Journal of Trace Elements in Medicine and Biology 52 (2019) 111–117

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Investigation of twelve trace elements in herbal tea commercialized in Brazil

Raquel Fernanda Milani[a,b], Letícia Kis Silvestre[a], Marcelo Antonio Morgano[a,*], Solange Cadore[b]

[a] Institute of Food Technology, Av. Brasil 2880, Jd. Chapadão, P.O. Box 139, 13070-178, Campinas, SP, Brazil
[b] Institute of Chemistry, University of Campinas, P.O. Box 6154, 13083-970, Campinas, SP, Brazil

ARTICLE INFO

Keywords:
Herbal tea
Trace elements
Dietary intake
Exposure assessment
ICP-MS

ABSTRACT

In this study twelve trace elements were investigated in herbal tea commercialized in Brazil. Boldo, Chamomile, Mate and Peppermint tea samples were acquired in Brazil local markets and both herbs and their infusions were evaluated. Trace elements were classified in two groups: poorly (Al, As, Ba, Cd, Cr, Fe, Pb and Se) and moderately (Cu, Mn, Ni and Zn) extractable. This patterned showed that even levels above threshold established by Brazilian and MERCOSUR regulations were observed in herbal tea (Cd and Pb in 89% and 78% of mate and peppermint tea samples, respectively), their infusions did not presented toxic levels. The estimative of exposure and dietary intake revealed important values for a daily consumption of a single cup of herbal infusion and an unique composition was also verified for herbal tea samples: although age and origin was unavailable, multivariate analysis classified the samples in four distinct groups.

1. Introduction

Tea (*Camellia sinensis*) is one of the most consumed non-alcoholic beverages in world and herbal tea also constitute a good source of minerals, vitamins and antioxidant compounds [1]. Herbal tea infusions are commonly associated with healthy benefits such as the reduction of arterial hypertension and cholesterol and better immunologic response [2].

Trace elements composition in herbal tea is highly related to the soil constitution, climate, plant ability to accumulate nutrients and environmental pollution [3,4]. Besides some trace elements (for example, chromium, copper, iron, manganese, selenium and zinc) exhibit an important role in human nutrition, at high levels they may present risk for human health including low oxygen absorption in blood, kidney and liver diseases [5,6]. For non-essential trace elements, such as aluminum, arsenic, barium, cadmium, lead and nickel, even low levels may cause toxic effects which include cancer, skin, gastrointestinal and neurological disorders [5,7].

In a previous study of our research group, a direct method was evaluated to determine trace elements in herbal tea infusions and soft drinks by ICP-MS. Low values were found for inorganic contaminants, such as arsenic (< 0.46-7.73 $\mu g\ L^{-1}$); cadmium (< 0.053- 1.05 $\mu g\ L^{-1}$) and lead (< 0.39-0.42 $\mu g\ L^{-1}$) whilst manganese demonstrated the nutritional importance of these beverages, ranging from 94 to 7641 $\mu g\ L^{-1}$ [8]. Trace elements were also evaluated in tea leaves and their infusions using inductively coupled plasma-mass spectrometry (ICP-MS). Although arsenic and lead were found in levels above the threshold established by Brazilian [9] and MERCOSUR [10] regulations, these inorganic contaminants exhibited a poor extraction from tea leaves to their infusions and safe levels were determined in tea infusions [11].

A study of micro and macroelements in several plants such as tea, coffee, mate, rooibos, honeybush and chamomile was reported by Malik et al. [12]. The authors found high levels of B, Ca, Cu, Mn, Mg and Zn in mate tea; B, Ca, Cu, Fe and P in chamomile tea and low values of elements in rooibos and honeybush infusions. Aluminum was found in high levels in tea and mate samples: 123–551 $mg\ kg^{-1}$ and 0.055–1.73 $mg\ L^{-1}$ in raw materials and infusions, respectively. In the same year, Özcan et al. [13] reported a study of mineral content in herbs and herbal tea beverages. The authors evaluated the mineral extraction behavior using two procedures (infusion and decoction) at three times (10, 15 and 20 min) and verified that both procedures reach an efficient mineral extraction at 10 min. Suliburska & Kaczmarek [2] performed a complete study with herbal tea purchased in Poland. The research group results demonstrated that chamomile and peppermint infusions are good source of minerals such as copper, iron and zinc.

More recently, Gómez-Nieto et al. [1] performed a study of nine elements in herbal teas commercialized in Spain using atomic absorption spectroscopy. The authors found levels within the international agencies thresholds except for Cd in thyme and chamomile samples, reaching 0.58 and 0.50 $mg\ kg^{-1}$, respectively. Zivkovic et al. [14] proposed an alternative analytical method for manganese and barium

* Corresponding author.
E-mail address: morgano@ital.sp.gov.br (M.A. Morgano).

https://doi.org/10.1016/j.jtemb.2018.12.004
Received 10 September 2018; Received in revised form 13 November 2018; Accepted 7 December 2018

determination in peppermint tea using laser induced breakdown spectroscopy and the authors verified a good agreement between the proposed method and the ICP OES conventional determination. de Oliveira et al. [15] evaluated Al, As, Cd, Cr and Pb in tea and herbal tea samples from several countries, including USA, China, Brazil, Canada, India, Pakistan and Peru. The authors found relevant values for Al intake for children and adults who consume larges amounts of black tea.

Although studies of mineral content in tea and medicinal herbs have been performed worldwide, to the best of our knowledge, few studies were reported in literature concerning trace elements levels in both herbal tea and their infusions. Thus, the main objectives of this study were to i) investigate the levels of twelve trace elements (Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn) in herbal tea and their extraction to their infusions; ii) estimate the daily intake of micronutrients and the dietary exposure to inorganic contaminants by daily consumption of one cup of herbal tea and iii) classify the herbal tea samples using multivariate analysis. Samples of boldo, chamomile, mate and peppermint tea were purchased in Brazil and trace elements levels were quantified by ICP-MS.

2. Materials and methods

2.1. Reagents

Water and nitric acid were purified by reverse osmosis (Gehaka, São Paulo, Brazil, 18.2 MΩ cm) and sub-boiling distiller (Berghof, Eningen, Germany), respectively. Analytical curves were prepared using 1000 mg L^{-1} certified standard solutions (Merck, Darmstadt, Germany) in the following ranges: 0.1–100 µg L^{-1} for As, Cd, Cr, Cu, Ni, Pb and Se; 10-2000 µg L^{-1} for Al, Ba, Fe, Mn and Zn in 0.2% (v/v) HNO_3. A 250 µg L^{-1} (v/v) internal standard solution was obtained by dilution of 100 mg L^{-1} certified standard solutions of Sc, Ge and Y (Specsol, São Paulo, Brazil) in 0.2% (v/v) HNO_3 for all elements, excepting for As, prepared in 2.5% (v/v) isopropyl alcohol.

