Mineral Composition of *Lippia alba* (Mill.) N.E. Brown Leaves

Pedro S. dos Reis,[a,b] Idalia Helena S. Estevam,[a] Wagna P. C. dos Santos,[c]
Maria Graças A. Korn,[b] Jorge M. David,[b] Juceni P. David,[d] Rennan G. O. Araújo,[b]
Maria Fernanda Pimentel[e] and Sérgio L. C. Ferreira[b]*

[a]*Departamento de Ciências Exatas e da Terra, Universidade do Estado da Bahia,*
41195-001 Salvador-BA, Brazil

[b]*Grupo de Pesquisa em Química e Quimiometria, Universidade Federal da Bahia,*
40170-290 Salvador-BA, Brazil

[c]*Departamento de Química, Centro Federal de Educação Tecnológica da Bahia,*
40301-015 Salvador-BA, Brazil

[d]*Departamento do Medicamento, Faculdade de Farmácia, Universidade Federal da Bahia,*
40170-290 Salvador-BA, Brazil

[e]*Departamento de Engenharia Química, Universidade Federal de Pernambuco, Av. Arthur de Sá s/n,*
Cidade Universitária, 50740-521 Recife-PE, Brazil

Neste trabalho descreve-se a composição mineral das folhas de *Lippia alba* (Mill.) que são utilizadas na preparação de chá utilizado na medicina popular. Assim, as folhas da espécie foram coletadas em cidades brasileiras e as amostras foram digeridas usando ácido nítrico e peróxido de hidrogênio em placa aquecedora e, em seguida, foram analisadas empregando espectrometria de emissão óptica com plasma acoplado indutivamente (ICP OES). Para avaliação da exatidão foi utilizado o material de referência certificado, folhas de maçã - NIST SRM 1515. Os resultados demonstraram que cálcio, magnésio e fósforo têm conteúdo médio de 1.950, 2.856 e 2.335 mg 100 g^{-1} e variações de concentrações de 767-3.887, 314-8.591 e 247-9.214 mg 100 g^{-1}, respectivamente. Os micro-elementos, bário, zinco, cobre, ferro, manganês e níquel têm conteúdos médios de 2,16; 2,95; 0,94; 11,2; 4,25 e 0,09 mg 100 g^{-1}, para variações de concentrações de 0,66-7,1; 1,35-6,3; 0,33-1,7; 0,98-34; 0,82-7,4 e 0,03-0,15 mg 100 g^{-1}, respectivamente. Os dados foram também avaliados usando análise de componentes principais (PCA).

Leaves of *Lippia alba* (Mill.) N.E. Brown are used for the preparation of tea infusions that are employed in folk medicine. In this paper, the mineral composition of *Lippia alba* (Mill.) leaves, collected from different Brazilian cities, was evaluated. The samples were digested using nitric acid and hydrogen peroxide on a hot plate and were analyzed using inductively coupled plasma optical emission spectrometry (ICP OES). SRM 1515 apple leaves from NIST were used for checking accuracy. The results demonstrated that calcium, magnesium and phosphorus have average contents of 1,950, 2,856 and 2,335 mg 100 g^{-1}, respectively, and concentration ranges of 767-3,887, 314-8,591 and 247-9,214 mg 100 g^{-1}, respectively. The microelements barium, zinc, copper, iron, manganese and nickel have average contents of 2.16, 2.95, 0.94, 11.2, 4.25 and 0.09 mg 100 g^{-1}, respectively, and concentration ranges of 0.66-7.1, 1.35-6.3, 0.33-1.7, 0.98-34, 0.82-7.4 and 0.03-0.15 mg 100 g^{-1}, respectively. The data were also evaluated using principal component analysis (PCA).

Keywords: *Lippia alba*, mineral composition, multivariate analysis

*e-mail: slcf@ufba.br

Introduction

The use of native plants as herbal medicine is a tradition in Brazilian culture. The use of plants began long ago, probably originating with indigenous tribes and slaves. The latter group likely adapted their native recipes to incorporate local plants and procedures. Many of these plants are still used as leaf infusions and in other medicinal preparations. Studies evaluating the organic and mineral composition of leaves used for preparation of teas are quite relevant and several publications have examined this subject.[1-6]

Lippia alba (Mill.) N.E. Brown, popularly known as "erva-cidreira", is an aromatic shrub that belongs to the Verbenaceae family. The genus *Lippia* encompasses about 200 species that grow in the wild in Central and South America, and in Africa. *L. alba* possesses sedative properties; it has also been used as an infusion, such as tea in Brazilian folk medicine, against intestinal and gastric illnesses.[7] Interestingly, the composition of *L. alba* essential oils showed variations related to the part of the plant employed in the distillation, the plant's state of development and its geographic location, the characteristics of the soil, the climate, and other local conditions.[8] From these reports, at least four *L. alba* chemotypes have been identified in terms of the main monoterpene constituents in the essential oils; each of these chemotypes has a distinct odor.[8,9] One of the chemotypes is characterized by a high content of 1,8-cineole, limonene, and carvone; the second has a limonene, carvone, and myrcene signature; the third is represented mainly by geranial and neral, and the fourth is rich in linalool.[8,9] Although an extensive body of work dealing with essential oils of *L. alba* exists, there have been no studies examining the relative presence of metals in the different chemotypes of this species so far.

