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Unlocking the elemental signature of European tea gardens: Implications for tea traceability

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ABSTRACT

This study presents a comprehensive analysis of the elemental profiles of tea leaves coming from plants grown in several European gardens, with a focus on the bioaccumulation of essential and potentially toxic trace elements in relation to processing and location of tea garden. Samples were collected from various gardens across Europe, including Portugal, the Azores, Germany, the Netherlands, and Switzerland. Elemental analysis was conducted on fresh tea leaves, dried leaves, and leaves processed for the production of green and black tea, along with soil samples from the root zones of tea plants. The results reveal no significant differences in elemental content based on the processing of tea leaves. However, distinct elemental profiles were observed among tea leaves of plants grown in gardens from different European regions. Utilizing chemometric and machine learning tools, the study highlights the potential of these elemental profiles for enhancing the traceability of tea products.

1. Introduction

Tea, derived from the leaves of *Camellia sinensis* (*var. sinensis* or *var. assamica*) (Wight, 1962), stands as one of the most frequently consumed beverages across the globe, primarily cherished for its delightful fragrance, diverse flavor spectrum, and invigorating attributes. With historical origins rooted in ancient China, tea has evolved into a universally favored beverage, second only to water in terms of global consumption. Diverse in its varieties, from green to black, oolong to white, tea offers a rich tapestry of flavors shaped by an intricate interplay of environmental conditions and meticulous processing techniques and the unique chemistry of each tea variety contributes to its distinct flavor profile (Wong et al., 2022). Currently, as reported by the Food and Agriculture Organization of the United Nations (FAO), the annual global tea production exceeds USD 17 billion, while the worldwide tea trade is valued at approximately USD 9.5 billion (FAO, 2022). Projections suggest an annual increase in production by 2.1 % for black tea and 6.3 % for green tea by 2030 (FAO, 2022).

Beyond its taste and aroma, tea's consumption is linked with significant beneficial properties, which include its antioxidant attributes and potential health benefits (Cabrera et al., 2003). Essential elements, vital for human health, play a pivotal role in the chemistry of tea, impacting both its quality and its potential therapeutic attributes (Karak and Bhagat, 2010). Nevertheless, the accumulation of potentially toxic elements (PTEs) in the soil, stemming from excessive fertilizer use or anthropogenic contamination, may pose health risks to consumers (Hideaki et al., 1976). Tea plants are indeed susceptible to the bioaccumulation of toxic elements from the root, underscoring the need for vigilant monitoring and management practices to ensure the safety of tea products (Peng et al., 2018).

Tea is also a highly sought-after global commodity. In Asia, particular tea varieties cultivated in specific regions fall victim to counterfeiting by inferior commercial teas, making the ability to trace the origin and quality of tea of paramount importance. Recognizing the susceptibility of particular tea varieties to counterfeiting, numerous studies have delved into the exploration of elements or molecules with site-specific attributes, aiming to establish robust traceability measures and safeguards against the proliferation of inferior commercial teas in key tea-producing regions (Hong et al., 2017; Liu et al., 2019; Pons et al., 2021; Wei et al., 2020).

The aim of the following research is underscored by the growing importance of tea in Europe's emerging tea production landscape. Europe's contribution to the study of tea, while relatively nascent compared to the well-established traditions of Asia, holds great promise

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(Carloni et al., 2023a; Carloni et al., 2023b).

This research navigates this burgeoning European tea production landscape, with a particular focus on European tea gardens located in Portugal, the Azores, Germany, the Netherlands, and Switzerland. The study builds on the foundation of a recent publication, pioneering the specific investigation of elemental profiling in these European tea gardens (Girolametti et al., 2023). Its primary objectives encompass: 1) the elemental profiling of soil and tea leaves, shedding light on the intricate interplay of elements in the tea's growth environment; 2) comprehensive study on the effects of production processes on elemental profiles, elucidating how processing techniques impact the elemental composition of the final tea product; 3) to discern the geographical origin of tea by means of Principal Component Analysis (PCA) and machine learning algorithm (PLS-DA, LDA, SVM) as robust chemometric tools, contributing to the development of traceability models in the European tea landscape.

These objectives collectively advance the understanding of tea production, quality, and traceability in Europe, bridging the knowledge gap and contributing to the wider domain of tea research.

2. Materials and methods

2.1. Study area

This study was conceived and developed in collaboration with the Tea Grown in Europe Association (EuT). The study area for this research encompassed five tea gardens located across Europe, each with distinct characteristics regarding their geographic location, climate, and cultivation practices (Fig. 1 and Table 1). The Chá Camélia tea garden located in the north of Portugal covers 1 ha of land and specializes in the production of organic green tea, with a growing season ranging from March to October and temperatures between 2 and 35 °C. The Agrarian Development Service of São Miguel in the Azores manages two small plantations (located in Ribeira Grande and Sete Cidades) covering an

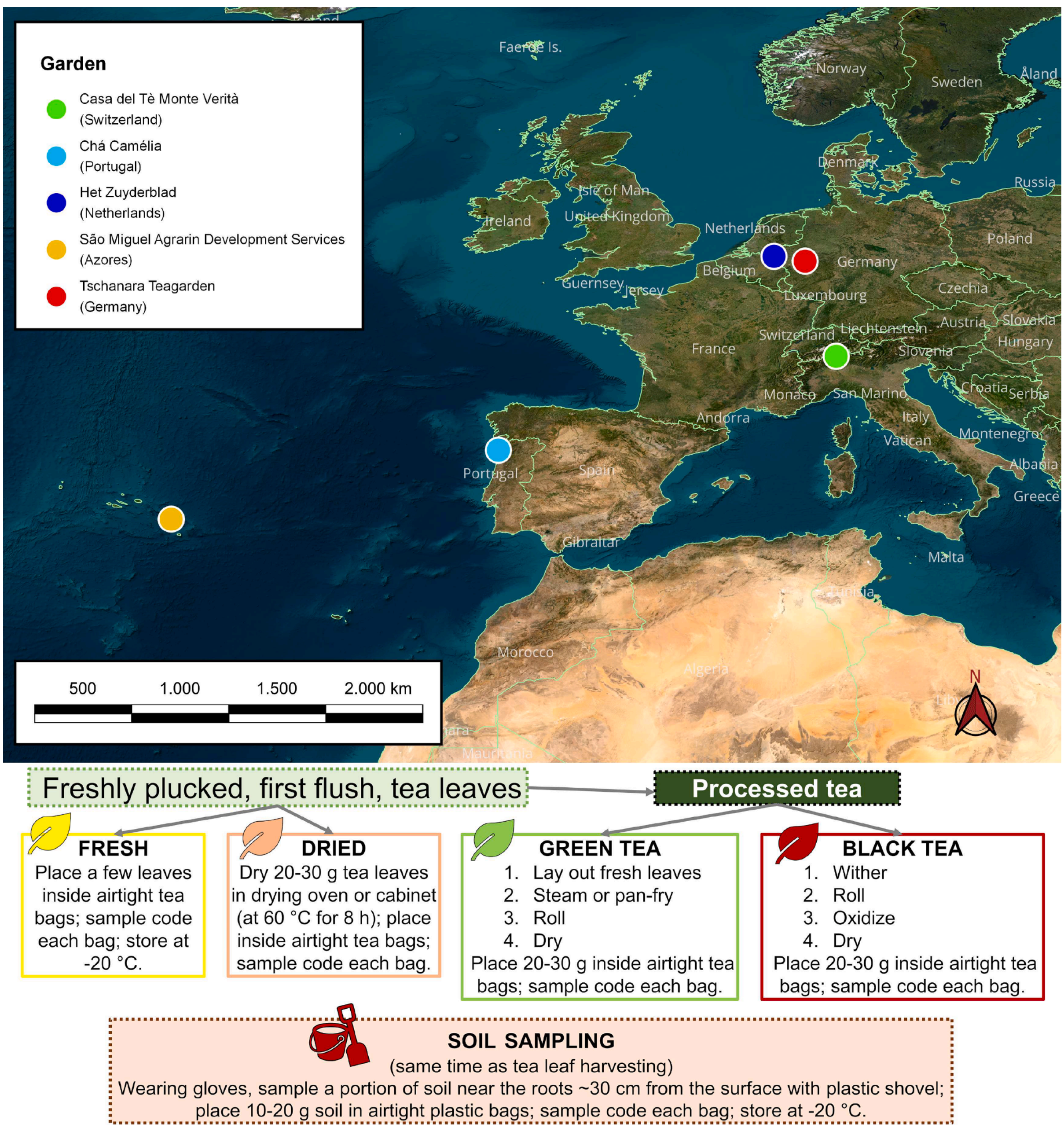

Fig. 1. Geographical distribution of European tea gardens included in this study and sampling flow chart.