2.2. Herbal tea samples

Thirty-six samples of herbal tea were purchased from markets in southeastern Brazil: Boldo (*Pneumus boldus Molina* leaves, n = 9); Chamomile (*Matricaria recutita L.* flowers, n = 9); Mate (*Ilex paraguariensis St. Hil.* leaves and stalks, n = 9) and Peppermint (*Mentha piperita L* leaves and branches, n = 9). Samples were acquired from 3 individual lots of 3 brands, totalizing 9 samples of each type of herbal tea. Infusions were prepared by brew the herbal tea bags, considering the proportion recommended by manufactures: 1 bag (ca. 1.5 g) for a 200 mL cup. Boiling purified water was added to the herbal tea bags and kept in contact for 3 min. The infusion was filtrated through a 0.25 mm polymeric membrane and acidified with purified nitric acid to obtain a 0.2% (v/v) acid solution.

2.3. Instrumentation

Trace elements Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn were determined using an ICP-MS (7700x, Agilent Technologies, Tokyo, Japan) operating with the follow conditions: RF power = 1550 W; Ar flow rate = 15 L min^{-1}; Ar auxiliary flow rate = 0.9 L min^{-1}; He flow rate = 5 and 10 mL min^{-1}; double pass spray chamber; micro-mist nebulizer with gas flow rate = 1.1 L min^{-1} and 0.3–1.0 s for integration time.

Isotopes ^{27}Al, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{80}Se, ^{111}Cd, ^{138}Ba, ^{206}Pb, ^{45}Sc, ^{72}Ge and ^{89}Y were monitored using conditions that attenuate mass interferences. For infusions, a discrete sampling introduction system (ISIS-DS) with 150 µL loop; 40 s for uptake and acquisition delay and rinse time during data acquisition was also applied.

2.4. Analytical methods

Trace elements were determined in herbal tea infusions applying a direct method [8]: infusions were acidified with HNO_3 to obtain a 0.2% (v/v) acid concentration and directly analyzed using ISIS-DS system.

Trace elements were analyzed in herbal tea after closed microwave decomposition (Start D, Milestone, Sorisole, Italy) at 170 °C (maximum), for 32 min, using the procedure described in our previous work [11]. Briefly, 0.2 g of herbal tea homogenized in a stainless steel mill (M-20, IKA, Staufen, Germany) was weighted in a digestion vessel and 3 mL of water and 5 mL of purified nitric acid were added. Final solutions were diluted to 25 mL in volumetric flasks using purified water.

2.5. Quality control and statistical analysis

For quality control, sample analysis was performed in triplicate and blank experiments followed the same procedure used for herbal tea samples. Analytical methods were validated based on INMETRO recommendations [16]. Limits of detection (LOD) and quantification (LOQ) were calculated as 3 and 10 times the standard deviation of 10 blank experiments and multiplied by the dilution factor used in sample procedure: 100x (250 mg / 25 mL) for herbal tea and 1x for infusions. The LOD and LOQ values ranged from 0.001 to 2.0 mg kg^{-1} and 0.004 to 6.7 mg kg^{-1}, respectively, for herbal tea, and from 0.09 to 10 µg L^{-1} and 0.053 to 35 µg L^{-1}, respectively, for infusion. Linearity of the analytical curves was determined by the correlation coefficient (Pearson or “r”) and obtained values were $r > 0.9999$.

Accuracy was verified using certified reference materials SRM 1547 Peach leaves (NIST, Maryland, USA) and INCT-TL-1 Tea leaves (Instytut Chemii i Techniki Jądrowej, Warszawa, Poland) and spiked experiments. Recoveries ranging from 77 to 118% and from 82 to 120% were verified for certified reference materials and spiked experiments, respectively. Both values were in agreement with AOAC [17]: 75–120%. Precision was evaluated considering the coefficient of variation (CV, in percentage) for 16 replicates of herbal tea and their infusions. Values ranged between 1–11% and 2–17%, respectively. Both values were in agreement with AOAC recommendation [17]: < 32% for 10 µg kg^{-1} and < 16% for 1 mg kg^{-1} [8,11].

Statistical one-way analysis of variance (ANOVA) and Tukey’s test were performed using XLSTAT software (Addinsoft, Paris, France). Principal component analysis (PCA) was executed using Pirouette software (Infometrix, Woodinville, WA, USA).

3. Results and discussion

3.1. Trace elements in herbal tea

The levels of Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn were determined in herbal tea samples and the values obtained are described in Table 1.

Despite the different visual appearance, according to the ANOVA and Tukey’s test results, trace elements levels were similar in boldo, chamomile and mate tea samples. Distinguish composition were verified for few elements, such as Ba, Mn and Zn. The highest levels of these elements were found in mate tea samples: 75.2, 1405 and 81 mg kg^{-1}, respectively. In general, peppermint tea samples presented the highest values for trace elements: Fe levels were up to 2-fold higher than the levels found in boldo, chamomile and mate tea samples (max. 1198 and 565 mg kg^{-1}, respectively).

Few studies are reported in the literature concerning trace elements levels in herbs and herbal tea. In general, trace elements levels found in our study are in agreement with the study reported by Malik et al. [12], which evaluated tea, coffee, mate, rooibos, honeybush and chamomile commercialized in Czech Republic. For mate tea samples, that authors found similar levels for Cu (7.98–12.7 mg kg^{-1}), Fe (83.4–88.1 mg kg^{-1}), Mn (309 - 114 mg kg^{-1}) and Ni (1.95–3.10 mg kg^{-1}) and high

Table 1
Trace elements levels in herbal tea samples.

