmed by the correlation matrix, where higher

Spearman coefficients are observed for the same variables (table B S1 text). Conversely, Pb in soils appears opposite to sand content and Pb phyto-available fraction (Fig. 4), which indicates lower Pb concentrations in sandy soils where, however, the phyto-available Pb fraction is more important than soils with higher clay contents. Moreover, Pb concentrations in soils are quite well correlated with C_{org} (%C_{org}) and H (%H) soils contents, while weakly correlated to nitrogen (%N), sulfur (%S) and C/N ratio (fig. 4 and B S1 text). Finally, Pb in soils have no relation with CEC and pH, as suggested by the near right angles between arrows (Fig. 4). Pb in tuber is positively correlated with fine size particles (clays and fine silts) and Pb concentrations in soils (Fig. 4). However, it presents poor correlation (near right angles between arrows) with most of the other variables (Fig. 4). BF is poorly to not correlated to soil granulometry while it is negatively correlated to soil chemistry with parameters such %H, %C_{org} or %N (Fig. 4).

Three clusters were identified with a k = 3, which correspond to the first and only maximum gap. $R^2_{clustering}$ is 0.58 and silhouette index is 0.35, which means well-identified clusters that could, however, overlap in some cases as highlighted by figure 4. Box plot comparing the 14 variables in figure 4, plus BF for the three clusters, are in figure D S1 text. Statistics are summarized in table C S1 text. Cluster 1 (C1) gathers 30 samples and is strongly associated with soil chemistry and to a lesser extent to Pb concentrations in soils and fine particles (clay and fine silts, Fig. 4). Cluster 2 (24 samples, C2) is linked to sand fraction (% Sand) and phyto-available Pb fraction while anti-correlated to soil chemistry, Pb concentrations in soils and tubers and fine particles (Fig. 4). Lastly, cluster 3 (C3), gathering 19 samples, is strongly correlated to Pb concentrations in tubers and to a lesser extent to Pb concentrations in soils and fine particles (clay and fine silts, Fig. 4). BF is also mostly associated to C3. C1 is exclusively represented by samples from SG while only three samples (over 24) found in C2 are from SG. C3 is shared between all locations.

3.5. Lead concentrations in tubers and relations to tuber colors and local names

Two colors of tubers were observed, yellow and white. Sixteen different varieties of bitter cassava and 1 variety of sweet cassava were identified in SG based on their local names. The difference between sweet and bitter cassava is explained by their cyanogen contents, sweet cassava varieties containing much less cyanogen than bitter ones [56]. In TS, 19 varieties of bitter cassava and one of sweet cassava were identified. Statistics for lead concentrations are gathered in table D S1 text.

Overall, yellow tubers are generally more concentrated than white ones (median: 0.479 and 0.237 $\mu g.g^{-1}$ dw, respectively) but not significantly (p-value=0.10).

In SG, Pb concentrations are significantly higher in yellow tubers compared to white ones (p-value = 5.4 $.10^{-3}$) with medians being respectively 0.365 $\mu g.g^{-1}$ and 0.170 $\mu g.g^{-1}$ (dw, Fig. 5A). Considering clusters, in C1 (SG only), yellow tubers (SG only) are significantly more concentrated in Pb compared to white tubers (p-value = 0.019, Fig. 5A). C2 presents too small populations to draw statistics, however, all individuals concentrations are higher than in C1 (Fig. 5A). Finally, yellow cassavas are more concentrated in C3 but not significantly (Fig. 5A).

In TS, Pb in yellow cassava tubers is moderately higher than in white tubers but not significantly (p-value = 0.3) and medians are respectively 0.644 and 0.558 $\mu g.g^{-1}$ (dw, Fig. 5A). In C2, no difference appears between yellow and white cassava tubers (p-value=0.51, Fig. 5A). Only one sample from yellow tuber in TS is in C3 so it is not possible to compare it to white cassavas. However, ranges of concentrations for both colors are the highest observed in cassavas for this study (Fig. 5A).

Fig. 5: Box plots of lead concentrations in cassava tubers classified by cluster and based on: A) bitter cassava tuber colors (white or yellow) and B) local names of varieties. Box plots of Bio-accumulation Factor (BF) based on: C) bitter cassava tuber color and D) local names of varieties. Data and statistics of all categories are summarized in S1 and S2 tables and table A S1 text. Letters (a, b, c) indicate distinct statistical classes (p-value <0.05) within clusters. This was not considered for panels B and D due to low number of samples in categories.

Lead concentrations in *cramanioc* (local creole, *awava* in pahikwene), a sweet cassava variety, are lower than in bitter cassava varieties, and it is significant compared to Pahikwene varieties such as *huvavrie* and *wakukwakwivui* (fig. 5B; p-values = 7.93 $.10^{-3}$ and 2.82 $.10^{-3}$, respectively). Within C2, *cramanioc* tends to have less lead compared to *simo* (a Wayãpi variety, Fig. 5B) despite absence of significance due to the small number of samples. *Sansan,* a white Pahikwene bitter variety, seems to have less lead than yellow bitter varieties from similar growing conditions (clusters C2 and C3, Fig. 5B), but not significantly (see table E S1 text for details of comparison tests). *Bugik*, *kalisha* and *wakukwakwivui* tend to be less concentrated in lead than *huvavrie*, *kunani* (local creole, *kunanvan* in pahikwene) and *suwikivuy,* all six being yellow bitter varieties (Fig. 5B) but not significantly. With comparable conditions, here C1, *suwikivuy* tends to be more concentrated than *bugik* and *kalisha*, but again not significantly (Fig. 5B). Finally, *tawa* (in wayãpi), a yellow variety from TS have a similar Pb content compared to *simo*, also a white variety from TS (Fig. 5B). In any case, those results must be mitigated due to limited data and more replications for each variety would be necessary to achieve better statistical accuracy.

3.6. Lead bio-accumulation factor (BF) in tubers.

Statistics for BFs are gathered in table F S1 text. BFs for yellow tubers in general (BF_{median} = 0.024) are quite like BFs for white tubers in general (BF_{median} = 0.015) with no significant differences (p-value = 0.40). In SG, yellow tubers accumulate significantly more lead than white ones (p-value = 0.03, Fig. 5C) while there is not significant difference in accumulation between colors in TS, despite higher median BF for yellow tubers (p-value = 0.44, Fig. 5C). Finally, it appears there is significantly more accumulation in TS than in SG for both white and yellow tubers (p-value = $8.0 .10^{-3}$ and 0.04, respectively, Fig. 5C).

In C1 (specific to SG), yellow tubers accumulate significantly more lead (p-value = 0.001) than white ones (Fig. 5C). Regarding C2, SG tubers cannot be compared due to too low populations. However, in this cluster there is no significant variation in accumulation between colors in TS (p-value = 0.28, Fig. 5C). Considering this, accumulation is similar between yellow and white tubers in this cluster (p-value = 0.14, Fig. 5C) with BF_{median} being respectively 0.016 and 0.035. For C3, there is no significant difference between yellow and white tubers (p-value = 0.15, Fig. 5C) but white tubers from TS accumulate significantly more than white ones from SG (p-value = 0.016, Fig. 5C). Differences in colors cannot be compared in TS for this cluster due to low populations, nevertheless, in general, yellow and white tuber seem to equally accumulate Pb in C3 (p-value = 0.30, Fig. 5C). Eventually, yellow and white tubers accumulate significantly less Pb compared to their respective colors in both C2 and C3 (all p-values < 0.05) whereas there is similar Pb accumulations between colors in C2 and C3 (all p-values > 0.05).

Globally, *cramanioc* seems to accumulate less Pb than other varieties (Fig. 5D), even though no significant differences were observed. Statistics suggests that among C2 *cramanioc* are in the lower range of accumulation (tables S5 and S8). Generally, no significant differences are observed between varieties, only trends (Table G S1 text). In C1, *suwikivuy* presents the highest Pb accumulation with higher BF_{median} than both *kalisha* and *bugik*, the latest showing a large range of accumulation though (Fig. 5D). *Suwikivuy* tubers seem to accumulate in the same ranges as *huvavrie* and *kunani* (Fig. 5D). Finally, *sansan* and *wakukwakwivui* accumulate lead in the same range as *kalisha* or *cramanioc* for instance, which seem to be less than *suwikivuy*, *huvavrie* and *kunani* (Fig. 5D).