Table 1
Classification of soil and tea samples collected from European tea gardens.

Garden	Location, country (altitude) (coordinates)	Group Code	Type (Process)	Variety	Propagation method	Cultivar	Sample Code
Chá Camélia	Fornelo, Portugal (90 a.s.l.) (41.332219 N, 8.641613 W)	PT1	Soil	*sinensis*	Seeds	Unknown	PSS
			Tea leaves (unprocessed)				PUS
			Tea leaves (dried)				PDS
			Tea leaves (green)				PGS
		PT2	Soil	*sinensis*	Cuttings	Unknown	PSC
			Tea leaves (unprocessed)				PUC
			Tea leaves (dried)				PDC
			Tea leaves (green)				PGC
São Miguel Agrarian Development Services	Ribeira Grande, Azores (50 a.s.l.) (37.825335 N; 25.513096 W)	AZ1	Soil	*assamica*	Cuttings	Unknown	ASR
			Tea leaves (dried)				ADR
			Tea leaves (black)				ABR
	Sete Cidades, Azores (266 a.s.l.) (37.866441 N; 25.769940 W)	AZ2	Soil	*assamica*	Cuttings	Unknown	ASS
			Tea leaves (dried)				ADS
			Tea leaves (black)				ABS
Casa del Tè Monte Verità	Ascona, Switzerland (320 a.s.l.) (46.158700 N, 8.764607E)	CH	Soil	*sinensis*	Cuttings	Unknown	SS0
			Tea leaves (unprocessed)				SU0
			Tea leaves (dried)				SD0
			Tea leaves (green)				SG0
			Tea leaves (black)				SB0
Tschanara Teagarden	Wirtsspezard, Germany (213 a.s.l.) (51.042362 N, 7.167825E)	DE1	Soil	*sinensis*	Seeds	Japanese	GSJ
			Tea leaves (unprocessed)				GUJ
			Tea leaves (dried)				GDJ
			Tea leaves (green)				GGJ
			Tea leaves (black)				GBJ
		DE2	Soil	*sinensis*	Seeds	Korean	GSK
			Tea leaves (unprocessed)				GUK
			Tea leaves (dried)				GDK
			Tea leaves (green)				GGK
			Tea leaves (black)				GBK
Het Zuyderblad	Soerendonk, Netherlands (29 a.s.l.) (51.293099 N, 5.560886E)	NL	Soil	*sinensis*	Seeds	Unknown	NS0
			Tea leaves (unprocessed)				NU0
			Tea leaves (dried)				ND0
			Tea leaves (green)				NG0
			Tea leaves (black)				NB0

area of approximately 0.4 ha, producing white tea without the use of fertilizers or pesticides. The Casa del Tè Monte Verità garden in Switzerland, features 1400 *Camellia sinensis* plants, with a growing season running from April to September, ranging between −2 and 34 °C, and harvesting green, black, and white tea. In Germany, the Tschanara Teagarden located in Odenthal-Scheure cultivates unique cultivars, producing white, yellow, green, oolong, and black teas. The temperature in this garden ranges between −13 to 38 °C and the cultivation season is from May to September. The Het Zuyderblad tea garden located in the south of the Netherlands covers 1 ha of farmland and harvesting and processing of tea are done by hand or with traditional machinery on a small scale. The growing season lasts between April and September, with temperatures ranging between −7 and 25 °C.

2.2. Sampling activity and pre-treatment

A total of 34 samples were collected, consisting of 26 tea leaves and 8 soil samples. The characteristics of each sample are listed in Table 1. Each tea sample consisted of three aliquots of first flush tea leaves collected from a large number of plants that were hand-plucked during the harvesting period between March and June 2023, depending on the harvesting season of each country. The tea leaves were then handled as reported in Fig. 1, in order to analyze fresh, dried and processed tea leaves. The tea growers were asked to follow the recommendations reported in the flow chart of Fig. 1 in order to standardize as much as possible the sampling and processing of leaves and soil. The processing steps for the mentioned teas are as follows: Black tea undergoes withering of the tea leaves, followed by hand-/electric-rolling, fermentation/aeration, and drying. Green tea is produced through heat enzyme inactivation of the leaves (steaming or wok-/pan-firing), followed by hand-/electric-rolling and drying (Carloni et al., 2023a). The corresponding soil samples were collected as reported in Fig. 1, near the plant roots at a depth of approximately 30 cm. Each sample was accurately introduced into plastic bags to prevent metal contamination and shipped to Italy, where they were kept frozen until analysis.

2.3. Treatment and analysis

The analytical procedures were conducted in an ISO 5 clean room laboratory. High-purity water (Elix and Milli-Q systems, Bedford, MA, USA) and reagents (ultrapure grade, Carlo Erba, Milan, Italy) were used for trace analysis. To prevent cross-contamination, samples were handled with tools cleaned using a 1:10 HCl solution to remove metals residues (Illuminati et al., 2010).

According to the NY/T 1377–2007 guideline, 10.0 g of soil were mixed with 25 mL of Milli-Q water and stirred thoroughly. The resulting solution was allowed to settle for 30 min before analysis. The pH levels of the supernatant were determined using a pH meter (XS pH50 VioLab, XS Instruments, Concordia sulla Secchia, Italy). Electrical conductivity (EC) was measured on the supernatant of 10.0 g of dried soil dissolved in 20 mL of Milli-Q water using a portable conductometer (COND 6, XS Instruments, Concordia sulla Secchia, Italy). Loss-on-ignition (LOI) was measured as a proxy for organic matter (OM). Approximately 1 g of previously dried soil was placed in an oven at 450 °C for 4 h, and the LOI was quantified gravimetrically. Each analysis was performed on 3 aliquots *per* sample.

Samples pretreatment for elemental analysis followed the procedure described in Girolametti et al. (2023). Briefly, about 1 g of each sample was placed into PTFE liners, followed by the addition of 3 mL HNO_3 and 3 mL H_2O_2. Microwave digestion was performed using a MARS-5 (CEM, Matthews, NC, USA) digester, with one sample serving as a temperature control. After digestion, samples were stabilized with 4 mL of ultrapure water, stored at + 4 °C, and shielded from light until analysis.

The elemental determination (Ag, Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Pd, Si, Sn, V, Zn) was conducted using an ICP-OES instrument (Agilent 5800, Agilent Technologies, Santa Clara, CA, USA), with high-purity argon (99.999 % purity, Ar 5.0 grade) employed as the carrier gas and internal standard (IS). Each sample was analyzed in triplicate ($n = 3$). The operating instrumental conditions are detailed in Table S1.

For accurate quantification, readings were taken at three different wavelengths for each element, and the optimal one, based on absence of spectral interferences and recovery percentage of certified reference materials (see below) was selected as shown in Table S2. Method linearity (R^2), limit of detection (LOD), and limit of quantification (LOQ) were determined for each element (Table S2) (European Medicines Agency, 2022). A procedure blank, where >LOD, was subtracted from the results.