Element	Herbal tea: Mean (range), mg kg^{-1} Boldo (n = 9)	Chamomile (n = 9)	Mate (n = 9)	Peppermint (n = 9)
Al	153 (53-254)^{a}	513 (174-1268)^{b}	542 (367-690)^{b}	990 (681-1451)^{c}
As	0.064 (0.025-0.108)^{a}	0.107 (0.027-0.228)^{a}	0.062 (0.026-0.100)^{a}	0.160 (0.088-0.199)^{b}
Ba	30.9 (20.3-42.8)^{b}	13.7 (5.30-25.1)^{a}	75.2 (60.9-89.3)^{c}	25.5 (22.0-30.6)^{b}
Cd	0.018 (0.006-0.045)^{a}	0.14 (0.090-0.251)^{b}	0.572 (0.246-0.774)^{c}	0.039 (0.027-0.077)^{b}
Cr	1.34 (0.04-2.82)^{a}	1.77 (1.23-2.98)^{a}	1.87 (0.80-3.91)^{a}	3.31 (2.37-4.59)^{b}
Cu	3.1 (2.6-3.5)^{a}	9.2 (7.7-11.6)^{b}	11.0 (9.5-12.2)^{c}	14.8 (13.1-19.9)^{d}
Fe	139 (60-283)^{a}	356 (164-565)^{b}	280 (103-437)^{ab}	995 (799-1198)^{c}
Mn	111 (83-140)^{a}	57 (46-73)^{a}	1405 (1155-1811)^{b}	90 (72-112)^{a}
Ni	0.66 (0.50-1.06)^{a}	0.96 (0.40-1.78)^{a}	2.98 (2.14-4.12)^{c}	1.79 (1.30-2.18)^{b}
Pb	0.15 (0.06-0.25)^{a}	0.35 (0.16-0.62)^{b}	0.39 (0.14-0.82)^{b}	0.71 (0.54-0.95)^{c}
Se	0.032 (0.024-0.051)^{a}	0.047 (0.026-0.066)^{ab}	0.053 (0.025-0.113)^{ab}	0.064 (0.048-0.108)^{b}
Zn	15 (11-16)^{a}	24 (18-36)^{a}	81 (40-105)^{b}	24 (21-26)^{a}

[a, b, c,d] Mean values between different columns with the same letter are not significantly different at $p > 0.05$, according to the Tukey's test.

levels for chamomile tea samples: Cu = 29.1 ± 8.3 mg kg^{-1} and Ni = 2.87 ± 0.45 mg kg^{-1}, respectively. In our study, however, high values for Al and Zn were observed, ranging from 367 to 690 mg kg^{-1} and 40–105 mg kg^{-1}, respectively, being 2.5-fold higher than those reported by Malik et al. [12].

Arpadjan et al. [18] performed a study for arsenic, cadmium and lead in chamomile and peppermint purchased in Bulgaria and found higher Cd and Pb levels than the present study, ranging from 22 to 256 µg kg^{-1} and from 288 to 2580 µg kg^{-1}, respectively. For arsenic, similar levels were observed, ranging from 82 to 225 µg kg^{-1}. Özcan et al. [13] reported a study of several trace elements in herbs and herbal teas purchased in Turkey. The herbal teas included peppermint (*Mentha piperita L.*) and chamomile tea (*Matricaria chamomilla*) and the authors verified similar results for Ba and Cd in peppermint tea and for Cd, Cu and Zn in chamomile tea. In general, Özcan et al. [13] found lower values for trace elements in herbal tea samples than those reported in the present study, except for Se (1.65 mg kg^{-1}) in peppermint tea and for As (16.4 mg kg^{-1}), Cr (11.19 mg kg^{-1}), Ni (3.71 mg kg^{-1}) and Pb (1.31 mg kg^{-1}) in chamomile tea.

A study of mineral and trace elements in herbs consumed for medical purpose in Poland was reported by Pytlakowska et al. [19]. In general, similar or lower levels than our study were observed for Al, Ba, Fe and Mn in peppermint (*Mentha x piperita*) and chamomile (*Matricaria chamomilla L.*). For Cu and Zn, high levels were reported by the authors in peppermint samples, ranging from 1.49 to 1.75 and 55.2 to 75.3 mg kg^{-1}, respectively.

More recently, Borges et al. [20] proposed a method for Cd and Cr determination in mate samples using an absorption spectrometric technique. The authors found levels between 0.28–2.06 mg kg^{-1} and 0.27–2.37 mg kg^{-1}, respectively in samples purchased in Brazil. These values were higher than those found for Cd in the present study, which ranged from 0.246-0.774 mg kg^{-1} and similar for Cr (0.80–3.91 mg kg^{-1}). These results may be due to difference in soil nutrients, rainfall incidence and altitude in herbal tea cultivated area [12]. The origin of herbal tea is often unspecified and herbal tea sachet is constituted by a pool of herbal leaves and/or flowers from different locations [11].

Concerning the inorganic contaminants, Brazilian and MERCOSUR regulations set maximum limits for arsenic (0.6 mg kg^{-1}), cadmium (0.4 mg kg^{-1}) and lead (0.6 mg kg^{-1}) in tea, yerba mate and other infused vegetables [9,10]. The obtained results showed that for some samples these thresholds were achieved: Cd in mate samples (89%) and Pb in chamomile (11%); mate (11%) and peppermint (78%) samples.

3.2. Multivariate analysis

Multivariate analysis is an important tool to interpret analytical data. Principal components analysis (PCA) algorithm allows classifying samples based on chemical composition. In this study, data was organized in a matrix (36 × 12): lines and columns corresponding to the herbal tea samples and the trace elements, respectively. Auto escalated pre-processing was used and no samples were considered outliers.

Two principal components can explain 79.3% of the total variance (Factor 1 = 40.6%; Factor 2 = 38.7%) and were chosen for further analysis. The first principal component (Factor 1) is related to the trace elements Al (0.4163), As (0.3556), Cu (0.3896), Fe (0.4305) and Pb (0.4052) whereas the second principal component (Factor 2) is related to Ba (0.4158), Cd (0.4351), Mn (0.4473), Ni (0.4190) and Zn (0.4403). The values in the parentheses correspond to the loading values. The results are presented in Fig. 1.

From Fig. 1 four groups were clearly recognized:

- Group 1 (Mate tea samples) associated with Ba, Cd, Mn and Zn levels (elements with high loading values in Factor 2);
- Group 2 (mainly Boldo tea samples) associated with low Ba, Cd, Mn and Zn levels (elements with low loading values in Factor 1);
- Group 3 (Chamomile tea samples) associated with As, Cr and Se levels (elements with loading values near to 0.3 in Factor 1);
- Group 4 (Peppermint tea samples) associated with Al, Cu, Fe, Pb levels (elements with high loading values in Factor 1)

PCA classified correctly herbal tea samples, except for one chamomile tea sample which present similar chemical composition with boldo tea samples. These results are significant due to origin and age of herbal tea when harvested was not available.