4. Discussion

4.1. Fate of lead during cassava transformation

At all scales, whether locally or combined, final products lead concentrations are always lower or ranging within tuber concentrations scattering which suggest no lead enrichment during the transformation steps (Fig. 3). Indeed, final dry products, cassava semolina, flatbreads and burnt flatbreads present the same range of concentration as raw tubers. For each transformation chain, steps have similar median or mean lead concentrations, with no significant differences and/or no variation of concentrations is observed. This suggests that tools, even metallic cooking utensils, used during the different steps of raw cassava tubers transformation, such as mechanical press from used tins, roasting pans bought in Brazil or Suriname, are not responsible of the lead levels measured in cassava end-products. Those results contradict findings made by Barbosa et al. [23], that implied the enrichment of lead in cassava products through transformation processes in communities along the Tapajós River, Brazil. One possible explanation is that processing equipment is not standardized across Amazonian regions. Although the processing tools used in the Oyapock region do not appear to contribute to Pb contamination, differences in the composition, manufacture, or provenance of processing equipment used elsewhere in Amazonia could potentially explain the contrasting observations. Concentrations are higher in general in TS compared to SG for each transformation step, which also suggests that differences in tools and techniques between TS and SG do not change final products' concentrations. Instead, the higher ranges and the higher concentrations recorded in TS' transformation chain are likely due to higher concentrations in tubers. Those results, combined with the absence of evidence of lead inputs by tools used, point at cassava tubers as the primary source of lead in transformed cassava products in Eastern French Guiana.

At all locations, while cassava juices contain lead (Fig. 3B), lead concentrations in grated cassava before and after squeezing show no differences in concentration (Fig. 3C and 3D). Unlike cyanides [56], squeezing does not appear to efficiently extract lead from cassava tubers . On the other hand, starch and roasted starch samples appear to be significantly less concentrated in lead compared to other products (Fig. 3A). Indeed, their concentrations are more than one order of magnitude lower. This raises the question of whether starch, as a food product, contains little to no lead because this metallic element does not bind to starch molecules, or because it is the result of lead leaching during starch production, consisting of cassava juice decantation with multiple water rinsing steps [27].

Water and starch in cassava tubers represent between 25 to 90 % of their total weight [57-59]. Lead speciation in cassava tubers or roots in general is poorly known, but as for other plant roots, we can speculate that phyto-available lead (which enters the roots) would be bound to reactive groups (*e.g.*, carboxyl and hydroxyl groups) from cell walls or would precipitate in extracellular compartments [60-61]. Only a small quantity of the lead taken up by tubers would be able to enter the cells [60-61]. This could explain why lead concentration in cassava remains steady during its transformation. Indeed, if most of the lead is bound to organic acid at the cell walls, the destruction of cells during grating and drainage would not change lead speciation. This bounded lead would remain in the pulps, while (a fraction of) precipitated lead and lead within cells would be evacuated with the juice (characterized by very low lead content, see Fig. 3B). Finally, starch is stored in amyloplast within root cells [62], which could explain the *quasi-absence* of lead in this product. To better determine those mechanisms, more studies about lead speciation in cassava tubers would be needed.

4.2. Level in food and implications for human consumption related to lead exposure

In all sub-sections, implications of lead level in cassava-based products were assessed with the benchmark dose lower confidence limit for neurotoxicity (BMDL, 0.50 $\mu g.kg^{-1}.d^{-1}$) as used in Carneiro et al. [24]. The goal here is to illustrate the amount of cassava-based products only, necessary to be consumed to reach this benchmark dose. Pahikwene and Wayãpi people consume other products in addition to cassava-based ones [27, 63], like wild games, that are additional sources of lead [14-15]. However, our study does not have the design to address this. Indeed, this would require a detailed characterization of exposure scenarios, including consumption patterns, exposure frequency and duration determined through food frequency questionnaires, anthropometric details, bioavailability assumptions and uncertainty treatments. Although the estimates of consumption quantities of cassava-derived foods, *i.e.,* cassava semolina (section 4.2.1), cassava flatbread and beer (section 4.2.2), cassava juice, starch and sweet cassava (section 4.2.3), provide exposure and public health context, they do not account for actual consumption patterns, cumulative exposure from other dietary and environmental sources, inter-individual variability, or uncertainty.

4.2.1. levels in cassava semolina

What is called *farinha* in Brazil, *couac* or *kwak* in French Guiana and cassava semolina in this study are the same products. In Brazil, Barbosa et al. [23] reported a median lead concentration of 0.19 $\mu g.g^{-1}$ (dw) in cassava semolina, ranging between 0.09 and 0.38 $\mu g.g^{-1}$ (mean ± SD = 0.19 ± 0.10 $\mu g.g^{-1}$ dw, n=6). Carneiro et al. [24] observed a median lead concentration of 0.25 $\mu g.g^{-1}$ (dw, n=30), ranging between 0.05 and 0.98 $\mu g.g^{-1}$. Those results are like the values measured in this study (table 1). These studies consistently indicate that cassava semolina from different Amazonian regions contains comparable Pb concentrations, suggesting that elevated Pb levels are not restricted to the Oyapock basin but may represent a broader regional issue. As highlighted by Carneiro et al. [24], these concentrations warrant a health risk consideration from a public health perspective, and cassava-based food has previously been identified as a possible source of human lead exposure in Amazonian communities [15, 23-24]. Using 0.252 $\mu g.g^{-1}$ (dw), an adult weighing 65 kg could eat up to 130 g of cassava semolina every day before exceeding BMDL. The most vulnerable part of the populations are pregnant women and children. Then for a child weighing 15 kg, the BMDL would be reached with the consumption of 30 g per day of cassava semolina.

4.2.2. Levels in flatbreads and cassava beer

Two types of flatbreads are considered in this study. Those used as a staple food, widely consumed in TS [63] and occasionally in SG [27], which are thinner, often sundried. Based on their median concentration (Fig. 3A), an adult weighing 65 kg, for instance, could eat up to 325 g each day before exceeding BMDL. In TS, the range of concentrations in flatbreads for consumption is quite wide, and despite the few samples collected, one reaches 0.99 $\mu g.g^{-1}$ (dw, Fig 3D). There, an adult weighing 65 kg could eat up to 203 g a day with the median concentration (0.16 $\mu g.g^{-1}$ dw, Fig. 3D), while it could be 33 g per day using the highest concentration (Fig. 3D). For a child weighing 15 kg, the consumption of 47 g per day, would be enough with the median concentration whereas it would be 8 g per day with the highest.

Finally, there is a second type of flatbread, thicker and burnt during cooking. It is only prepared in TS and used to brew cassava beer. Concentrations are in the same range as other transformation steps from TS. They are mixed with water to make the beverages, and all the liquid samples collected were below the threshold for juice and nectar, of 30 µg.L^{-1} (Fig. 3B; [52,54]). Moreover, only one is above the limit for mineral water (10 µg.L^{-1} [52, 54], Fig. 3B). Median lead concentration for this type of cassava beer is 2.51 µg.L^{-1} (Fig. 3B) and it would mean that an adult weighing 65 kg could drink up to 12.9 L to reach the BMDL without any other sources of lead in his diet. Cassava beers made with boiled tubers have similar concentrations to the other type of cassava beer (Fig. 3B), but only two samples were collected.

4.2.3. levels in other products (starch, sweet cassava tubers, other tubers, cassava juice)

Cramanioc are sweet cassava and can widely consumed (*e.g.* McKey et al. [56]). Despite a limited number of samples (n=5), it seems that this strain of cassava has a lead median concentration strictly below the threshold of 0.19 µg.g^{-1} (dw, [52-54]), reaching 0.14 µg.g^{-1} (Fig. 5B). Considering this preliminary concentration, an adult of 65 kg for instance, could eat up to 232 g each day using this median concentration and BMDL.

Sweet potatoes and yams do not present elevated levels of lead in comparison to cassava tubers (Fig. 3A), even though the number of samples is not the same. Both are used to give color to cassava beer. Given their lead concentrations and the amount used, they are not a source of this element in cassava beer. However, the limited number of samples could underestimate the range of Pb concentrations. Literature (*e.g.*, Shin et al. [64] and Padham et al. [65]) suggests that yam tubers could accumulate as much Pb as cassava tubers. Conversely, sweet potatoes are known to have low Pb accumulation and concentration (*e.g.*, Luis et al. [66]), which is in line with concentrations measured in this study. Physiological and morphological arguments seem difficult to invoke as yams produce stem tubers (*i.e.*, tubers *sensu stricto*), whereas sweet potatoes, like cassava, develop tuberous roots [67]. However, cultivation techniques could also play a role because yams are grown entirely underground in soil, like cassava, whereas sweet potatoes are cultivated partially above the soil rather than being completely buried.

Cassava juice is not consumed as a beverage like cassava beer or any other type of refreshment. Instead, it is used to cook meat or fish and as seasoning or condiment [27]. It would be necessary to consume daily 4.1 L of juice to reach BMDL for an adult weighing 65 kg, with no other Pb sources in his daily diet.