Accuracy was assessed using three certified reference materials (CRMs): Dogfish Muscle Certified Reference Material for Trace Metals (DORM-2, National Research Council, Canada), Wheat Flour (SRM 1567b, NIST, USA), and New York/New Jersey Waterway Sediment (SRM 1944, NIST, USA). A recovery level always between 91 and 107 % indicated the reliability of the method applied.

2.4. Bioaccumulation factor (BAF)

To evaluate the accumulation of elements from the soil to the tea leaves, bioaccumulation factor (BAF) was used:

$$BAF = C_{tealeaves} / C_{soil}$$

Where $C_{tea\ leaves}$ is the element concentration on dried tea leaves (mg kg^{-1}) and C_{soil} is the concentration of the same element in dried soil (mg kg^{-1}). A BAF ≤ 1 indicates a condition of non-accumulation from the soil to the leaf, while a BAF > 1 indicates that the single element is accumulated in tea leaf (Peng et al., 2018).

2.5. Statistical analysis

The data, presented as the mean ± standard deviation (sd) in units of mg kg^{-1}, underwent thorough statistical analysis within the RStudio software (R Version 4.3.2). In case of values < LOD, they were considered half the LOD value.

Redundancy analysis (RDA) was employed to extensively examine the impact of pH, electrical conductivity, and organic matter on the composition of elements within the soil.

One-way analysis of variance (ANOVA) was employed to evaluate statistically significant differences in average elemental content in tea samples which underwent different treatment. Post hoc comparison was investigated with Tukey test at 95 % confidence level.

Furthermore, the relationships between the elements in tea leaves and those within the soil were explored comprehensively through the utilization of both a correlogram and heatmap. Spearman's correlation coefficient and significance test served as the metrics for establishing the statistically significant correlations (set at $p < 0.05$, $p < 0.01$ or $p < 0.001$).

In addition, Principal Components Analysis (PCA) was conducted on previously standardized data to explore the impact of the distribution of elemental content within both the soil and the tea leaves on the overall variability observed in the dataset.

In this investigation, we employed Partial Least Squares Discriminant Analysis (PLS-DA) with standard scaling as machine learning tool to explore the association between predictor variables (X, representing the elemental content in tea leaves) and response variables (Y, denoting the country of origin). The model was constructed using five latent components without the inclusion of orthogonal components. Standard scaling was applied to both X and Y, facilitating a standardized evaluation of their respective contributions to the model. To evaluate the significance and reliability of the model, we employed permutation testing, a technique that involved randomizing the relationship between X and Y to determine if the observed results were statistically meaningful. This analysis yielded p-values of 0.05 for both the proportion of explained variance (pR^2Y) and predictive ability (pQ^2), indicating the model's statistical significance. Additionally, Variable Importance in Projection (VIP) values were calculated to assess the contribution of each predictor variable to the model.

The Linear Discriminant Analysis (LDA) technique was employed to classify tea samples based on their elemental profiles. After training the LDA model with predictor variables (elemental composition) and response variables (country of origin), the model's performance was evaluated using a confusion matrix, which summarized the classification accuracy of the LDA model. Additionally, statistical tests were conducted to assess the significance of the model's predictive capabilities.

Additionally, we employed the Support Vector Machine (SVM) algorithm to classify tea samples and predict their country of origin. The SVM model was configured with specific parameters: SVM-Type set to classification, SVM-Kernel utilizing a radial kernel, and a cost parameter of 1. These parameters were chosen to optimize the model's performance in classifying the tea samples.

3. Results

3.1. Elemental content in soil

In soil samples, the pH ranged from 4.3 (observed in the Japanese cultivar of Tschanara Teagarden, Germany) to 5.7 (registered in Het Zuyderblad, the Netherlands), with an overall mean value of 4.9 ± 0.4. The average recorded EC value was 216 ± 143 μS, ranging from 33.4 μS in Ribeira Grande (Azores) to 367 μS in Chà Camèlia (Portugal). Organic matter content varied from a minimum of 3.3 % in Het Zuyderblad Garden (the Netherlands) to a maximum of 32.8 % in Chà Camèlia (Portugal), with a mean recorded value of 11 ± 10 % (Table S3).

The average concentration (mean ± sd, min–max, mg kg^{-1}) of elements in soil samples followed the order Al (98384 ± 54680, 17098–193324) > Fe (15197 ± 6808, 2643–23285) > K (3796 ± 2045, 764–7738) > Mg (3214 ± 2010, 350–6583) > Ca (2190 ± 1691, 366–5149) > Si (585 ± 114, 477–820) > Mn (513 ± 426, 41–1115) > Sn (157 ± 72, 26–244) > Zn (90 ± 38, 26–145) > Na (54 ± 53, 15–177) > Pb (38 ± 24, 3–71) > Cu (33 ± 27, 10–91) > V (31 ± 19, 10–57) > Cr (22 ± 15, 9–45) > Ni (17 ± 12, 2–29) > Pd (11 ± 9, 1–22) > As (10 ± 6, 2–20) > Co (7 ± 4, 0.9–11.1) > Cd (0.5 ± 0.4, 0.1–1.0) > Ag (0.4 ± 0.4, 0.07–1.03) > Hg (0.11 ± 0.05, 0.4–1.9) (Fig. 2). Samples coming from the same garden (Germany and Portugal) exhibited a similar elemental profile, while greater differences were recorded for the two samples collected in the Azores as they originate from two different gardens, located 30 km apart and at different altitudes (Table 1). The soil from the garden located in the Netherlands (Het Zuyderblad) always exhibited lower concentrations compared to other soils for all elements, except for