3.3. Trace elements in herbal tea infusions and extraction from the herbs to the infusions

Herbal tea infusions were prepared brewing the herbal tea bags in the recommended proportion by manufacturers: 1 bag (ca. 1.5 g) for a 200 mL cup. The levels of Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn in herbal tea infusions and the percentage of extraction from the herbal tea to the infusions are presented in Table 2.

In general, trace elements levels in herbal infusions revealed low variation for boldo, chamomile and peppermint infusions, according to the ANOVA and Tukey's test results. Nevertheless for Cd, Cu and Fe, a significant difference was observed: Cd in chamomile infusions reached 0.24 µg L^{-1} whilst it was not quantifiable in boldo and peppermint infusions; Cu and Fe average levels were above 26.1 µg L^{-1} and 32.4 µg L^{-1} in chamomile and peppermint infusions, respectively.

Similarly to the herbal tea bags, few studies were dedicated to trace elements investigation in herbal tea infusions. In the study performed by Malik et al. [12], Al, Cu, Fe and Ni levels in chamomile and mate infusion were higher than those observed in our study, being Al levels

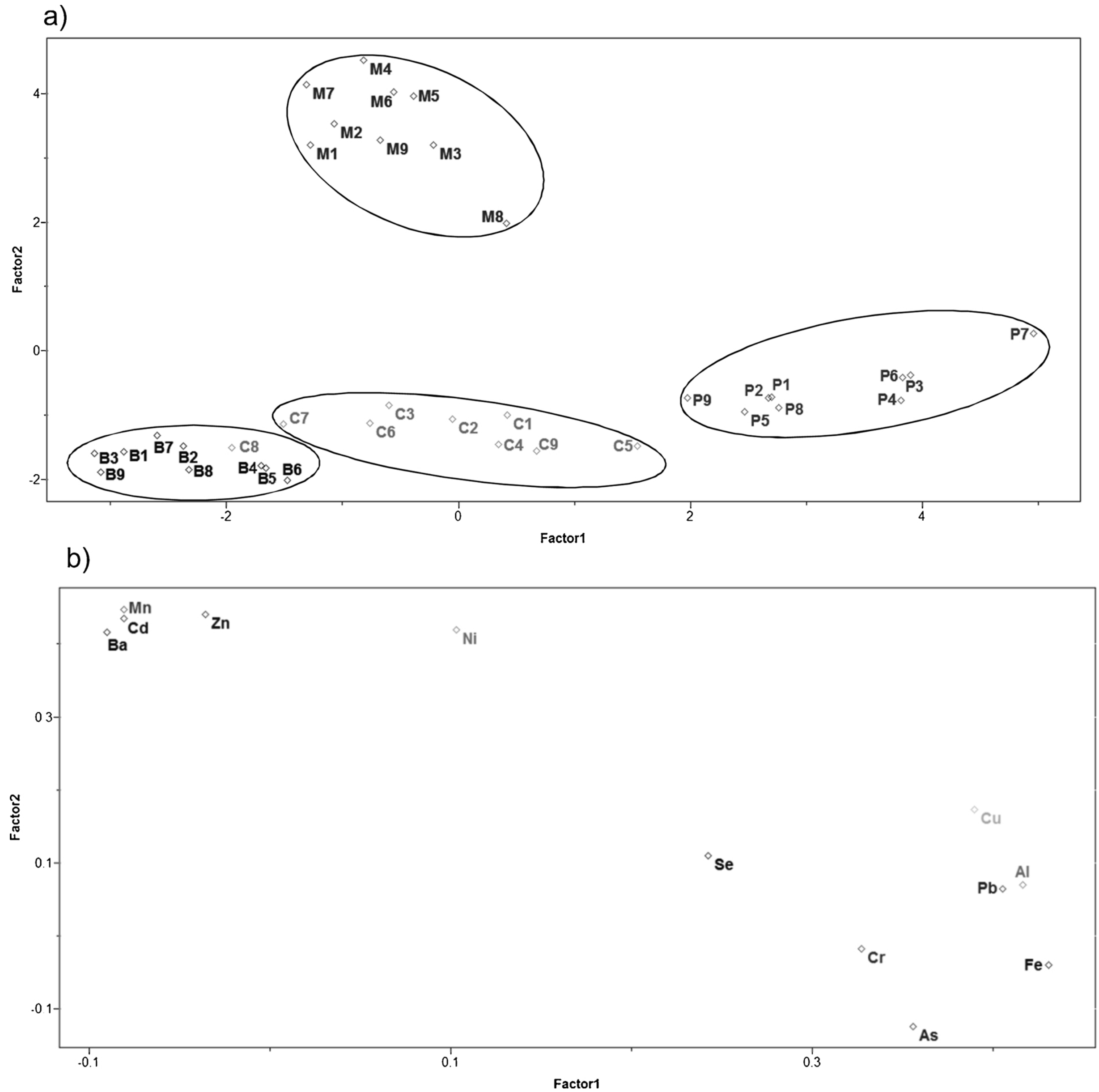

Fig. 1. PCA for trace elements levels in herbal tea samples (**B** = Boldo tea; **C** = Chamomile tea; **M** = Mate tea; **P** = Peppermint tea) **1a**) Score plot; **1b)** Loading plot.

Table 2
Trace elements levels in herbal tea infusions and % of extraction from herbal teas to their infusions (200 mL).