In recent years, multivariate data analysis, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), has been increasingly used for the evaluation of analytical results from foods and others matrices.[10-22] In a recent paper,[20] sixteen trace metallic analytes (Ba, Ca, Ce, Co, Cr, Cu, Fe, K, La, Mg, Mn, Na, Ni, P, Sr and Zn) in acid digests of herbal teas were determined. The data were subjected to chemometric evaluation in an attempt to classify the herbal tea samples. The plants were classified into five groups by principal component analysis (PCA).

Principal component analysis is a well-known, unsupervised, exploratory technique used for visualizing information in large data sets. PCA can be used to simplify data by reducing the number of variables into a smaller number of orthogonal variables (principal components, PCs). The PCs are linear combinations of the original variables and maximize the variability contained within them, thereby displaying most of the original variability in a smaller number of dimensions. Each variable has a loading on each PC. The loading reflects both how much the variable contributed to that PC and how well that PC takes into account the variation of that variable over the data points. Thus, the graphical presentation of the pair-wise components (score plots) allows the natural grouping of the samples to be observed, indicating the similarity among the samples and allowing different groups of samples to be identified. The loadings can be used to understand how much each variable contributes to the meaningful variation in the data and to interpret variable relationships.

In this paper, the leaf mineral content (Ba, Zn, Cu, Fe, Mn, Ni, Ca, Mg and P) of 28 *L. alba* specimens were evaluated; of these 27 samples were collected in the Bahia State (Brazil) and one was from the city of Rio de Janeiro. The data were subjected to principal component analysis to verify eventual similarities among the samples as well as to determine correlations between the elements and outliers detection.

Experimental

Instrumentation

An inductively coupled plasma optical emission spectrometer with an axially viewed configuration (ICP OES, VISTA PRO, Varian, Mulgrave, Australia) was used for the content determination of Ba, Zn, Cu, Fe, Mn, Ni, Ca, Mg and P. The ICP OES system was equipped with a solid-state CCD that allows simultaneous measurements from 167 to 785 nm. The instrumental parameters used were as follows: RF generator, 40 MHz; applied power, 1300 W; plasma gas flow-rate, 15.0 L min^{-1}; auxiliary gas flow-rate, 1.5 L min^{-1}; nebulizer gas flow-rate, 0.7 L min^{-1}, and sample uptake rate, 0.8 mL min^{-1}. The sample introduction system employed was a concentric nebulizer and a cyclonic spray chamber. The analysis was carried out at the following wavelengths (nm): Ca II (317.933), Fe II (259.939), Mg II (285.213), P I (214.914), Cu I (324.754), Ba II (455.397), Mn II (257.610), Ni II (221.645) and Zn II (213.857), where "I" represents an atomic line and "II" an ionic line. In most cases, the wavelengths of the analytical lines were selected considering the most prominent line; secondary lines were used as an alternative to prevent possible interferences. All analyses were performed in triplicate and were checked by standard additions.

Reagents and reference solutions

All reagents were of analytical grade unless otherwise stated. Ultrapure water was obtained from an EASYpure RF (Barnstedt, Dubuque, IA, USA). Nitric acid and hydrogen peroxide were of Suprapur quality (Merck, Darmstadt, Germany). Laboratory glassware was kept overnight in a 10% v/v nitric acid solution. The glassware was rinsed with deionized water and dried in a dust free environment before use. Commercially available metal standard solutions (1,000 ± 0.002 mg L⁻¹) (Titrisol, Merck) were prepared for the standard working range. Analytical curves were obtained for concentrations from 6.0 to 100.0 mg L⁻¹ for Ca, Mg and P and from 0.05 to 10.0 mg L⁻¹ for Ba, Zn, Cu, Fe, Mn and Ni.