Finally, starch, whether dry (roasted, it is called *tapioca*) or wet (called *empoi*), contains low level of lead (Fig. 3A), as already mentioned in section 4.1. This product is widely used in Brazil and

French Guiana [27]. It is the key ingredient that gives *tacacá*, a regional dish, its viscous texture, added to hot beverages like coffee, and is the main ingredient in pancake-like flatbreads called *tapioca* (or *beiju*) in Brazil, where it is a common snack. Given the measured lead levels in starch, those products are unlikely to represent a significant source of human exposure to lead.

4.3. Lead uptake from soils to tubers

4.3.1. The role of soil composition

Among the three clusters identified in this study (Fig. 4), lead accumulation is as follows, C3> C2> C1, (Fig. D S1 text). Tubers in this C3 grow in a soil rich in clay and fine silt fractions, resulting in high Pb content in tubers. Fine fractions could contain clay minerals (*e.g.*, kaolinite), soil organic matter, Fe/Al oxides (*e.g.*, hematite, goethite and gibbsite). Interestingly, this content in fine particles is equal in C1, with similar Pb concentrations in soils. However, both accumulation and Pb in tubers are lower than both C3 and C2 for the first and only C3 for the second. Then, it turns out that other parameters are limiting accumulation in cassava tubers. Indeed, in C1, N, C_{org}, H and CEC are higher than those in C2 and C3, especially C_{org} and N, which can be twice as high. The homogeneous values in pH and CEC explain their low role in Pb uptake.

C2 is quite different from C1 and C3 because it presents almost half the amount of Pb concentrations in soil, but Pb accumulation is intermediate, as already mentioned. This cluster is outlined by high sand contents (Fig. 4), the lowest level of C_{org}, and a high fraction of phyto-available Pb (Fig. 4). The first two explain the low lead concentrations in soils. The phyto-available Pb fraction is also slightly higher in C3 than in C1. This emphasized the role of organic matter (C_{org}) in mitigating lead accumulation in tubers. C_{org} is a bulk analysis in this study. Consequently, it would be interesting to further investigate organic matter (OM) speciation, whether it is fulvic or humic acid, or whether micro-organisms, fungi or mycorrhizae for instance as they are known to play a significant role in metal uptake by roots (*e.g.*, Sharma & Dubey [60]; Li et al. [61]).

To summarize, H, N and C_{org} (and more generally soil organic matter) are the main parameters controlling Pb uptake by cassava tuber from soil. In every condition observed in this study, low contents of these elements promote Pb uptake while high contents reduce it. In addition, granulometry plays an indirect role as fine particles, by their composition (oxy-hydroxydes of Fe) and texture, could explain the increasing Pb or other metallic elements levels in soils (*e.g.* Horbe et al., [68]) Associated with low C_{org}, low H and low N contents, conditions are maximized for optimal uptake due to more Pb in soils. Thus, having low C_{org}, low H and low N contents, associated with coarse/sandy soil particles, dramatically decreases the amount of Pb in soils, mitigating Pb level in tuber despite favorable uptake. Those kinds of conditions were widely observed in this study, with a high variability at field scale. Conditions from C3 and C1 or C3 and C2 could be observed in the same parcel at short distances from one another. Ultimately, this spatial heterogeneity may contribute to mitigating elevated Pb concentrations in tubers despite the final products remaining above thresholds [52-54]. Figure 6 summarized the different factors affecting lead uptake from soil to cassava tubers and its migration path until final products.

Figure 6: Migration path of Pb from soils to final products. Local names are in French Creole or Portuguese when relevant. Decrease in Pb concentrations observed for cassava juice and cassava beer is mostly due to dilution because they are liquids. Icons are original illustrations derived from photographs taken by the authors.

Few parameters were not investigated in this study. For instance, tuber size was not measured and variations in Pb concentrations due to size differences could not be considered. As well as growth time impact on Pb uptake, even though all tubers from this study stayed in the ground between 8 to 10 months. Finally, other parameters could be investigated to understand the processes responsible for Pb uptake, such as Pb speciation, both with soil bearing phases and soil water, soil OM speciation (as already mentioned) and fallowing time (*i.e.*, the length between two slashing events on the same field) and its impacts on soil properties and physiology for instance.

4.3.2. Comparison with other areas

In villages from Zamfara State, Nigeria, BF ranged between 0.00018 to 0.0038 [22]. Tuber concentrations are between 0.15 ± 0.19 $\mu g.g^{-1}$ (average, dw) in the non-mining area and 0.10 ± 0.66 $\mu g.g^{-1}$ (average, dw) to 2.53 ± 8.60 $\mu g.g^{-1}$ (average, dw) in mining areas [22]. Globally, concentrations in tubers are in the same range or higher than in our study, while Pb soil concentrations are much more concentrated (68.5 ± 1.5 $\mu g.g^{-1}$ to 341.5 ± 2.9 $\mu g.g^{-1}$) in Udiba et al. [22]. Where the Pb soil is the highest, Pb in tuber is higher than in French Guiana, however, BF were low (0.00018 to 0.0038 [22]).

For cassava from the Ecuadorian Amazon, Barraza et al. [69] published an average BF of 0.34 ± 0.15 with tuber concentrations in the same order of magnitude as our study (average = 1.3 ± 0.5 $\mu g.g^{-1}$, dw). Kříbek et al. [8] found higher lead concentrations in cassava tubers grown in a noncontaminated area (Copper Belt, Zambia) compared to a contaminated area, averaging at 1.8 $\mu g.g^{-1}$ (dw, BF = 0.16) and 0.7 $\mu g.g^{-1}$ (dw, BF = 0.06), respectively. Pb in soil (average 11 $mg.kg^{-1}$ in both areas) are like TS, as well as Pb tuber concentrations and BFs while soil pH is more acid in Kříbek et al. [8] and C_{org} is similar to our study low values.

In the latest studies, cassava varieties are not known, as well as some soils parameters. Consequently, it is not possible to discuss the roles of soil parameters compared to cassava varieties on Pb uptake, here both could remain as important. Moreover, it appears that there is a width range of accumulations but BFs are always below 1, including in our study. Then, cassava tubers are not hyperaccumulators (BF>1) of Pb. However, concentrations are generally high, higher than the international recommended threshold [52-54], which could raise some concern for human health, not only in the Amazon Region, but in most of the tropical areas.

4.3.3. Impact of cassava varieties

In general, independently of geographical origin, yellow bitter cassava tubers seem to have a broader range of Pb accumulation and concentration than white bitter cassava tubers (Fig. 5A and 5C). This pattern is more evident in SG, where yellow tubers present both higher accumulation and concentrations than white tubers. Distribution between favorable and less favorable conditions for Pb uptake is balanced in SG, so differences in soil parameters (*e.g.*, Pb level, C_{org}, N, H) cannot alone explain the observed variations. In comparable conditions, in C1 (Fig. 5A and 5C), yellow cassava varieties appear to accumulate more Pb and exhibit higher Pb concentrations than white ones. It is also the case in C3 (Fig. 5A and 5C). A similar pattern is observed in TS when ~~no~~ clusters are not considered, but yellow cassavas (n=10) are less represented than white ones (n=18), which could increase the weight of outliers. Indeed, in similar growing conditions, there is no difference in Pb concentrations between yellow and white cassava varieties in TS (Fig. 5A and 5C). Moreover, there are clear differences between TS and SG, and cassavas of both colors appear to accumulate more Pb and be more concentrated in TS than in SG (Fig. 5A and 5C). These results suggest that cassava varieties and/or farming practices may contribute to differences in Pb uptake. Finally, this is consistent with to be supported by the differences in accumulation and concentration observed on varieties based on local names (Fig. 5b and 5D).

Overall, these observations suggest that variations among cassava varieties may contribute to differences in Pb uptake. However, the interpretation of varietal differences should be approached with caution due to the unequal representation of local varieties. Because this study was conducted under real farming conditions rather than a controlled experimental design, the sample size for each variety reflected its frequency of cultivation across the surveyed cassava fields, including its distribution among fields and relative abundance. As several varieties are less common than others, this limits the robustness of comparisons among varieties. Future studies based on replicated field plot experiments with balanced replication across genetically characterised varieties grown under controlled conditions (*e.g.,* Castiglione et al. [70]; Yang et al. [71]), will be necessary to evaluate the extent to which varietal differences contribute to Pb accumulation independently of environmental conditions.