Element	Boldo (n = 9)		Chamomile (n = 9)		Mate (n = 9)		Peppermint (n = 9)	
	Mean (range) ($\mu g\ L^{-1}$)	Extraction (%)	Mean (range) ($\mu g\ L^{-1}$)	Extraction (%)	Mean (range) ($\mu g\ L^{-1}$)	Extraction (%)	Mean (range) ($\mu g\ L^{-1}$)	Extraction (%)
Al	< 20^{a}	0^{A}	33 (< 20-87)^{a}	0.71 (0-1.6)^{A}	163 (85-334)^{b}	4.0 (2.2-6.5)^{B}	11 (< 20-52)^{a}	0.14 (0-0.49)^{A}
As	< 0.46^{a}	0^{A}	< 0.46^{a}	0^{A}	< 0.46^{a}	0^{A}	< 0.46^{a}	0^{A}
Ba	11 (6-17)^{a}	4.7 (3.2-6.0)^{A}	13 (4-26)^{a}	13 (6.2-19)^{B}	80 (58-110)^{b}	14 (9.2-24)^{B}	23 (15-36)^{a}	12 (7.2-21)^{B}
Cd	< 0.05^{a}	0^{A}	0.11 (< 0.05-0.24)^{b}	11 (0-14)^{C}	0.28 (0.07-0.60)^{c}	6.7 (1.7-13)^{B}	< 0.05^{a}	0^{A}
Cr	< 0.29^{a}	0^{A}	< 0.29^{a}	0^{A}	1.02 (0.29-2.34)^{b}	8.3 (0-17)^{B}	0.31 (< 0.29-0.83)^{a}	1.3 (0-3.3)^{A}
Cu	6.4 (4.6-7.9)^{b}	28 (20-35)^{B}	30.8 (19.4-39.1)^{d}	45 (27-50)^{C}	1.7 (< 1.7-2.7)^{a}	2.0 (0-3.1)^{A}	26.1 (21.1-32.3)^{c}	24 (19-30)^{B}
Fe	12.2 (4.2-19.0)^{a}	1.3 (0.71-1.9)^{B}	32.4 (8.8-57.4)^{b}	1.3 (0.50-2.1)^{B}	16.8 (9.2-29.4)^{a}	0.88 (0.45-1.2)^{AB}	36.9 (19.1-63.2)^{b}	0.49 (0.24-0.79)^{A}
Mn	122 (91-183)^{a}	15 (12-17)^{A}	106 (69-153)^{a}	24 (17-30)^{B}	2312 (1551-3651)^{b}	22 (16-30)^{B}	141 (96-231)^{a}	20 (15-28)^{B}
Ni	2.4 (< 2.1-4.8)^{a}	46 (0-68)^{A}	2.7 (< 2.1-8.6)^{a}	26 (0-81)^{A}	11.3 (9.1-19.1)^{c}	51 (40-70)^{A}	6.3 (4.4-8.3)^{b}	47 (38-56)^{A}
Pb	< 0.39^{a}	0^{A}	0.15 (< 0.39-0.88)^{ab}	5.2 (0-31)^{A}	0.25 (< 0.39-0.73)^{ab}	7.2 (0-27)^{A}	0.39 (< 0.39-0.76)^{b}	7.3 (0-19)^{A}
Se	< 0.50^{a}	0^{A}	< 0.50^{a}	0^{A}	< 0.50^{a}	0^{A}	< 0.50^{a}	0^{A}
Zn	< 35^{a}	0^{A}	38 (< 35-58)^{bc}	22 (0-28)^{C}	70 (< 35-171)^{c}	12 (0-22)^{B}	35 (< 35-49)^{b}	19 (0-29)^{BC}

[a, b, c,d, A,B,C] Mean values between different columns with the same letter are not significantly different at $p > 0.05$, according to Tukey's test.

10-fold higher (292 μg L^{-1}) in chamomile infusions and 2-fold higher (220–386 μg L^{-1}) in mate infusions using herbal tea purchased in Czech Republic. For Zn, Malik et al. [12] reported very similar levels to those found in our investigation: 418 and 235 μg L^{-1} for mate and chamomile infusions, respectively.

Suliburska & Kaczmarek [2] studied herbal tea samples purchased in Poland and the results revealed that chamomile and peppermint infusions are source of minerals such as Cu, Fe and Zn. The results reported by this research group were higher than those found in our investigation: Cu and Fe levels were 4-fold higher, being around 115 and 100 μg L^{-1}, respectively and Zn levels were 2-fold higher, being around 70 μg L^{-1}. Recently, Schulzki et al. [21] reported a study with tea (*Camellia sinensis L. Kuntze*) and herbal/fruit infusions purchased in Germany. The researchers reported similar levels to inorganic contaminants As, Cd and Pb in chamomile, mate and peppermint infusions and high values for Al and Cu in chamomile and peppermint infusions, being 214 and 252 μg L^{-1} and 71.3 and 66.9 μg L^{-1}, respectively.

The difference between the levels found in our study and those reported in literature may due to different infusions processing habits such as the ratio and contact time between herbal tea and water [22]. Regarding the inorganic contaminants, Brazilian and MERCOSUR regulations set maximum limits for arsenic (0.05 mg kg^{-1}), cadmium (0.02 mg kg^{-1}) and lead (0.05 mg kg^{-1}) in non-alcoholic beverages [9,10]. Inorganic contaminants in all samples were found within the thresholds established.

Concerning the trace elements extraction from herbal tea bags to their infusions, the analytes can be classified considering the extraction percentage: poorly extractable (< 20%), moderately extractable (22–55%) and highly extractable (> 55%) [22,23]. From Table 2 data, in general, trace elements were classified in two groups: poorly extractable (Al, As, Ba, Cd, Cr, Fe, Pb and Se) and moderately extractable (22–55%: Cu, Mn, Ni and Zn).

Overall, the lowest extraction percentages were found in boldo infusions, being < 15% except for Cu and Ni, 28 and 46%, respectively. In contrast, in chamomile infusions were found the highest values for Cu and Ni, 50 and 81%, respectively. Mate infusions presented a distinctive pattern extraction for Al, Cd, Cr and Zn, according to ANOVA and Tukey's test results, being the average extractions 4.0, 6.7, 8.3 and 12%, respectively. In peppermint infusions, all trace elements were quantifiable except As, Cd and Se and the highest value for Pb was found in these samples (19%).

Several nutritional components have been reported to affect the element extraction from herbal and medicinal. Pinto [24] reported in a recent study that flavonoids contribute to 30% of tea leaves being EGCG (epigallocatechin 3-gallate) the most abundant in green tea. Chelating components that may bound the element in matrix of herbal plants, the formation of insoluble complexes and/or chemical proprieties such as pH of water and herbal infusions were also reported as factors that may influence the leachability rates [22,23,25]. Contact time between boiling water and tea leaves were also evaluated by Milani et al. [8], and no significance increasing in trace elements (Al, Ba, Cr, Cu, Fe, Mn, Ni, Zn) levels were observed. In this study, the authors considered four different contact times with boiling water (3, 5, 10 and 15 min) and a bag with tea leaves (ca. 1.5 g) and a linear behavior was observed in trace elements extraction.

Table 3
Estimative of micronutrients intake and inorganic contaminants exposure, considering the daily consumption of one cup of tea (200 mL) by an adult (body weight, bw, 70 kg).