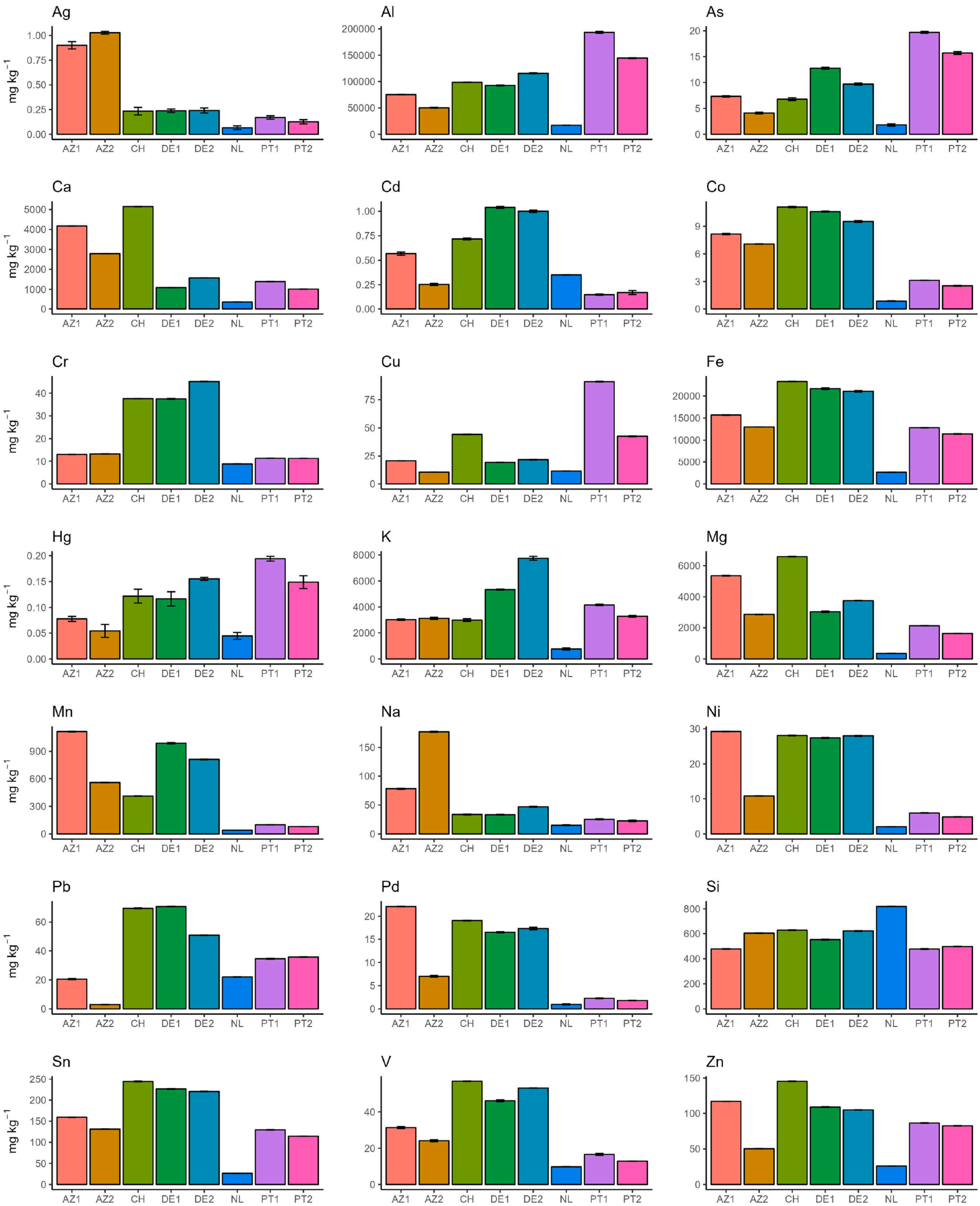

Fig. 2. Elemental content in soils coming from European tea gardens.

Si.

The redundancy analysis (RDA) applied to soil elemental profile, with pH, EC and LOI as constraining variables, showed that the pH variable was associated only with Si, at the Dutch and the Sete Cidades garden from the Azores (NS0 and ASS, Table S4), suggesting that the other studied elements were likely to be found in higher concentrations in soils with lower pH values (Fig. 3). On the other hand, EC and LOI were associated with higher content of As, Al, Hg and Cu, in correspondence with the Portuguese garden compared to other sites, regardless of the propagation method used for the corresponding tea plants (PSS and PSC, Fig. 3, Table S4).

Concerning thresholds limits set by Commission Decision (EU) 2022/

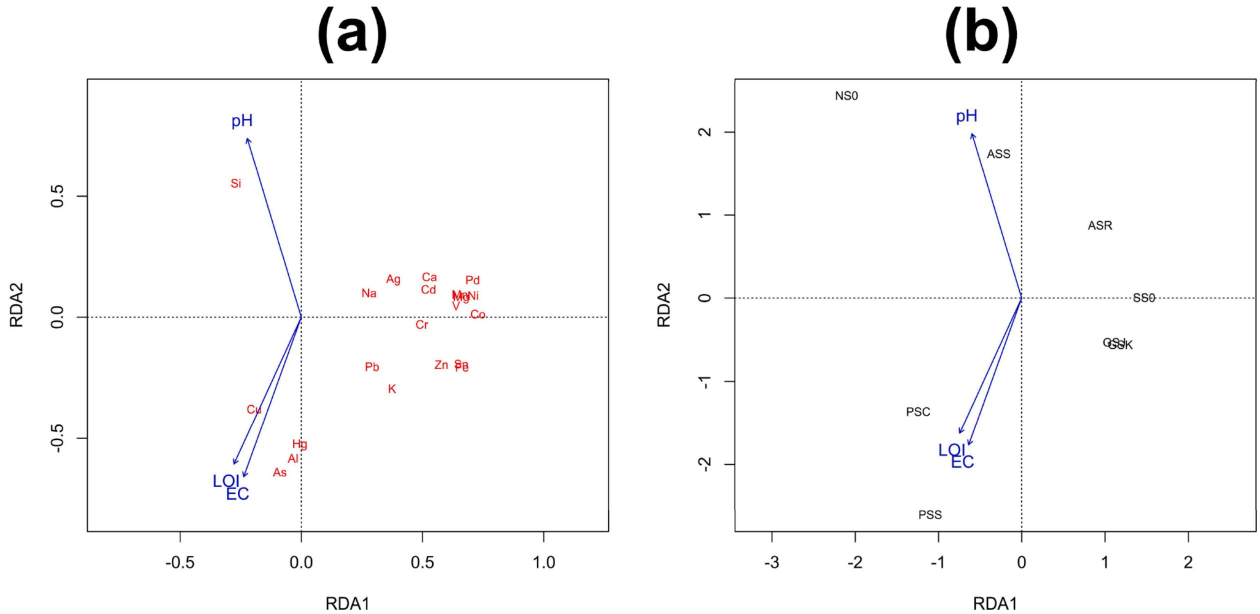

Fig. 3. Redundancy Analysis (RDA) of: (a) variables; (b) observations and constraining variables.

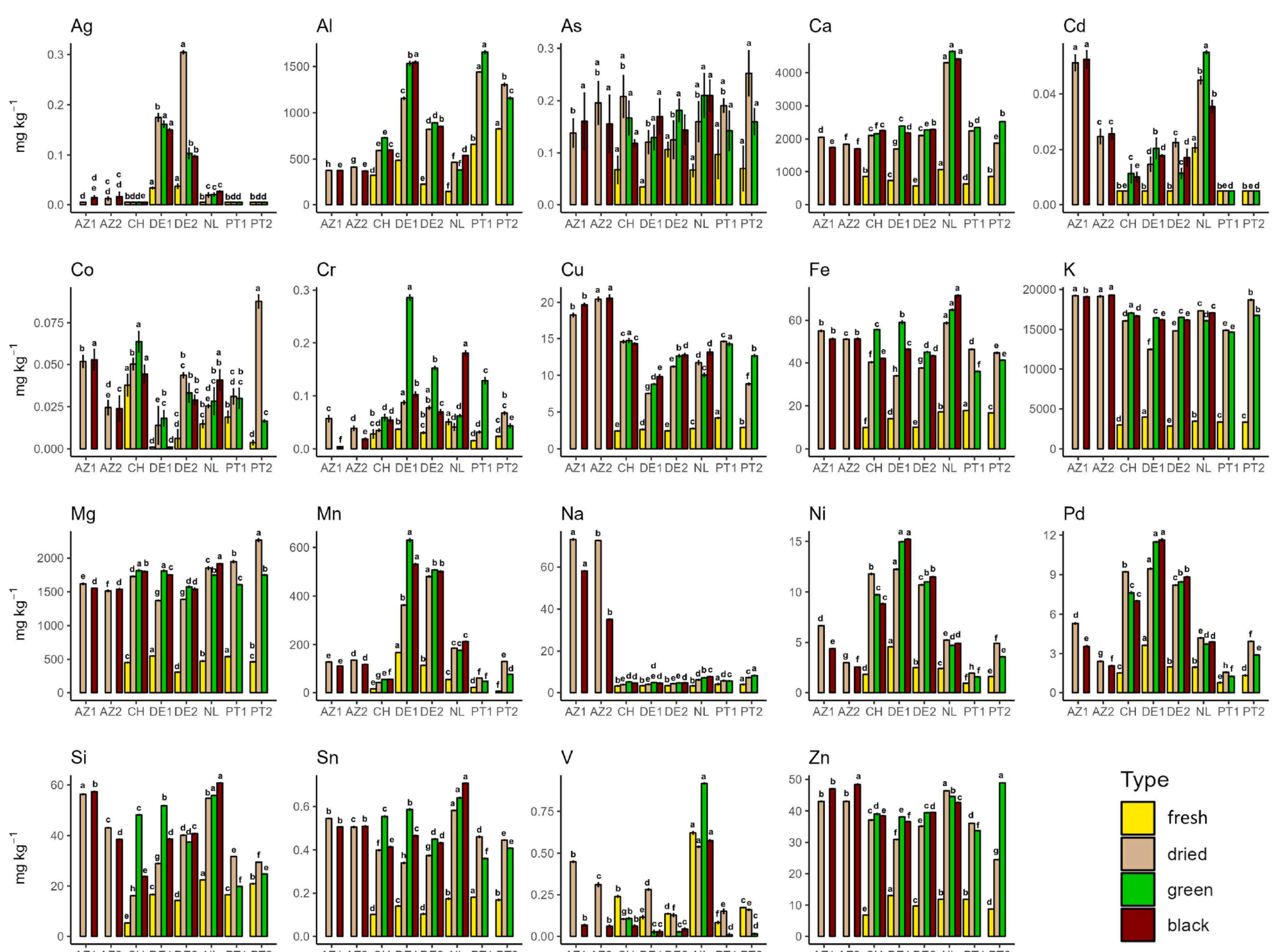

Fig. 4. Elemental content (mg kg^{-1}) in tea leaves coming from European tea gardens. Different letters above the bars indicate statistically significant difference ($p < 0.05$) among samples coming from different gardens which underwent the same treatment process.