4.4. Discussions around the origin of lead

4.4.1. Type of soils and soil compositions

According to literature, soils in eastern French Guiana are mostly acrisols and ferrasols with some gleysols in wetlands [72-73]. In our study, sampling did not occur in wetlands, so it is unlikely to have gleysols, whereas sand and clay contents and organic carbon concentrations, for instance, are consistent with acrisols and ferrasols [72-73]. Most of SG soils, for instance, are similar in terms of CEC, clay and C_{org} contents to the gibbsite, and geric ferrasols from Amapá, Brazil [73]. Both types of soil are mostly composed of kaolinite, quartz, iron/aluminum (hydr)oxides (*e.g.*, goethite, hematite and gibbsite) and traces of micas (mostly muscovite) and smectite/illite [72-73]. In TS, soils are developed over a monzonite and granodiorite gneiss from Paleoproterozoic (around, 2.1 Ga) with dykes of microgabbro of the same era (1.8-1.9 Ga [74]). Considering the type of soil and geological settings, TS soils might be quite old, close or over 1 million years old or more [75-76]. In SG, parental rocks are also monzogranite from Paleoproterozoic (around 2.1 Ga) with more resent basalt dykes (198-189 Ma) and a young unidentified sedimentary formation [74]. Considering this, soil in SG might be younger than in TS [75-76].

4.4.2. Possible origin of lead

As mentioned in section 4.4.1, TS soils are old soils developed on monzonite and gneiss bedrock. They present a wide range of Pb isotopes ratios (Fig. ~~6~~7) but $^{206}Pb/^{204}Pb$ (Fig. 7A) are in the same range as old soils (>300 ka) developed on granitoid background [77] and in line with ratios observed during granitoid weathering process [68, 77-80] (Fig. 7A and 7B). In those environments, Pb first originated from degradation of accessory minerals enriched in radiogenic Pb (206, 207 and

208) such as allanite, apatite, and sphene [80]. Consequently, Pb in soil samples is enriched in radiogenic Pb and $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ are higher. Then, with time, weathering of feldspars (main minerals in plutonic rocks) increases the amount of non-radiogenic ^{204}Pb, lowering $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ ratios in bulk soil [77-80]. Figure 6 confirms this mixture of geogenic sources, accessory minerals on one hand, but out of range, and feldspars and galena on the other hand. Soil samples are converging towards ratio from K-Feldspar of Paleoproterozoic granite (Fig. 7A and 7B), even though it is from another plutonic system [77], which emphasizes the old weathering process. Variability observed in figure 6 for soils could be explained by the difference in mineralogical compositions between samples.

Fig. 7: Lead isotopic composition of soils, cassava tubers and foods measured in TS and adapted from Maurice et al. [15] compared to potential lead sources. A) $^{207}Pb/^{204}Pb$ vs. $^{206}Pb/^{204}Pb$; B) $^{208}Pb/^{207}Pb$ vs. $^{206}Pb/^{207}Pb$; C) $^{206}Pb/^{204}Pb$ vs. log (1/Pb). All error bars are 1SD. All data are from total digestion protocols. In C, galena [81], K-feldspar [77] and Pb shoots [15] are represented as dashed lines as concentrations are not given.

In figure 7A, soil samples are divided into two groups, separated because aligned with two slopes, covering the geogenic sources but one group ($^{207}Pb/^{206}Pb = 0.12 \times {}^{206}Pb/^{204}Pb + 13.62$) could also contain Pb inherited from aerosols and Pb shoots. Aerosols represent atmospheric Pb which is composed of natural and anthropogenic Pb from past leaded-gasoline burning, waste incineration and industrial activities (*e.g.*, Monna et al. [4]). Ratios from aerosols presented in figure 6 are not from TS, so it is not possible to conclude on aerosols origin and balance between natural and anthropogenic Pb but rather give an indication of Pb atmospheric. Weathering of lead ammunition can be another source of Pb in soils [82]. Most Pb from bullets is transformed into Pb carbonates (cerussite or hydro-cerussite) or in massicot (PbO, Pb oxide). In phosphorus-rich soils, Pb phosphates are formed preferably to Pb carbonates [82]. In French Guiana, there are no carbonates in soils, unlike phosphorus, present in significant content [83], so this specie of lead minerals and massicot could be expected in TS, even though data are necessary to confirm. Data in SG would help to explain the difference in Pb contents between SG and TS in soil and tubers.

Cassava tubers uptake the more mobile and labile forms of Pb. Consequently, they can be seen as a form of leaching in which Pb isotopic ratios give an insight of Pb origin in those mobile fractions compared to the bulk. Here, there is less radiogenic Pb than in bulk soils as highlighted by the lower $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ (Fig. 7A) and always shifted toward a feldspar pole (Fig. 7B). This highlights the geogenic contribution, which is coherent with later stages of weathering from feldspar [80]. This is also coherent with the role of clay minerals in Pb soil-transfer (see section 4.3.1) as clays are product of feldspar alteration. Moreover, it seems that atmospheric Pb and possibly weathered lead ammunition might be a meaningful source of Pb in cassava tubers (Fig. 7). However, it is difficult to distinguish between them and to risk a quantification of the two contributions. Atmospheric Pb presented here represents a wide range of sources, from France and Brazil, with both anthropogenic and natural origins. As they are not from TS itself, it is difficult to state their origin for this location. As data from atmospheric Pb are not from TS, it is difficult to state their origin. Finally, the contribution from weathered lead ammunition would depend on the density of them in a cropped plot, which was not estimated here, and on the phyto-availability of the different sources of Pb in soils.

Cassava-based foods and beverages could be considered as a more important stage of leaching. They seem to shift toward Pb ratios of Pb shoots and aerosols (Fig. 7A and 7B). Those contributions are also evident in figure 7C. In Maurice et al. [15], mostly cassava flatbreads, cassava semolina and cassava beers (from burnt cassava flatbreads) were sampled. Considering transformation processes to obtain those products (Fig. 2), Pb isotopic data suggests that Pb in squeezed grated cassava, thus probably the Pb bound to tuber cell walls (see section 4.1), contains more lead originating from aerosols and/or Pb ammunition than the whole tuber (Fig. 7).

To summarize, this highlights that phyto-available Pb in TS can be a mixture between late weathering of geogenic products, atmospheric Pb and weathered lead ammunition. Finally, considering the small number of samples, it is not possible to conclude on the effect of global anthropogenic activity (from contaminated aerosols), or hunting, on lead concentrations in TS soils. However, it is important to do more investigations on the soil labile fraction, especially to confirm the proportion of lead coming from aerosols and its origins. Indeed, even if aerosols in some places in the world present a major anthropogenic Pb contribution, TS is a remote village and other sources of Pb are more likely forest fires due to slash and burn agriculture.

5. Conclusions and implications for local populations

From a global health point of view, despite the high lead levels in cassava tubers and cassava-based foods, the major outcome of this work is to ~~demonstrate~~ suggest that, in French Guiana, traditional cassava processing does not appear to substantially enrich Pb concentrations in cassava-derived products. Rather, our results indicate that Pb concentrations in processed foods are largely inherited from the raw tubers, pointing to soils as the most likely source of contamination under the conditions investigated. This would relieve those involved in the production of cassava-based foods of any economic and operational burdens associated with replacing tools and adapting their methods to limit lead input in the diet. If this points to an environmental origin, it need not lead to futility. The possible very low level of lead in starch-based products is also important to highlight, as some of these are of cultural importance and might be particularly recommended for children and pregnant women.

This focus on cassava and cassava products should not hide one of the other probable lead sources in diet: wild game meat consumption [15]. To date, apart data from Richard-Hansen et al. [17], the contemporary consumption of game meat remains to be measured effectively, as some opinions consider an increase due to electrification and the augmentation of freezers allowing better conservation, while others consider a diminution due to social-economic changes.

However, this research raises questions about how current threshold (0.1 $\mu g.g^{-1}$ fw) for Pb dietary consumption are calculated by international authorities. These findings raise whether these thresholds adequately reflect the dietary and cultural habits of many indigenous and local populations around the world who have given cassava a central place in their diet.

Today, the Pahikwene population is highly concerned by other health issues such as diabetes [27, 84], and in light of the social and economic changes in these small communities, the evolution of the epidemiology of lead exposure, as well as its perception, is difficult to predict.

On the other hand, as lead concentrations in raw cassava appear to be the primary determinant of lead concentrations in derived products, processes driving lead accumulation in cassava, such as soil to tuber transfers, need to be better understood. Although cassava accumulates lead, it is not a hyperaccumulator species. However, further research is needed on the speciation of organic matter in soils to try to understand which parameters may limit lead transfer to tubers. Similarly, it would be interesting to investigate the speciation of lead, particularly in the fraction that accumulates in tubers, and to try to explore whether this fraction is indeed linked to atmospheric lead deposits and, if so, determine their origins in the areas under study.