Sample collection and storage

During the period of August 2004 to September 2005, 27 leaf samples were collected in 12 different cities in the Bahia State and one sample in the city of Rio de Janeiro, in Brazil. The respective cities were Amélia Rodrigues (AMR), Camaçari (CAM), Cruz das Almas (CZA), Euclides da Cunha (EUC), Feira de Santana (FSA), Jequié (JEQ), Lauro de Freitas (LAF), Ribeira do Pombal (RIP), Salvador (SSA), Simões Filho (SIF), Tucano (TUC), Vera Cruz (VEC) and Rio de Janeiro (RIO). The individual samples of 100 g each were kept in separate polyethylene bags and stored in a refrigerator at 6 °C for less than one day.[23] Afterwards, the non-edible parts were removed, and the leaf samples were washed first with deionized water and then with 3% v/v Extran solution. The samples were dried in an air flow oven at 40 °C for 24 h, later homogenized in a blender and stored in polyethylene bags.

Humidity determination

The humidity of each sample was determined by mass loss after drying in an air flow oven at 40 ºC for 20 h, as recommended by the Association of Official Analytical Chemists.[24]

Sample digestion

About 0.5 g of leaf sample was accurately weighed into glass vessels, and 10.0 mL of concentrated nitric acid were added. The hot plate temperature was adjusted to 125 °C, the sample was digested for 30 min, and 3.0 mL of 30% m/m hydrogen peroxide were added. Heating was maintained to allow evaporation and yielded a final volume of *ca.* 5 mL. Later, the digested material was quantitatively transferred to a 50 mL volumetric flask and diluted with deionized water.

Validation of analytical method

The limit of detection (LOD) and limit of quantification (LOQ) for each element evaluated were determined based on the IUPAC recommendation, where LOD = (3s)/S, LOQ = (10s)/S, where s is the relative standard deviation of the measurements of a blank solution and S represents the slope of the analytical curve used for quantification. The results are presented in Table 1.

The accuracy of the method used for determination of the elements was confirmed by the analysis of a certified reference material produced by the National Institute of Standard and Technology (Gaithersburg, MD, USA), namely NIST apple leaves STM 1515. This material was submitted to the same procedure employed for the leaves samples. The results obtained are in agreement with the certified values (Table 1).

Table 1. Limits of detection (LOD), limits of quantification (LOQ) and accuracy check results of ICP OES with NIST SRM -1515 apple leaves

Element	LOD*	LOQ*	Certified value	Determined value
Calcium (%)	26	88	1.526 ± 0.015	1.514 ± 0.018
Magnesium (%)	14	48	0.271 ± 0.008	0.267 ± 0.009
Phosphorus (%)	3.6	12.1	0.159 ± 0.011	0.166 ± 0.004
Barium ($\mu g\ g^{-1}$)	0.10	0.30	49 ± 2	46.6 ± 0.7
Copper ($\mu g\ g^{-1}$)	0.01	0.03	5.64 ± 0.24	5.3 ± 1.4
Zinc ($\mu g\ g^{-1}$)	0.03	0.008	12.5 ± 0.3	12.3 ± 0.4
Manganese ($\mu g\ g^{-1}$)	0.13	0.44	54 ± 3	51.0 ± 0.8
Nickel ($\mu g\ g^{-1}$)	0.001	0.004	0.91 ± 0.12	1.02 ± 0.14
Iron ($\mu g\ g^{-1}$)	0.27	0.89	83 ± 5	85.0 ± 4.7

*(mg 100 g^{-1}).

Results and Discussion

The percentage humidity for all 28 samples varied from 64.50 to 86.80%, averaging 76.81%. These results also demonstrated that the mineral content of the *L. alba* depends fundamentally of the humidity of the leaf analyzed.

The contents of Ba, Zn, Cu, Fe, Mn, Ni, Ca, Mg and P in the 28 leaf samples were determined (Supplementary Information). Calcium, Mg and P were present at the highest concentrations; their average content was 1,950, 2,856 and 2,335 mg 100 g^{-1}, respectively, while the concentration ranges were 767-3,887, 314-8,591 and 247-9,214 mg 100 g^{-1}, respectively. The micro-elements Ba, Zn, Cu, Fe, Mn and Ni had an average content of 2.16, 2.95, 0.94, 11.2, 4.25 and 0.09 mg 100 g^{-1}, respectively, and concentration ranges of 0.66-7.1, 1.35-6.3, 0.33-1.7, 0.98-34, 0.82-7.4 and 0.03-0.15 mg 100 g^{-1}, respectively. These results match previous reports in the literature for metal content determination in tea leaves,[22,25] demonstrating that *L. alba* leaves can be a source of Ca, Mg and P in the human diet.

Data were evaluated using principal component analysis (PCA). A data matrix was constructed using the determined elements as columns and the leaf samples as rows. Principal component analysis was performed on auto-scaled data due to the different orders of magnitude of the concentration of the elements.