1244, the content of Cd, Cr, Cu, Hg, Ni Pb, Zn was always below the limits set for growing media (1.3, 100, 200, 0.45, 40, 100, 300 mg kg^{-1}, respectively). Special attention should be given to As since the limit values (10 mg kg^{-1}) were surpassed in 38 % of samples.

3.2. Elemental content in tea leaves

In tea leaves, the average elemental content (mean ± sd, min–max, mg kg^{-1}) was registered in the order K (14051 ± 5951, 2838–20643) > Ca (2067 ± 1022, 572–4647) > Mg (1428 ± 544, 306–2266) > Al (741

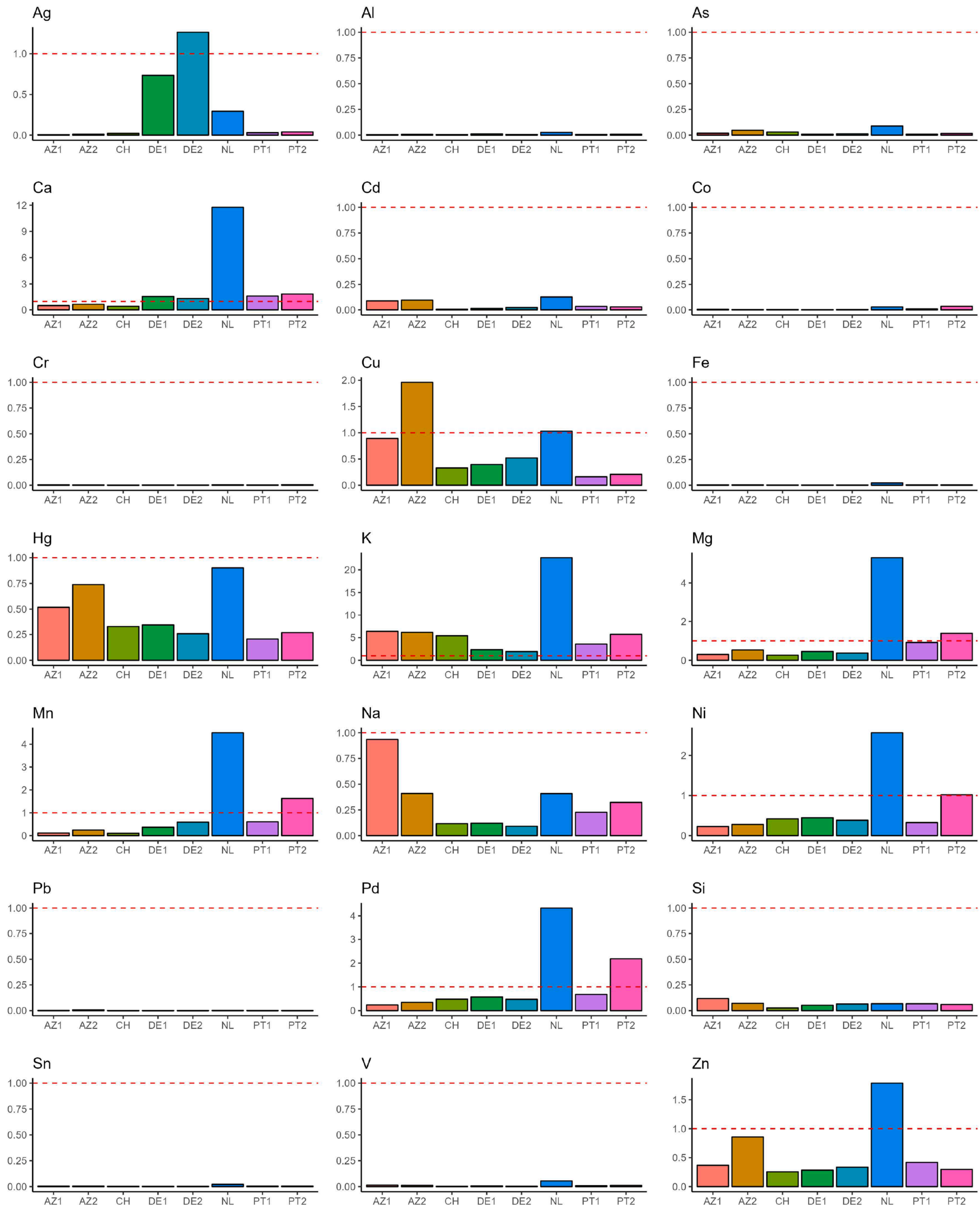

Fig. 5. Single element bioaccumulation factor (BAF) from soil to tea leaf. Red line is plotted at BAF = 1.0. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

± 438, 144–1656) > Mn (190 ± 180, 6–630) > Fe (42 ± 17, 10–72) > Si (36 ± 17, 5–74) > Zn (34 ± 14, 7–52) > Na (16 ± 22, 3–73) > Cu (12 ± 6, 2–21) > Ni (6 ± 4, 0.9–15.2) > Pd (5 ± 3, 0.8–11.6) > Sn (0.4 ± 0.2, 0.1–0.7) > V (0.2 ± 0.2, 0.01–0.92) > As (0.14 ± 0.05, 0.03–0.25) > Cr (0.07 ± 0.06, <LOD-0.286) > Ag (0.05 ± 0.07, <LOD-0.304) > Hg (<LOD) > Co (0.03 ± 0.02, <LOD-0.087) > Pb (<LOD) > Cd (0.02 ± 0.02, <LOD-0.054) (Fig. 4). Due to the removal of water content after the drying process, fresh tea leaves exhibited a lower elemental content compared to dried and to green and black processed tea leaves. V is the only element that seemed not to be affected by this process.

To consider the overall effect of different processes applied in European tea gardens, a comparison of the elemental content based on different tea leaves, dried and processed (green and black) was conducted grouping the samples coming from different tea gardens. The results indicated that the observed differences were not statistically significant ($p > 0.05$, Table S5). This suggests that the type of tea processing is not a significant representative factor influencing elemental content in European teas.

Occasional instances were observed where specific elements consistently varied in concentration among the different tea leaves. For example, Ag in the dried leaves of the Korean cultivar from Tschanara Teagarden (Germany), Co in the dried leaves of Sete Cidades garden (Azores), or Cr in the green processed leaves of the Japanese cultivar from Tschanara Teagarden (Germany). Nevertheless, no consistent pattern was identified, and this variability seemed to be associated with specific elements in particular gardens rather than a generalized trend.

Contrastingly, considering tea leaves coming from different tea gardens that underwent the same treatment process, the elemental profile exhibited statistically significant differences ($p < 0.05$) among gardens (Fig. 4).