Lastly, exploring whether other agricultural techniques could limit Pb absorption due to their high organic matter content is an interesting perspective. Finally, the question of cassava varieties is crucial, but very complex to clarify, as well as the intricate question of the length of stay of tubers in the soil (variety dependent, but not only). It is difficult to provide concrete answers to these questions from this study, but there appears to be trends worth exploring, bearing in mind that although some varieties might be of specific interest regarding lead contamination, promoting varietal and diet diversity remains a strategy for coping with environmental hazards.

Overall, this study provides new insights into Pb transfer from soils to cassava-derived foods in eastern French Guiana. However, further work combining Pb speciation, controlled experiments, detailed dietary surveys and biomonitoring will be necessary to confirm the mechanisms proposed here and to better assess their implications for public health.

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Data Availability Statement

Data is available in SI Excel file.

R code is available upon request.

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Supplementary data

S1 Table: Raw data for Trois-Sauts. Excel file gathering all data coming from Trois-Sauts and used in this study.

S2 Table: Raw data for Saint-Georges-de-l'Oyapock. Excel file gathering all data coming from Saint-Georges-de-l'Oyapock and used in this study.

S1 Text. Supplementary data for the paper. This file contains information regarding transformations tools used during cassava processing, general statistics, the evolution of lead concentrations in processed products, factors influencing Pb uptake from soils to tubers and data for comparisons of lead concentrations in tuber based on tuber colors and vernacular names.

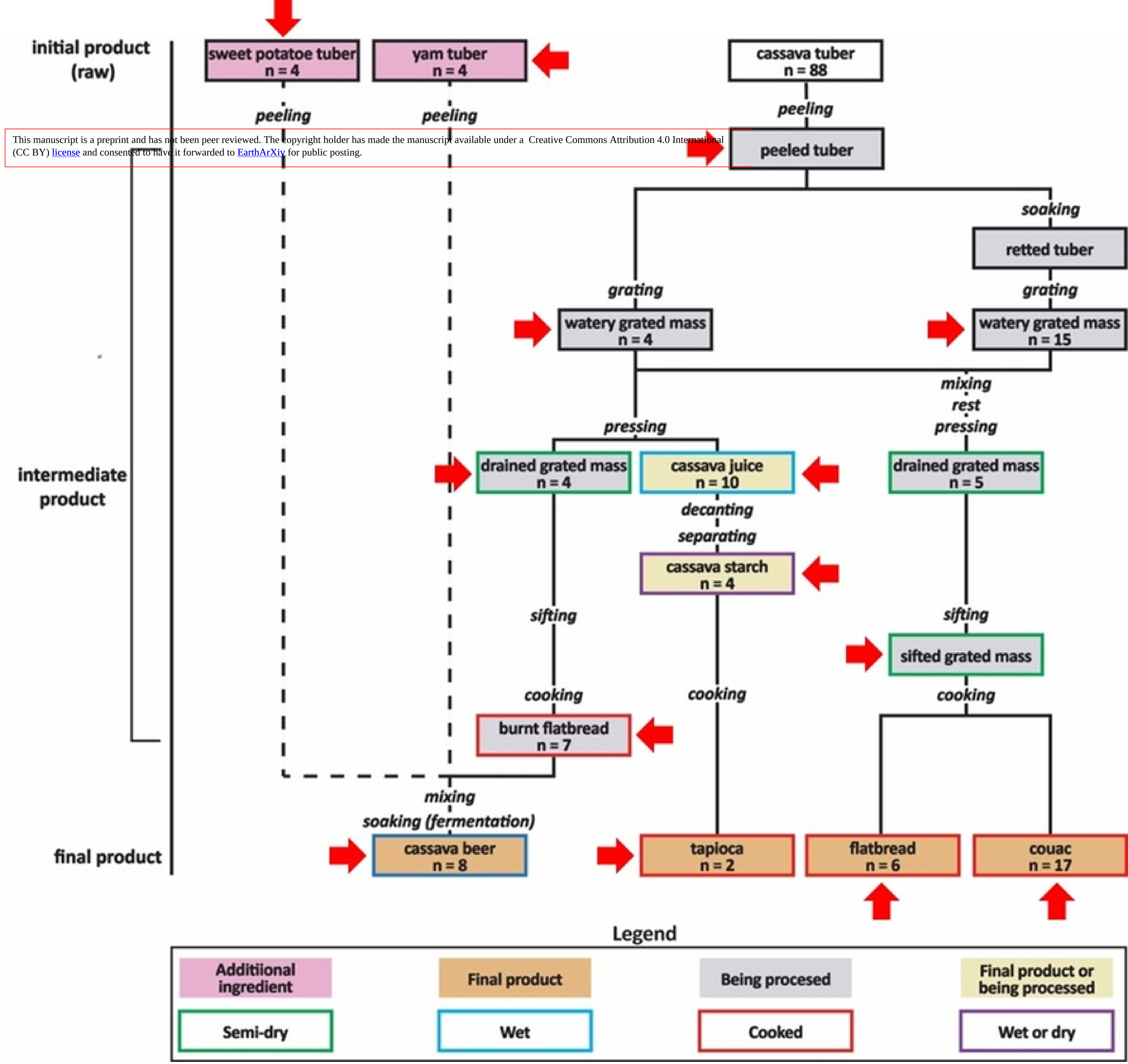

initial product (raw)
intermediate product
final product
sweet potatoe tuber n = 4
yam tuber n = 4
cassava tuber n = 88
peeling
peeling
peeling
peeled tuber
soaking
retted tuber
grating
grating
watery grated mass n = 4
watery grated mass n = 15
mixing
rest
pressing
pressing
drained grated mass n = 4
cassava juice n = 10
drained grated mass n = 5
decanting
separating
cassava starch n = 4
sifting
sifting
sifted grated mass
cooking
cooking
cooking
burnt flatbread n = 7
mixing
soaking (fermentation)
cassava beer n = 8
tapioca n = 2
flatbread n = 6
couac n = 17
Legend
Additiional ingredient
Final product
Being procesed
Final product or being processed
Semi-dry
Wet
Cooked
Wet or dry