Trace Element			Boldo	Chamomile	Mate	Peppermint
Inorganic contaminants	Monthly intake					
	Cd	Mean (range), μg kg^{-1} bw	< 0.021	0.047 (< 0.021-0.103)	0.12 (0.030-0.257)	< 0.021
		% PTMI	< 0.09	0.19 (< 0.09-0.41)	0.48 (0.12-1.03)	< 0.09
	Weekly intake					
	Al	Mean (range), μg kg^{-1} bw	< 2.0	3.3 (< 2.0-8.7)	16.3 (8.5-33.4)	1.1 (< 2.0-5.2)
		% PTWI	< 0.10	0.16 (< 0.10-0.43)	0.82 (0.43-1.67)	0.06 (< 0.10-0.26)
	Daily intake					
	As	Mean (range), μg kg^{-1} bw	< 0.007	< 0.007	< 0.007	< 0.007
		% $BMDL_{05}$	< 0.23	< 0.23	< 0.23	< 0.23
	Ba	Mean (range), μg kg^{-1} bw	0.16 (0.09-0.24)	0.19 (0.06-0.37)	1.14 (0.83-1.57)	0.33 (0.21-0.53)
		% $BMDL_{05}$	< 0.002	< 0.002	< 0.002	< 0.002
	Ni	Mean (range), μg kg^{-1} bw	0.03 (< 0.03-0.07)	0.04 (< 0.03-0.12)	0.16 (0.13-0.27)	0.09 (0.06-0.12)
		% TDI	0.29 (< 0.25-0.57)	0.32 (< 0.25-1.0)	1.35 (1.08-2.27)	0.75 (0.52-0.99)
	Pb	Mean (range), μg kg^{-1} bw	< 0.006	0.006(< 0.006-0.013)	0.007(< 0.006-0.010)	0.007(< 0.006-0.011)
		% $BMDL_{01}$	< 0.05	0.05 (< 0.05-0.10)	0.06 (< 0.05-0.09)	0.06 (< 0.05-0.09)
Micronutrients	Cu	Mean (range), μg kg^{-1}	6.4 (4.6-7.9)	30.8 (19.4-39.1)	1.7 (< 1.7-2.7)	26.1 (21.1-32.3)
		% DRI	0.07 (0.05-0.09)	0.34 (0.22-0.43)	0.02 (< 0.02-0.03)	0.29 (0.23-0.36)
		% DV	0.03 (0.02-0.04)	0.15 (0.10-0.20)	0.01 (< 0.01-0.01)	0.13 (0.11-0.16)
	Cr	Mean (range), μg kg^{-1}	< 0.29	< 0.29	1.00 (0.29-2.30)	0.31 (< 0.29-0.83)
		% DRI	< 0.08	< 0.08	0.29 (0.08-0.66)	0.09 (< 0.08-0.24)
		% DV	< 0.02	< 0.02	0.08 (0.02-0.19)	0.03 (< 0.02-0.07)
	Fe	Mean (range), μg kg^{-1}	12.2 (4.2-19.0)	32.4 (8.8-57.4)	16.8 (9.2-29.4)	36.9 (19.1-63.2)
		% DRI	0.01 (0.003-0.01)	0.02 (0.01-0.04)	0.01 (0.01-0.02)	0.03 (0.01-0.05)
		% DV	0.01 (0.002-0.01)	0.02 (0.01-0.03)	0.01 (0.01-0.02)	0.02 (0.01-0.04)
	Mn	Mean (range), μg kg^{-1}	122 (91-183)	106 (69-153)	2312 (1551-3651)	141 (96-231)
		% DRI	0.5 (0.4-0.8)	0.5 (0.3-0.7)	10.1 (6.7-15.9)	0.6 (0.4-1.0)
		% DV	0.6 (0.5-0.9)	0.5 (0.4-0.8)	11.6 (7.8-18.3)	0.7 (0.5-1.2)
	Se	Mean (range), μg kg^{-1}	< 0.50	< 0.50	< 0.50	< 0.50
		% DRI	< 0.15	< 0.15	< 0.15	< 0.15
		% DV	< 0.07	< 0.07	< 0.07	< 0.07
	Zn	Mean (range), μg kg^{-1}	< 35	38 (< 35-58)	70 (< 35-171)	35 (< 35-49)
		% DRI	< 0.05	0.05 (< 0.05-0.08)	0.10 (< 0.05-0.24)	0.05 (< 0.05-0.07)
		% DV	< 0.02	0.03 (< 0.02-0.04)	0.05 (< 0.02-0.11)	0.02 (< 0.02-0.03)

PTMI (Provisional Tolerable Monthly Intake): Cd = 25 μg kg^{-1} bw; **PTWI** (Provisional Tolerable Weekly Intake): Al = 2 mg kg^{-1} bw; **$BMDL_{05}$** (Benchmark Dose Lower Limit): As = 3.0 μg kg^{-1} bw (for inorganic arsenic) [28]; **TDI** (Tolerable Daily Intake): Ni = 12 μg kg^{-1} bw [27]; **$BMDL_{01}$**: Pb = 12 μg kg^{-1} bw [29]; **$BMDL_{05}$**: Ba = 63 mg kg^{-1} bw [30]; **DRI** = Daily recommend intake based on a caloric intake of 2000 calories: Cr = 35 μg/100 g, Cu = 900 μg/100 g, Fe = 14 mg/100 g, Mn = 2.3 mg/100 g, Se = 34 μg/100 g, Zn = 7 mg/100 g [33]; **DV** = Daily value based on a caloric intake of 2000 calories: Cr = 120 μg/100 g, Cu = 2 mg/100 g, Fe = 18 mg/100 g, Mn = 2 mg/100 g, Se = 70 μg/100 g, Zn = 15 mg/100 g [32].

3.4. Dietary intake and estimative of exposure

The dietary intake and the estimative of exposure was performed considering a daily consumption of a cup of tea (200 mL) by a 70 kg adult (bw = body weight). This postulation agreed with the established in Camargo & Toledo study [26], who determined a mean daily consumption of 263.34 mL of tea by the population of Campinas (Brazil). The results are presented in Table 3.