3.3. *Influence of soil characteristics on the elemental profile of tea leaves*

The bioaccumulation factor (BAF) was measured on the single element and its value (min–max) followed the order K (1.9–22.7) > Ca (0.4–11.8) > Mg (0.3–5.3) > Pd (0.2–4.3) > Mn (0.1–4.5) > Ni (0.2–2.6) > Cu (0.2–2.0) > Zn (0.3–1.8) > Hg (0.2–0.9) > Na (0.091–0.94) > Ag (0.006–1.27) > Si (0.026–0.12) > Cd (0.007–0.13) > As (0.009–0.088) > V (0.002–0.055) > Co (0.001–0.035) > Al (0.005–0.027) > Fe (0.002–0.022) > Sn (0.001–0.022) > Cr (0.0009–0.0060) > Pb (0.0004–0.0084) (Fig. 5).

K was the only element with a BAF > 1 in every garden. Other values > 1 were represented by Ag in the Korean cultivar of Tschanara Teagarden (Germany), Ca in gardens located in Portugal, Netherlands and Germany, Cu in Sete Cidades (Azores) and Het Zuyderblad (the Netherlands) gardens, Mg, Mn, Ni and Pd in Chà Camèlia (Portugal) garden (only in samples deriving from cuttings) and Het Zuyderblad (the Netherlands). Lastly, Zn showed a BAF > 1 in Het Zuyderblad (the Netherlands).

The analysis of the correlation matrix indicated a positive correlation among clusters of elements in the soil, with the exception of Si (Figure S1a). These clusters included Ag-Na (cluster 1), Ca-Mg-Mn-Ni-Pd-K-Zn-Fe-Sn-Co-V-Pb-Cd-Cr (cluster 2), and Cu-As-Al-Hg (cluster 3). In contrast, the correlation matrix for tea leaves exhibited a more intricate pattern, featuring both positive and negative correlations, such

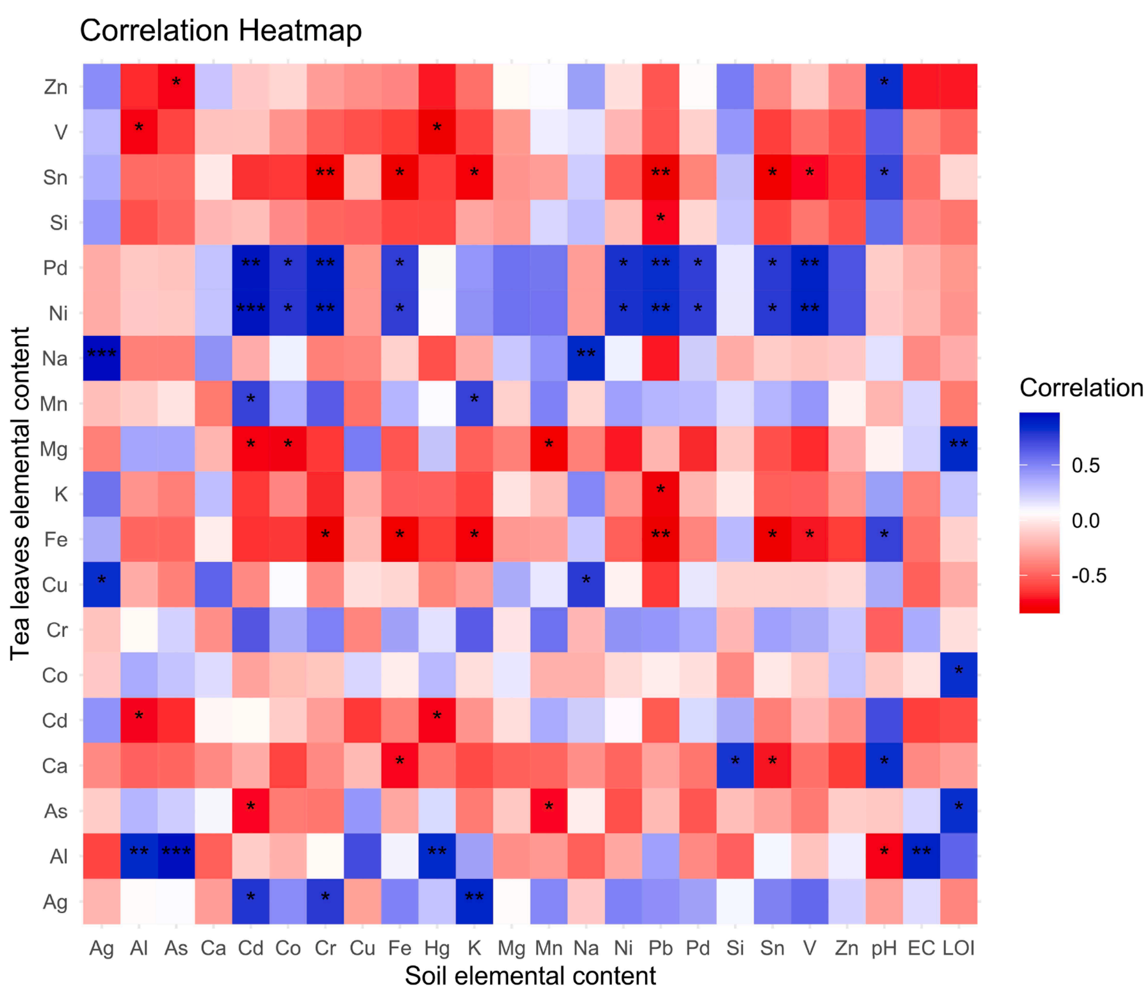

Fig. 6. Correlation heatmap relative to elemental content in soil and tea leaves. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

as Al-Zn and Al-Cd (Figure S1b).

A correlation matrix analysis was performed also between elements and other parameters in soil vs. elements in tea leaves. The results showed that all the elements, except for Cr, present in tea leaves are significantly affected ($p < 0.05$) by the composition of the soil intended as elemental profile, pH, EC or organic matter content (Fig. 6). Notably, only some elements exhibited significant linear positive correlations between tea leaves and soil content, including Pd ($p < 0.05$), Ni ($p < 0.05$), Na ($p < 0.05$) and Al ($p < 0.01$). Conversely, Sn ($p < 0.05$) and Fe ($p < 0.05$) in tea leaves showed negative linear correlations with Sn and Fe in soil, respectively.

Principal Component Analysis (PCA) was conducted for both soil and tea leaf elemental profiles, taking into account the elemental composition, and the resulting biplots of PC1 vs. PC2 are presented in Fig. 7.

In the soil analysis, four significant components were identified, explaining 94.98 % of the total variance (Fig. 7a). On the basis of elemental composition, the first two PCs (cumulative variance 74.78 %) associated soils coming from the same garden, such as the Portuguese and German ones, while separated soils originated from different gardens. The tea gardens exhibited distinctive separation, occupying diverse quadrants. Gardens from the Azores (Ribeira Grande and Sete Cidades) deviated from this pattern, positioning themselves in Quadrants II and III, respectively. The score plot revealed that element cluster 1 was associated with the Azores gardens, element cluster 2 with gardens located in Germany, Switzerland, and Ribeira Grande (Azores), while element cluster 3 was linked to the garden in Portugal (Table S6).