Figure 2

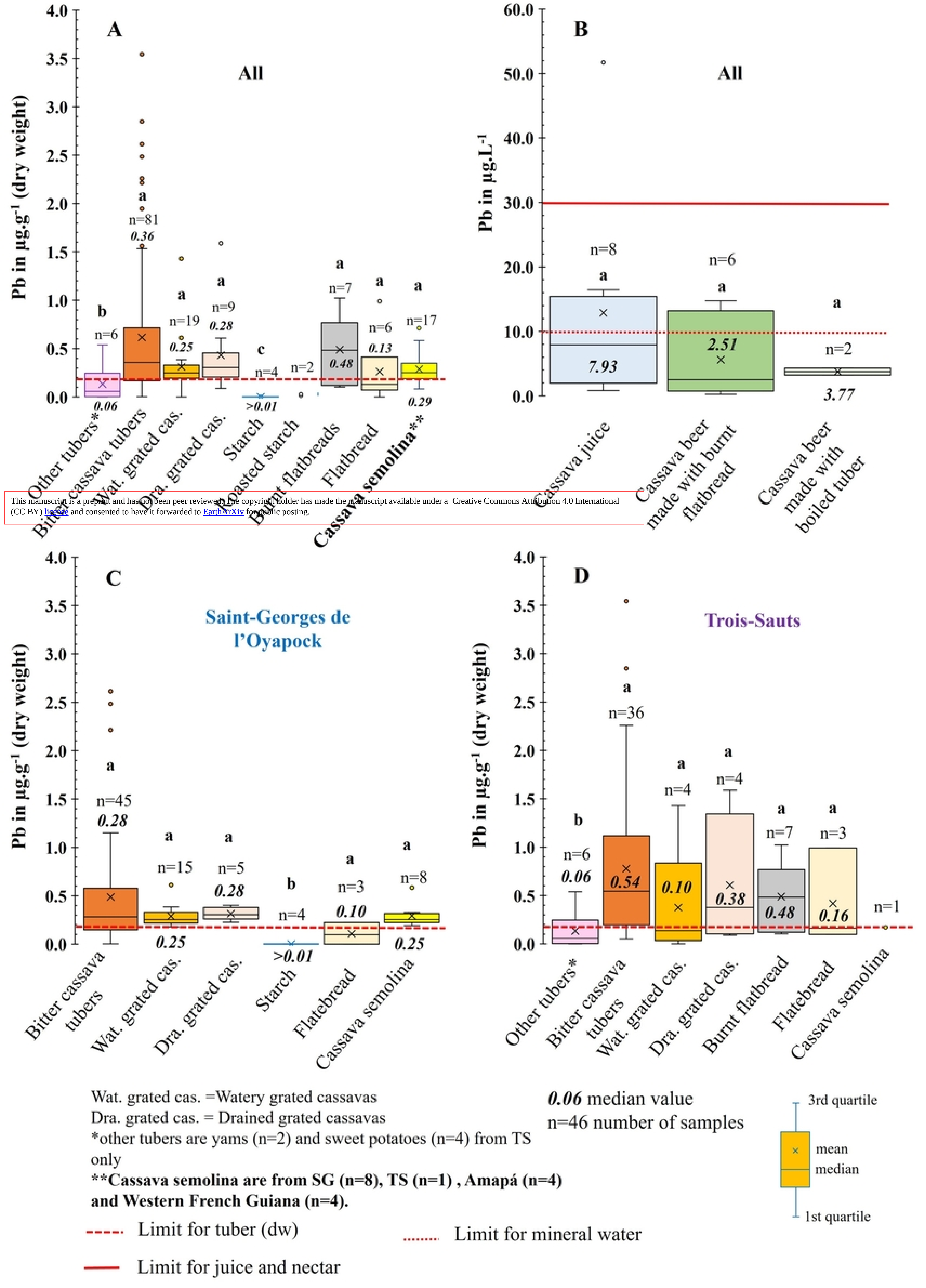
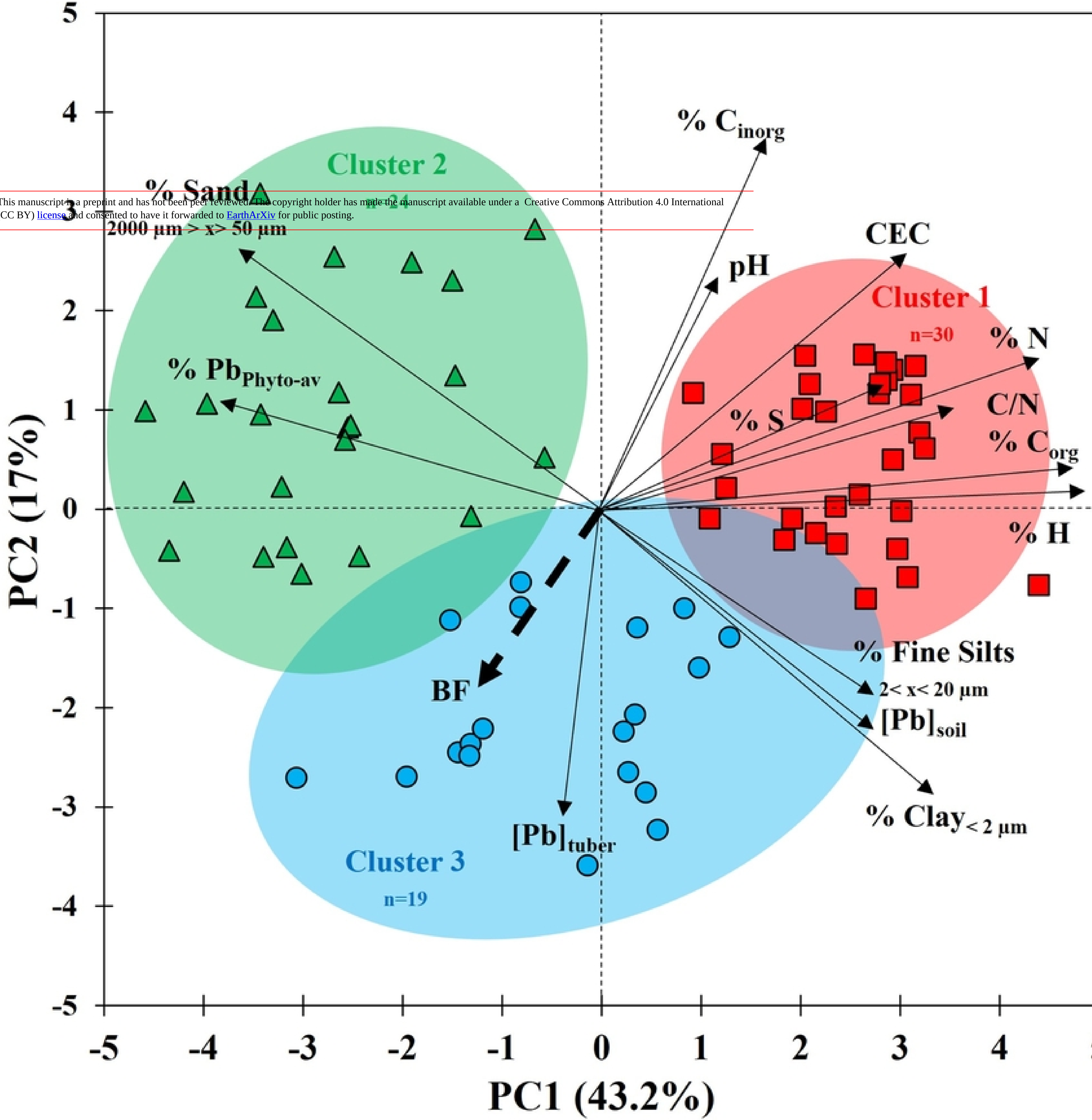

A
All
Pb in µg.g-1 (dry weight)
Other tubers*
Bitter cassava tubers
Wat. grated cas.
Dra. grated cas.
Starch
Roasted starch
Burnt flatbreads
Flatbread
Cassava semolina**
B
All
Pb in µg.L-1
Cassava juice
Cassava beer made with burnt flatbread
Cassava beer made with boiled tuber
C
Saint-Georges de l'Oyapock
Pb in µg.g-1 (dry weight)
Bitter cassava tubers
Wat. grated cas.
Dra. grated cas.
Starch
Flatebread
Cassava semolina
D
Trois-Sauts
Pb in µg.g-1 (dry weight)
Other tubers*
Bitter cassava tubers
Wat. grated cas.
Dra. grated cas.
Burnt flatbread
Flatebread
Cassava semolina
Wat. grated cas. =Watery grated cassavas
Dra. grated cas. = Drained grated cassavas
*other tubers are yams (n=2) and sweet potatoes (n=4) from TS only
**Cassava semolina are from SG (n=8), TS (n=1) , Amapá (n=4) and Western French Guiana (n=4).
0.06 median value
n=46 number of samples
3rd quartile
mean
median
1st quartile
Limit for tuber (dw)
Limit for mineral water
Limit for juice and nectar

Figure 3



Cluster 2
n=24
% Sand
2000 µm > x> 50 µm
% Pb Phyto-av
% C inorg
pH
CEC
Cluster 1
n=30
% N
C/N
% S
% C org
% H
% Fine Silts
2< x< 20 µm
[Pb] soil
% Clay < 2 µm
BF
[Pb] tuber
Cluster 3
n=19
PC2 (17%)
PC1 (43.2%)
5
4
3
2
1
0
-1
-2
-3
-4
-5