The estimative of inorganic contaminants exposure for a cup of tea consumption by a 70 kg adult were compared to the threshold values established by European Food Safety Authority (EFSA), U.S. Environmental Protection Agency (US EPA) and The World Health Organization (WHO). For Ni, the Tolerable Daily Intake (TDI) $= 12\,\mu g\,kg^{-1}$ bw [27]; for Al, the Provisional Tolerable Weekly Intake (PTWI) $= 2\,mg\,kg^{-1}$ bw, for As, Benchmark Dose Lower Limit ($BMDL_{05}$) for inorganic arsenic $= 3.0\,\mu g\,kg^{-1}$ bw, for Cd, Provisional Tolerable Monthly Intake (PTMI) $= 25\,\mu g\,kg^{-1}$ bw [28]; for Pb, $BMDL_{01} = 12\,\mu g\,kg^{-1}$ bw [29] and for Ba, $BMDL_{05} = 63\,mg\,kg^{-1}$ bw [30].

Overall, the estimative of inorganic contaminants exposure revealed safe levels. The highest levels were observed for mate tea infusions: the daily consumption of a cup of mate tea can reach 1.03% of PTMI for Cd, 1.67% of PTWI for Al, 2.27% of TDI for Ni and 0.09% $BMDL_{01}$ for Pb. For As and Ba, the contribution to the $BMDL_{05}$ was below 0.23% and 0.002%, respectively for all types of herbal tea. Although the $BMDL_{05}$ was established for inorganic arsenic, our estimative based on arsenic total levels is consistent with the results reported in Yuan, Gao, He and Jiang study [31], who verified that inorganic As species were predominant in tea infusions.

The micronutrients daily intakes for a cup of tea consumption by a 70 kg adult were compared to the values established by U.S Food and Drug Administration (FDA) and the Brazilian Health Regulatory Agency (ANVISA). FDA fixed the daily value (DV) based on a caloric intake of 2000 calories for Cr = 120 µg/100 g, Cu = 2 mg/100 g, Fe = 18 mg/100 g, Mn = 2 mg/100 g, Se = 70 µg/100 g, Zn = 15 mg/100 g [32] while ANVISA established the daily recommend intake based (DRI) on a caloric intake of 2000 calories for Cr = 35 µg/100 g, Cu = 900 µg/100 g, Fe = 14 mg/100 g, Mn = 2.3 mg/100 g, Se = 34 µg/100 g, Zn = 7 mg/100 g [33].

Low contributions were observed for daily intake of Fe, Se and Zn, being below 0.24% DRI and 0.11% DV. For Cu and Cr, the highest contributions were observed for chamomile and mate infusions, ranging between 0.20 - 0.43% and 0.19 - 0.66%, respectively. Mate infusions were verified to be an outstanding manganese source: 15.9% of the DV and 18.3% of the DRI can be reached by the daily consumption of a single cup of herbal tea.

4. Conclusions

A study of twelve trace elements in herbal tea and their infusions was performed using ICP-MS. Even though the information about the age and the origin was not available, principal component analysis (PCA) allowed to classify the herbal tea in four groups, considering similarities of their trace elements composition. Although inorganic contaminants Cd and Pb were found above the maximum limits set by Brazilian and MERCOSUR regulations in some herbal tea samples, the results demonstrated that the infusion procedure provides low trace elements extraction from the herbal tea. The estimative of inorganic contaminants (Al, As, Ba, Cd, Ni and Pb) exposure revealed safe levels. For micronutrients, low contributions ($< 0.24\%$) were observed to the daily intake of Fe, Se and Zn, while Cu and Cr highest contributions were observed in chamomile and mate infusions, reaching 0.43% and 0.66%, respectively. Mate infusions were verified to be a outstanding manganese source: 16% of the daily value can be reached by the daily consumption of a cup of herbal tea by an adult.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This work was funding by the FAPESP (process 2012/19142-7) and INCTAA (process CNPq 573894/2008-6 and FAPESP 2008/57808-1). F.F. Silva and Agilent Technologies Brazil are grateful acknowledged for analytical support.