In the tea leaf analysis, five significant components were extracted, explaining 85.07 % of the total variance (Fig. 7b). On the basis of elemental composition, the first two PCs (cumulative variance 55.25 %) demonstrated discernible separation of tea leaves coming from different

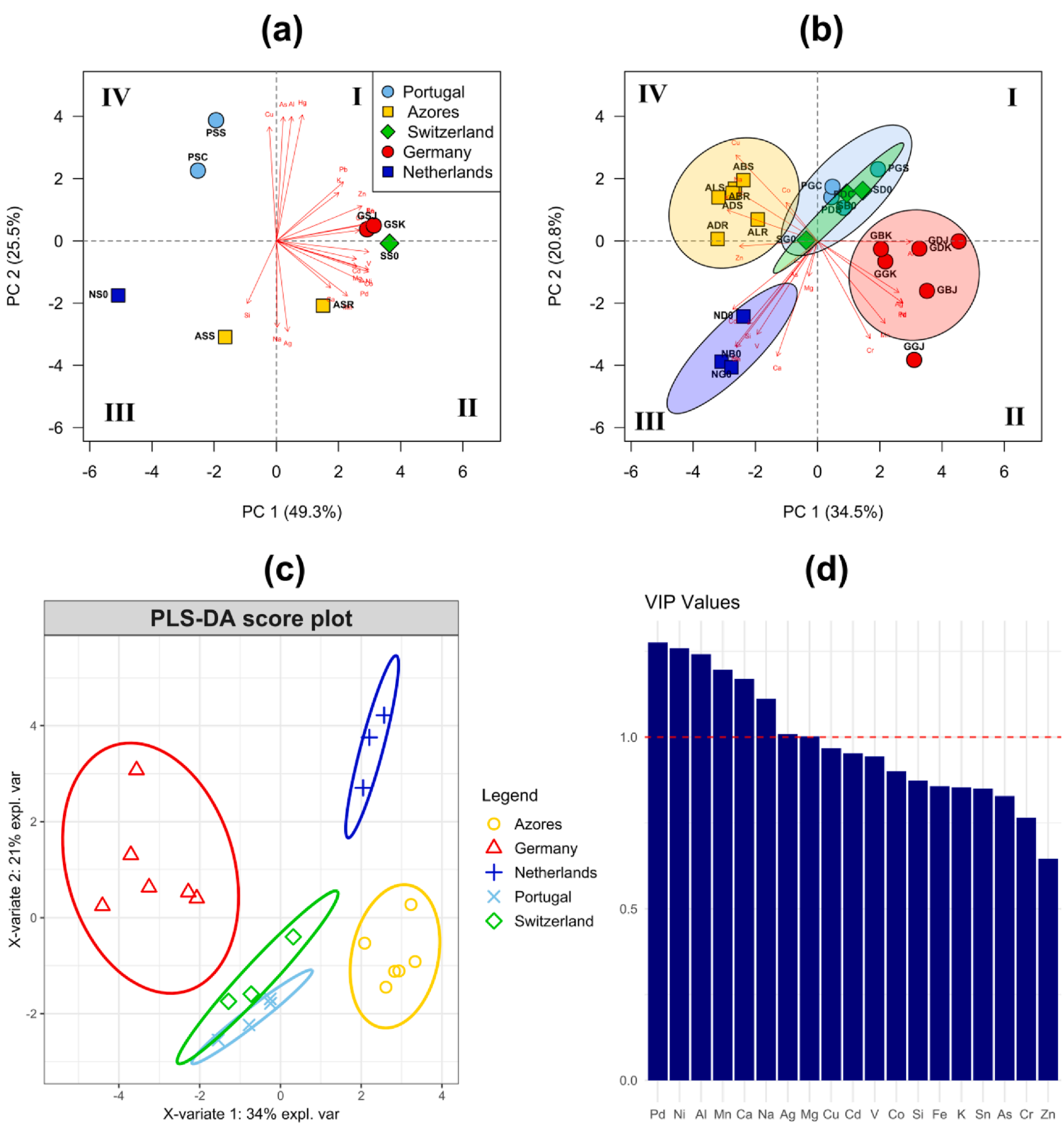

Fig. 7. Biplot of PC1 vs. PC2 in soil (a) and tea leaves (b). PLS-DA score plot (c) and VIP values bar plot (d). Ellipses in (b) and (c) were plotted at 95% confidence level.

gardens across different quadrants. Specifically, Chà Camèlia (Portugal) and Casa del Tè Monte Verità (Switzerland) were positioned in Quadrant I, Tschanara Teagarden (Germany) in Quadrant II, Het Zuyderblad (the Netherlands) in Quadrant III, and São Miguel Agrarian Development Service (Azores) in Quadrant IV. The corresponding score plot highlighted distinct associations: i) German tea leaves exhibited higher concentrations of Al, Ag, Pd, Ni, Mn, and Cr; ii) Dutch tea leaves were characterized by elevated levels of As, Mg, Cd, Si, V, Ca, Sn, and Fe; and iii) tea leaves from the Azores were linked to increased levels of Zn, Co, K, Na, and Cu (Table S6). Considering the biplots obtained from the combination of the significant principal components (PC1-PC5, Figure S2), a better separation of the gardens from Portugal and Switzerland occurred on PC3 vs PC5 biplot.

The PLS-DA model demonstrated strong performance, explaining approximately 93.6 % of the cumulative variance in X and 87.7 % in Y (Fig. 7c). The cumulative predictive ability (Q^2) of 79.3 % indicates the model's efficacy in predicting responses. The Root Mean Square Error of Estimation (RMSEE) was low (0.119), suggesting a good fit between observed and predicted responses.

In our exploration of the elemental composition of tea leaves using the Variable Importance in Projection (VIP) analysis, certain elements stood out as particularly crucial in predicting the country of origin (Fig. 7d). Specifically, Ag (1.009), Al (1.242), Ca (1.170), Mg (1.002), Mn (1.196), Na (1.112), Ni (1.259) and Pd (1.276), with a VIP > 1, played a significant role in accurately predicting the country of origin in our model, emphasizing their key contributions to the observed geographical distinctions in tea leaves.

The LDA model successfully classified tea samples based on their elemental composition, achieving 100 % accuracy. The summary of the confusion matrix indicates a total of 22 cases analyzed across 5 countries. A chi-squared test for independence of factors yielded a significant result (Chisq = 88, df = 16, p-value = 5.834×10^{-12}), suggesting strong evidence against the null hypothesis of no association between predicted and actual country of origin.

The SVM model also achieved a classification accuracy of 100 % (6 Azores, 6 Germany, 3 Netherlands, 4 Portugal, 3 Switzerland), indicating that all samples were correctly classified.

4. Discussion

4.1. Soil

The observed pH values within the soil samples, ranging from 4.3 to 5.7, align with the acknowledged acidic nature of soils in tea plantations. Tea plants, characterized as acidophilic crops, thrive optimally within a pH range of 4.0 to 5.5, with a natural soil acidification process highlighted by an annual rate of 0.071 (Yang et al., 2018). The distinctive physiological attributes of tea plants actively contribute to this acidification. According to Zhang et al., 2020, several mechanisms likely underpin the observed soil acidification in tea plantations (Zhang et al., 2020a): 1) the roots of tea plants release organic acids (e.g., oxalic acid, malic acid, and succinic acid), actively participating in soil acidification; 2) fertilization practices, integral for maximizing tea leaf yield, induce the nitrification of ammonium from nitrogen fertilizers, generating H^+ ions and expediting soil acidification (Yemane et al., 2007); 3) prolonged nitrogen fertilizer application may culminate in the accumulation of exchangeable Al^{3+}, and subsequent hydrolysis of Al^{3+} releases H^+ ions, intensifying soil acidification.

In our study, RDA showed that the analyzed elements, inclusive of toxic metals(oids) (e.g., Hg, Pb, Cd, As, Ni, Cr, …), exhibit an affinity for soils with lower pH values. This unveils an additional layer of concern, suggesting a potential compounding impact on soil dynamics. The explanation lies in the enhanced phytoavailability and heightened migration capacities of metals in acidic soils, making them more easily assimilated by the tea plant's roots. Numerous studies have consistently demonstrated that higher soil pH values lead to a reduction in both the availability of heavy metals in the soil and their concentrations in tea leaves (Wen et al., 2018; Zhang et al., 2018).