Figure 4

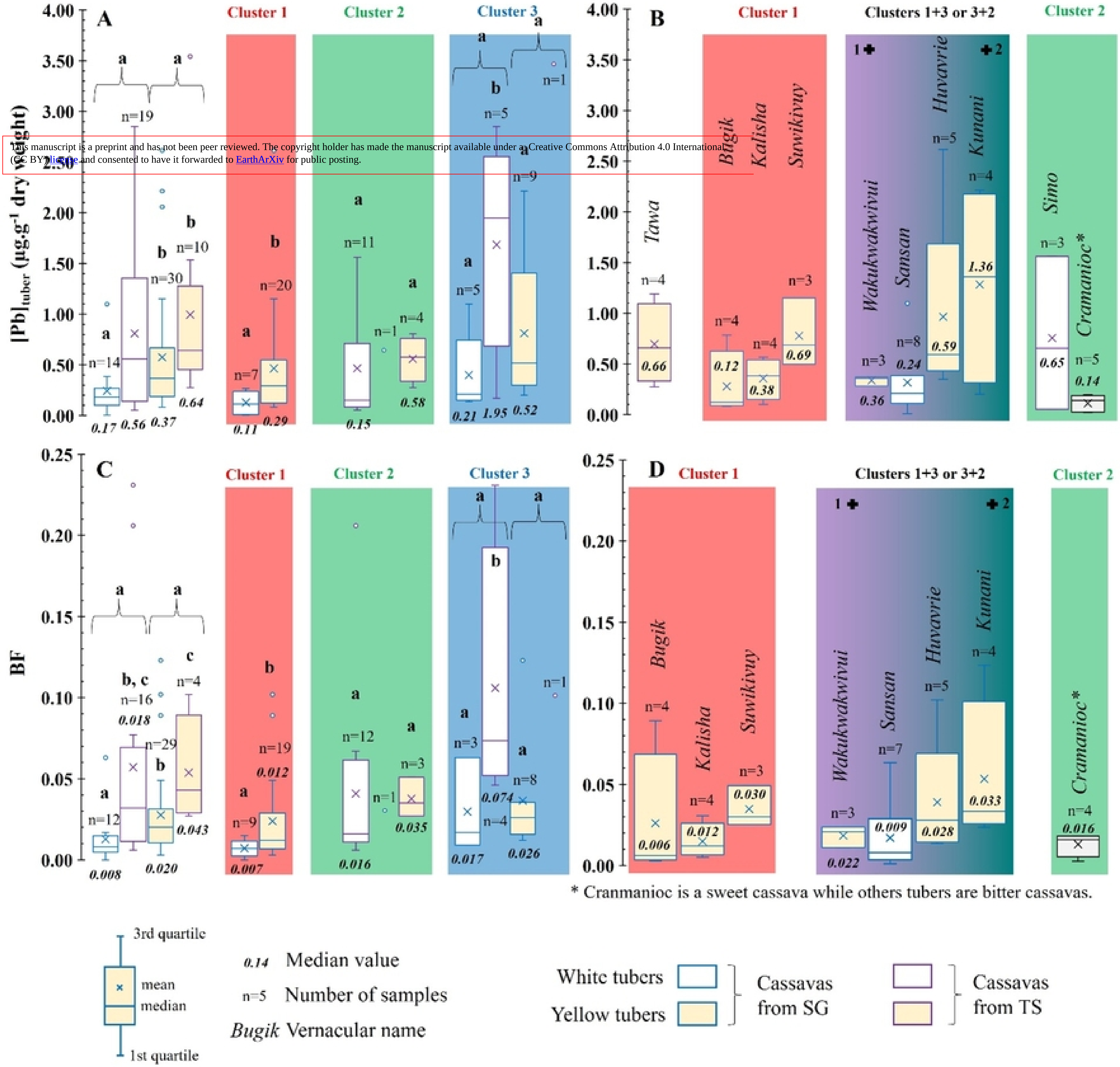

A
B
C
D
Cluster 1
Cluster 2
Cluster 3
Clusters 1+3 or 3+2
$[Pb]_{tuber}$ ($\mu g.g^{-1}$ dry weight)
BF
Tawa
Bugik
Kalisha
Suwikivuy
Wakukwakwivui
Sansan
Huvavrie
Kunani
Simo
Cramanioc*
* Cranmanioc is a sweet cassava while others tubers are bitter cassavas.
3rd quartile
mean
median
1st quartile
0.14 Median value
n=5 Number of samples
Bugik Vernacular name
White tubers
Yellow tubers
Cassavas from SG
Cassavas from TS

Figure 5

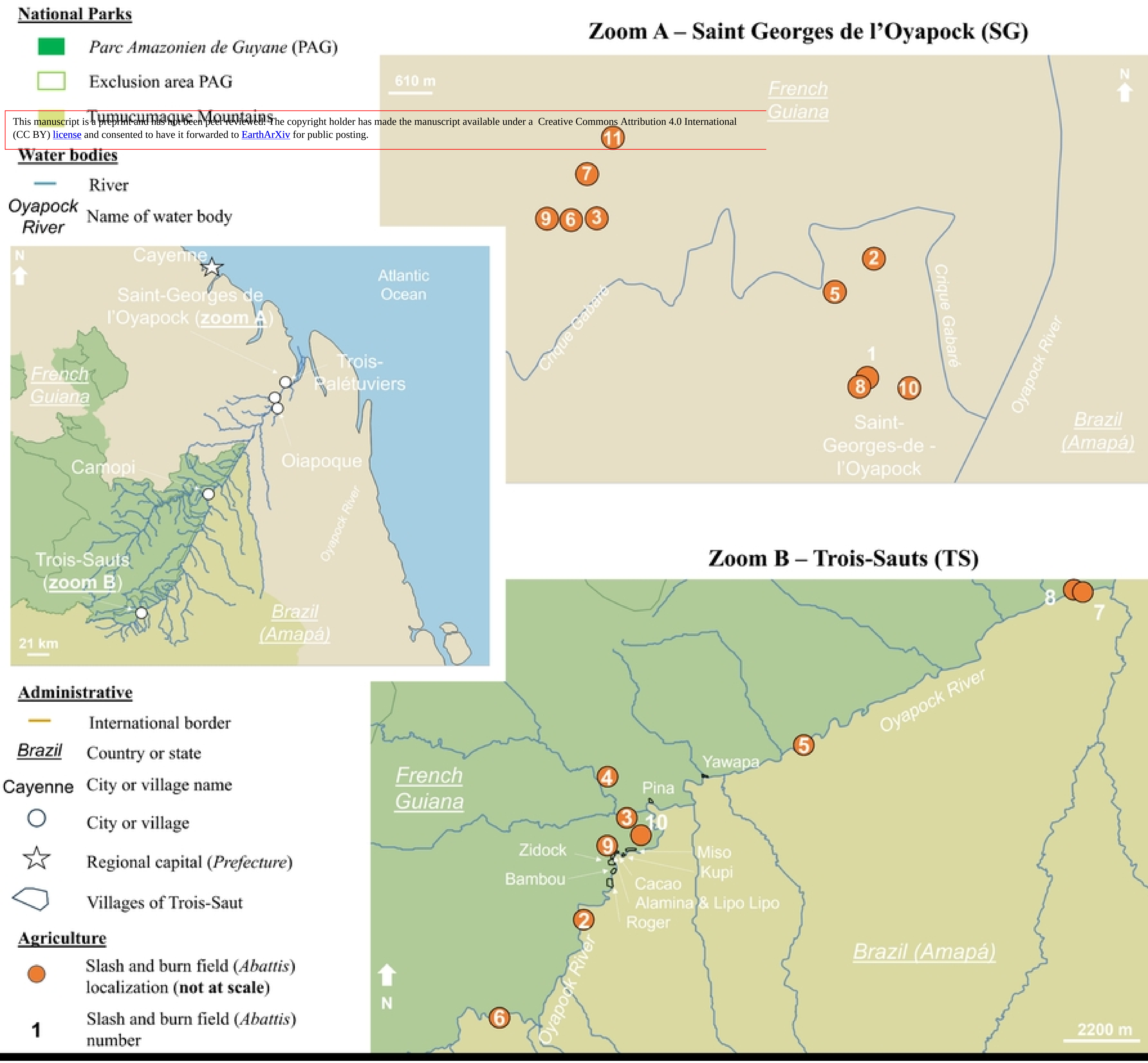
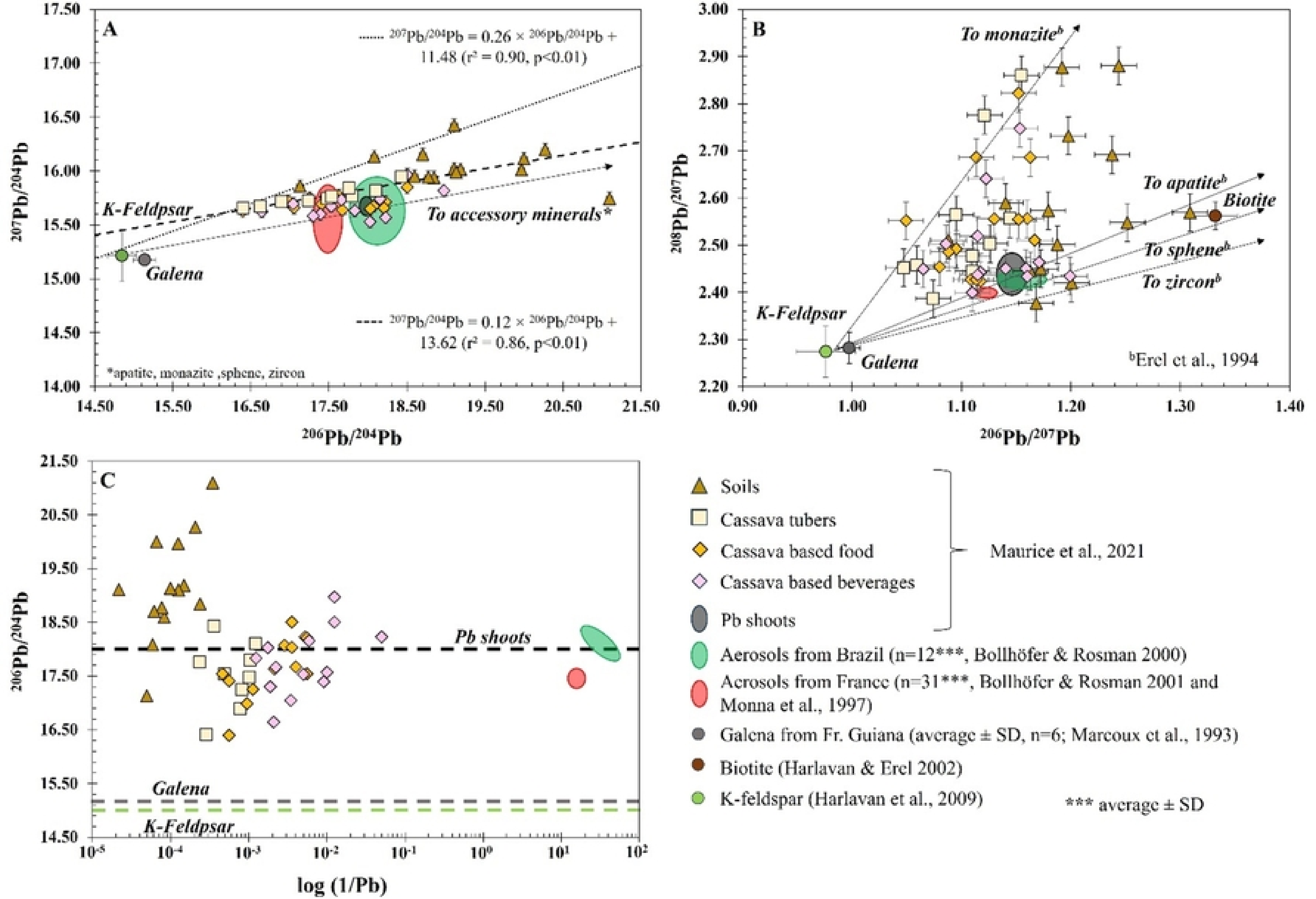