References

[1] B. Gómez-Nieto, M. Jesús Gismera, M.T. Sevilla, J.R. Procopio, Determination of essential elements in beverages, herbal infusions and dietary supplements using a new straightforward sequential approach based on flame atomic absorption spectrometry, Food Chem. 219 (2017) 69–75, https://doi.org/10.1016/j.foodchem.2016.09.121.
[2] J. Suliburska, K. Kaczmarek, Herbal infusions as a source of calcium, magnesium, iron, zinc and copper in human nutrition, Int. J. Food Sci. Nutr. 63 (2) (2012) 194–198, https://doi.org/10.3109/09637486.2011.617359.
[3] A. Szymczycha-Madeja, M. Welna, W. Zyrnicki, Multi-element analysis, bioavailability and fractionation of herbal tea products, J. Braz. Chem. Soc. 24 (5) (2013) 777–787.
[4] C. Copat, A. Grasso, M. Fiore, A. Cristaldi, P. Zuccarello, S.S. Signorelli, G.O. Conti, M. Ferrante, Trace elements in seafood from the Mediterranean sea: an exposure risk assessment, Food Chem. Toxicol. 115 (2018) 13–19, https://doi.org/10.1016/j.fct.2018.03.001.
[5] M. Abtahi, Y. Fakhri, G.O. Conti, H. Keramati, Y. Zandsalimi, Z. Bahmani, R.H. Pouya, M. Sarkhosh, B. Moradi, N. Amanidaz, S.M. Ghasemi, Heavy metals (As, Cr, Pb, Cd and Ni) concentrations in rice (*Oryza sativa*) from Iran and associated risk assessment: a systematic review, Toxin Rev. 36 (2017) 331–341, https://doi.org/10.1080/15569543.2017.1354307.
[6] T. Filippini, S. Cilloni, M. Malavoti, F. Violi, C. Malagoli, M. Tesauro, I. Bottecchi, A. Ferrari, L. Vescovi, M. Vincetti, Dietary intake of cadmium, chromium, copper, manganese, selenium and zinc in a Northern Italy community, J. Trace Elem. Med. Biol. 50 (2018) 508–517, https://doi.org/10.1016/j.jtemb.2018.03.001.
[7] M.A. Morgano, L.C. Rabonato, R.F. Milani, L. Miyagusku, S.C. Balian, Assessment of trace elements in fishes of Japanese foods marketed in São Paulo (Brazil), Food Control 22 (2011) 778–785, https://doi.org/10.1016/j.foodcont.2010.11.016.
[8] R.F. Milani, M.A. Morgano, E.S. Saron, F.F. Silva, S. Cadore, Evaluation of direct analysis for trace elements in tea and herbal beverages by ICP-MS, J. Braz. Chem. Soc. 26 (6) (2015) 1211–1217, https://doi.org/10.5935/0103-5053.20150085.
[9] Brazil, ANVISA – The Brazilian Health Regulatory Agency. Resolução RDC nº 42, de 29 de agosto de 2013, Regulamento Técnico MERCOSUL sobre Limites Máximos de Contaminantes Inorgânicos em Alimentos, 2013.
[10] MERCOSUR, Resolução GMC MERCOSUL n. 12/2011, (2011).
[11] R.F. Milani, M.A. Morgano, S. Cadore, Trace elements in Camellia sinensis marketed in southeastern Brazil: extraction from tea leaves to beverages and dietary exposure, LWT - Food Sci. Technol. 68 (2016) 491–498, https://doi.org/10.1016/j.lwt.2015.12.041.
[12] J. Malik, J. Szakova, O. Drabek, J. Balik, L. Kokoska, Determination of certain micro and macroelements in plant stimulants and their infusions, Food Chem. 111 (2008) S20–S25, https://doi.org/10.1016/j.foodchem.2008.04.009.
[13] M.M. Özcan, A. Ünver, T. Uçar, D. Arslan, Mineral content of some herbs and herbal teas by infusion and decoction, Food Chem. 106 (2008) 1120–1127, https://doi.org/10.1016/j.foodchem.2007.07.042.
[14] S. Zivkovic, J. Savovic, M. Kuzmanovic, J. Petrovic, M. Momcilovic, Alternative analytical method for direct determination of Mn and Ba in peppermint tea based on laser induced breakdown spectroscopy, Microchem. J. 137 (2018) 410–417, https://doi.org/10.1016/j.microc.2017.11.020.
[15] L.M. de Oliveira, S. Das, E.B. da Silva, P. Gao, J. Gress, Y. Liu, L.Q. Ma, Metal concentrations in traditional and herbal teas and their potential risks to human health, Sci. Total Environ. 633 (2018) 649–657, https://doi.org/10.1016/j.scitotenv.2018.03.215.
[16] INMETRO - Instituto Nacional de Metrologia, Normalização e Qualidade Industrial. Orientação Sobre Validação de Métodos Analíticos. DOQ-CGCRE-008. Rev. 04, (2011), pp. 1–20.
[17] AOAC (Association of Official Agricultural Chemists), Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals, (2013).
[18] S. Arpadjan, G. Çelik, S. Taşkesen, Ş. Gücer, Arsenic, cadmium and lead in medicinal herbs and their fractionation, Food Chem. Toxicol. 46 (2008) 2871–2875, https://doi.org/10.1016/j.fct.2008.05.027.
[19] K. Pytlakowska, A. Kita, P. Janoska, M. Połowniak, V. Kozik, Multi-element analysis of mineral and trace elements in medicinal herbs and their infusions, Food Chem. 135 (2012) 494–501, https://doi.org/10.1016/j.foodchem.2012.05.002.
[20] A.R. Borges, D.N. Bazanella, A.T. Duarte, A.V. Zmozinski, M.G.R. Vale, B. Welz, Development of a method for the sequential determination of cadmium and chromium from the same sample aliquot of yerba mate using high-resolution continuum source graphite furnace atomic absorption spectrometry, Microchem. J. 130 (2017) 116–121, https://doi.org/10.1016/j.microc.2016.08.010.

[21] G. Schulzki, B. Nüßlein, H. Sievers, Transition rates of selected metals determined in various types of teas (Camellia sinensis L. Kuntze) and herbal/fruit infusions, Food Chem. 215 (2017) 22–30, https://doi.org/10.1016/j.foodchem.2016.07.093.
[22] P. Pohl, A. Dzimitrowicz, D. Jedryczko, A. Szymczycha-Madeja, M. Welna, P. Jamroz, The determination of elements in herbal teas and medicinal plantformulations and their tisanes, J. Pharm. Biomed. Anal. 130 (2016) 326–335, https://doi.org/10.1016/j.jpba.2016.01.042.
[23] A. Szymczycha-Madeja, M. Welna, P. Pohl, Elemental analysis of teas and their infusions by spectrometric methods, Trends Anal. Chem. 35 (2012) 165–181, https://doi.org/10.1016/j.trac.2011.12.005.
[24] M. Pinto, Tea: a new perspective on health benefits, Food Res. Int. 53 (2013) 558–567, https://doi.org/10.1016/j.foodres.2013.01.038.
[25] M.A. Rostagno, N. Manchón, M. D'Arrigo, E. Guillamón, A. Vilares, A. García-Lafuente, A. Ramos, J.A. Martínez, Fast and simultaneous determination of phenolic compounds and caffeine in teas, mate, instant coffee, soft drink and energetic drink by high performance liquid chromatography using a fused-core column, Anal. Chim. Acta 685 (2011) 204–211, https://doi.org/10.1016/j.aca.2010.11.031.
[26] Camargo, M.C.F. M.C.R. Toledo, Chá-mate e café como fontes de hidrocarbonetos policíclicos aromáticos (HPAS) na dieta da população de Campinas, Food Sci. Technol. 22 (1) (2002) 49–53, https://doi.org/10.1590/S0101-20612002000100009.
[27] WHO - World Health Organization, Nickel in Drinking-water. Background Document for Preparation of WHO Guidelines for Drinking-water Quality, 30p (2005).
[28] WHO - World Health Organization. CF/11. Rio de Janeiro, Brazil, 2017.
[29] EFSA - European Food Safety Authority, Scientific Opinion on Lead in Food, 151p (2010).
[30] US EPA - U.S. Environmental Protection Agency, Toxicological Review of Barium and Compounds, 87p (2005).
[31] C. Yuan, E. Gao, B. He, G. Jiang, Arsenic species and leaching characters in tea (Camellia sinensis), Food Chem. Toxicol. 45 (12) (2007) 2381–2389, https://doi.org/10.1016/j.fct.2007.06.015.
[32] FDA – U.S Food and Drug Administration, Guidance for Industry: a Food Labeling Guide (14. Appendix F: Calculate the Percent Daily Value for Appropriate Nutrients), (2013) (accessed 16 August 2018), http://www.fda.gov.br.
[33] Brazil. ANVISA - The Brazilian Health Regulatory Agency, Resolução RDC 360, de 23 de dezembro de 2003. Aprova Regulamento Técnico sobre Rotulagem Nutricional de Alimentos Embalados, tornando obrigatória a rotulagem nutricional, (2003).