In the context of regulatory compliance, our investigation assessed elemental concentrations in soil with reference to the established thresholds outlined in Commission Decision (EU) 2022/1244, which sets the EU Ecolabel criteria for growing media and soil improvers. The elements Cd, Cr, Cu, Hg, Ni, Pb, and Zn consistently demonstrated concentrations below the prescribed limits for growing media, which are 1.3, 100, 200, 0.45, 40, 100, and 300 mg kg^{-1}, respectively (European Union, 2022). Notably, 38 % of samples (3 out of 8 soils) exhibited As concentrations exceeding limits. In the Portuguese soil (Chà Camèlia tea garden), the As values exceeded the limit by 197 and 157 % for seeds and cuttings crops, respectively, while in the German soil (Japanese cultivar of Tschanara Teagarden), it was 128 % above the regulatory threshold. A study on As in European agricultural soils highlights its predominantly geological origin, particularly in clayey soils (Tóth et al., 2016). Despite this, pervasive anthropogenic arsenic pollution exceeds natural sources. Our study, in alignment with Tóth et al. (2016), reveals cases where As concentrations in soil surpassed regulatory limits. This underscores the necessity for further investigation into As specific sources, mechanisms, and regional variations.

4.2. Tea leaves

The elemental composition of tea leaves presented a complex interplay of factors, offering insights into bioaccumulation dynamics and the influence of processing methods.

Overall, the concentrations of the studied elements are in agreement with those of our previous study (Girolametti et al., 2023) and they are of similar magnitude to those from other studies carried out in Asia and Africa (Cao et al., 2010; Ghuniem, 2019; Peng et al., 2018; Pourramezani et al., 2019; Rashid et al., 2016; Salahinejad and Aflaki, 2010; Shen and Chen, 2008).

K plays a crucial role in activating enzymes, regulating water balance, supporting photosynthesis, and facilitating the synthesis of proteins and starch in tea plants. In tea leaves, it is the second element in concentration after N and it contributes to the tolerance of both abiotic and biotic stresses, enhancing the biochemical parameters and organoleptic quality of tea. In our study, K emerged as a dominant element, showcasing consistent bioaccumulation across diverse tea gardens. This prevalence confirms the pivotal role of K in tea plant physiology and metabolic processes (Li et al., 2018; Singh and Pathak, 2018).

Some studies have demonstrated that the manufacturing process is one of the main sources of some selective elements and micronutrients (Jakubczyk et al., 2022; Zhang et al., 2018). As reported in our previous study (Girolametti et al., 2023), it was confirmed that the processing methods for European green and black teas have limited influence on elemental content, with variations observed only in specific elements. The garden-specific nature of these variations emphasizes the nuanced contributions of specific processes applied in individual gardens rather than a generalized trend. This phenomenon suggests a potential enrichment or removal of specific elements during the production of green or black tea that requires further investigations with a focused approach. Notably, the drying process, while affecting overall elemental concentrations, did not uniformly impact all elements, with V standing out as particularly susceptible to removal with water, suggesting a potential depletion of this element during the drying process.

The observed correlations between soil and tea leaf elements provide insights into the complex dynamics of element uptake by tea plants. Positive correlations for Al, Ni, Na, and Pd, suggest that these elements are particularly susceptible to be absorbed by the tea plant.

Human exposure to Al through contaminated food has been associated with Alzheimer's disease (Tomljenovic, 2011). In soil, Al salts dissociate at pH < 5.5, forming complexes with phosphate that are absorbed by roots and transported to leaves. Tea plants, known as Al hyperaccumulators, may accumulate up to 10 times more Al in older leaves and this condition can be of particular concern for human health, as excessive tea consumption could more than double an individual's basic Al intake (Hayacibara et al., 2004). In our study, the strong correlation between Al in tea leaves and Al in soil aligns with the classification of tea plants as Al-hyperaccumulator species. Interestingly, despite the strong correlation, the bioaccumulation factor for Al in tea leaves was found to be less than 1. This indicates that, contrary to expectations, there was no significant bioaccumulation of this element in tea leaves.

Exploration of other bioaccumulation factors in tea leaves highlighted K as a paramount accumulator, reinforcing its significance in the elemental composition of tea. The distinctive BAF values observed for specific elements, such as Ag, Ca, and Cu, underscore the garden-specific nature of elemental uptake. The BAF > 1 observed for different elements in teas from the Dutch garden (Het Zuyderblad) may be attributed to the relatively lower elemental content recorded in the soil rather than a higher concentration in tea leaves.

Importantly, the extremely low BAF for As at 0.03 signifies minimal bioaccumulation of this potentially toxic element in tea plants. This finding is crucial, particularly in soils where As concentrations surpass regulatory limits, indicating that high As levels in soil are not completely transferred to tea leaves.

PCA of soil and tea leaf elemental profiles have added depth to our understanding. In the soil analysis, the separation of tea gardens into distinct quadrants underscores the influence of regional and garden-specific factors on elemental composition. The identification of significant components and their association with specific gardens further emphasize the unique elemental signatures of each tea-producing region. Furthermore, the dissimilar association of gardens in the PCA of soil and tea leaves suggest that, despite a shared origin, the elemental profiles in soil and tea leaves exhibit distinct signatures.

4.3. Implications for tea quality and traceability

The elemental profile revealed through our study holds paramount implications for tea quality and traceability. By providing crucial insights into the presence of both essential and toxic elements, this profile serves as a valuable tool for assessing the overall quality and safety of tea leaves (Karak and Bhagat, 2010).

It is noteworthy that, despite the critical role of elemental composition in determining tea quality, there is currently no uniform European regulation governing the maximum concentration of specific elements in tea leaves. While some national regulations exist, the absence of a standardized framework highlights the need for comprehensive guidelines to ensure consistency and safety across European tea products.

Building on our prior research, where we measured the single element Hazard Quotients (HQs) and the comprehensive Hazard Index (HI) related to the consumption of European teas (Girolametti et al., 2023), the present study reaffirms the safety of tea consumption. The promising results of the risk index underscore the favorable outlook for the future of European tea production, providing consumers with a reliable assurance of product safety.

The issue of counterfeit teas in the market adds a layer of complexity to the tea industry. Our application of multivariate statistical approaches, particularly Principal Component Analysis (PCA), emerges as a robust defense mechanism in recognizing the geographical origin of European teas. By leveraging the distinctive elemental signatures identified through PCA, this method offers a powerful tool for combating fraudulent practices, enhancing traceability, and safeguarding the authenticity of tea products. The application of chemometric approaches (PCA, dendrogram, and other statistical techniques) and machine learning algorithms (PLS-DA, LDA, SVM, KNN and others) has proven successful in tracing the origin of tea in Asian and African regions (Li et al., 2018; Marcos et al., 1998; Moreda-Piñeiro et al., 2003; Zhang et al., 2020b). The present study underscores the importance of extending this application in differentiating European teas by country. The successful delineation of distinct clusters, irrespective of the processing method, underscores the overriding influence of unique elemental profiles imparted by the geographical origin of the teas.

In our investigation, we have identified specific elements that prove effective for tracing the origins of European tea, including Ag, Al, Ca, Mg, Mn, Na, Ni and Pd. This application not only benefits consumers but also contributes to the overall integrity and reputation of European tea producers.

5. Conclusions

This comprehensive investigation into the elemental profiling of tea leaves and soil across European tea gardens has elucidated that processing methods exert limited influence on individual elemental content. While processing may not significantly alter elemental profiles, the distinct regional fingerprints provide valuable insights into the geographical origin of tea products.

The recognition of garden-specific elemental profiles not only facilitates quality control but also presents opportunities for utilizing elemental composition as a tool for product differentiation and traceability.

Future prospects include delving into a more nuanced exploration of major elements in tea brews derived from the tea leaves investigated in this study. Analyzing the elemental dynamics during the brewing process will provide additional layers of insight, contributing to the continuous refinement of our understanding of tea production and quality parameters.

CRediT authorship contribution statement

Federico Girolametti: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Anna Annibaldi:** Writing – review & editing. **Silvia Illuminati:** Writing – review & editing. **Elisabetta Damiani:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Conceptualization. **Patricia Carloni:** Writing – review & editing, Investigation, Conceptualization. **Behixhe Ajdini:** Writing – review & editing. **Matteo Fanelli:** Writing – review & editing. **Cristina Truzzi:** Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization, Writing – review & editing.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139641.

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