National Parks
Parc Amazonien de Guyane (PAG)
Exclusion area PAG
Tumucumaque Mountains
Water bodies
River
Oyapock River
Name of water body
Zoom A – Saint Georges de l'Oyapock (SG)
610 m
N
French Guiana
11
7
9
6
3
2
5
1
8
10
Crique Gabaré
Oyapock River
Saint-Georges-de - l'Oyapock
Brazil (Amapá)
Cayenne
Saint-Georges de l'Oyapock (zoom A)
Atlantic Ocean
Trois-Palétuviers
Oiapoque
Camopi
Trois-Sauts (zoom B)
21 km
Zoom B – Trois-Sauts (TS)
4
Pina
Yawapa
Zidock
Bambou
Miso
Kupi
Cacao
Alamina & Lipo Lipo
Roger
Brazil (Amapá)
2200 m
Administrative
International border
Brazil
Country or state
Cayenne
City or village name
City or village
Regional capital (Prefecture)
Villages of Trois-Saut
Agriculture
Slash and burn field (Abattis) localization (not at scale)
1
Slash and burn field (Abattis) number

Figure 1



A
$^{207}Pb/^{204}Pb = 0.26 \times ^{206}Pb/^{204}Pb + 11.48$ ($r^2 = 0.90$, p<0.01)
$^{207}Pb/^{204}Pb = 0.12 \times ^{206}Pb/^{204}Pb + 13.62$ ($r^2 = 0.86$, p<0.01)
K-Feldpsar
To accessory minerals*
Galena
*apatite, monazite ,sphene, zircon
$^{207}Pb/^{204}Pb$
$^{206}Pb/^{204}Pb$
B
To monazite[b]
To apatite[b]
Biotite
To sphene[b]
To zircon[b]
K-Feldpsar
Galena
[b]Erel et al., 1994
$^{208}Pb/^{207}Pb$
$^{206}Pb/^{207}Pb$
C
Pb shoots
Galena
K-Feldpsar
$^{206}Pb/^{204}Pb$
log (1/Pb)
Soils
Cassava tubers
Cassava based food
Cassava based beverages
Pb shoots
Maurice et al., 2021
Aerosols from Brazil (n=12***, Bollhöfer & Rosman 2000)
Aerosols from France (n=31***, Bollhöfer & Rosman 2001 and Monna et al., 1997)
Galena from Fr. Guiana (average ± SD, n=6; Marcoux et al., 1993)
Biotite (Harlavan & Erel 2002)
K-feldspar (Harlavan et al., 2009)
*** average ± SD

Figure 7

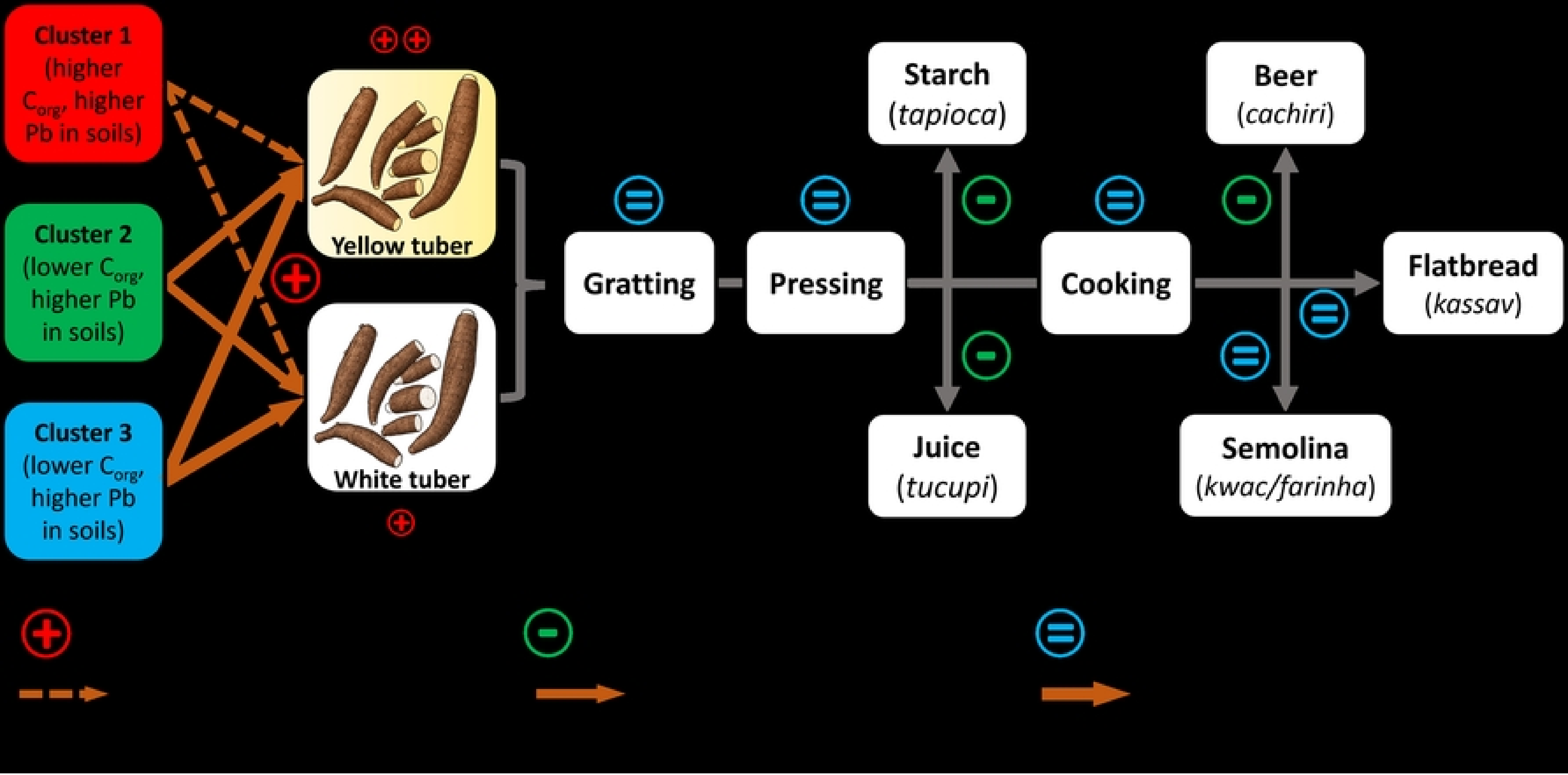

Cluster 1
(higher C_{org}, higher Pb in soils)
Cluster 2
(lower C_{org}, higher Pb in soils)
Cluster 3
(lower C_{org}, higher Pb in soils)
Yellow tuber
White tuber
Gratting
Pressing
Starch
(*tapioca*)
Juice
(*tucupi*)
Cooking
Beer
(*cachiri*)
Semolina
(*kwac/farinha*)
Flatbread
(*kassav*)

Figure 6