The results of the PCA revealed that the first three principal components explained only 63.35% of total variance. Figure 1 shows the loadings of the first two principal components. PC1 represents 29.36% of the variability of the data, and is mainly associated with Fe and Ba concentrations. These two elements are positively correlated and account for the major variability presented in data set. The score plot of the first two components is shown in Figure 2. Samples with high scores on PC1 (TUC1, TUC2, TUC3, TUC 4, RIO, SSA2, CZA, and RIP, all circled in Figure 2) have high concentrations of Fe and Ba (as can also be seen in Table S1). These samples are from a region where the industrial extraction of iron ore and barite occur.
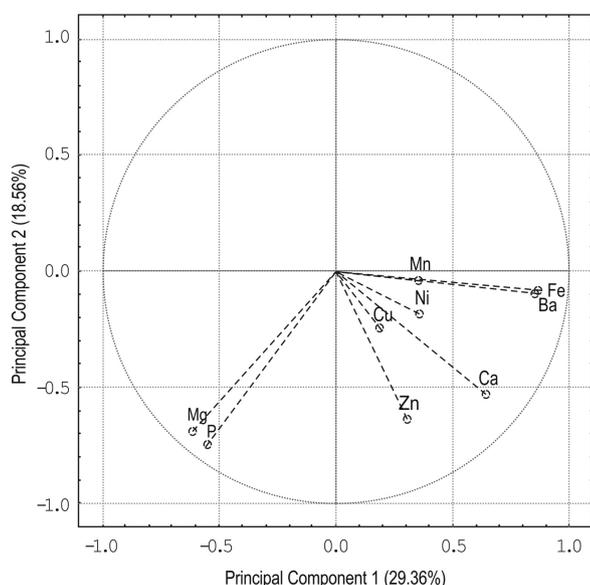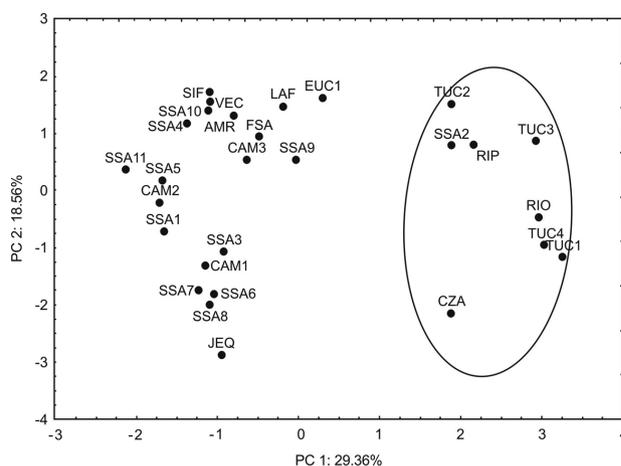
Figure 2. PCA results for the mineral content of *Lippia alba* leaves. The score plot is shown; objects are labeled according to the site of sample collection.

The second principal component (PC2, describing 18.56% of the total variance) is primarily associated with variations in P, Mg, Zn and Ca contents. All these variables present negative loadings in PC2. Thus, samples with negative scores have higher than average values for these elements. The score plot (Figure 2) does not show well-defined clusters.

Finally, the third principal component (PC3) explains only 15.43% of the total variance and is associated with Cu (positive value) and Mn (negative value). From the scores plot (not shown) there are no evident clusters or outliers.

Conclusions

This paper is the first to report the evaluation of the mineral composition of *Lippia alba* leaves. The samples analyzed indicate that *L. alba* contains Ca, Mg and P as macroelements as well as Ba, Zn, Cu, Fe, Mn and Ni as microelements.

The exploratory analysis using principal component was applied to verify eventual similarities among the samples and correlations between elements and the outliers

Figure 1. PCA results for the mineral content of *Lippia alba* leaves. The loading plot is shown. PC1 and PC2 explain 29.36% and 18.56% of the total variance, respectively.

detection. PCA helped to identify that the concentrations of the elements Ba and Fe are responsible for the major variability in the data set. Samples which presented high concentrations of these elements are from a region where the industrial extraction of iron ore and barite occur. The PCA results also provided no evidence of well defined clusters or outliers in this data set.

Supplementary Information

Supplementary information about mineral composition of leaf samples of *Lippia* is available free of charge as PDF file at http://jbcs.sbq.org.br.

Acknowledgments

References

1. Fernandes, S. C.; Osorio, R. E. M.; dos Anjos, A.; Neves, A.; Micke, G. A.; Vieira, I. C.; *J. Braz. Chem. Soc.* **2008**, *19*, 1215.

2. Lemos, V. A.; David, G. T.; Santos, L. N.; *J. Braz. Chem. Soc.* **2006**, *17*, 697.

3. Reto, M.; Figueira, M. E.; Filipe, H. M.; Almeida, C. M. M.; *Quim. Nova* **2008**, *31*, 317.

4. Gnoatto, S. C. B.; Schenkel, E. P.; Bassani, V. L.; *J. Braz. Chem. Soc.* **2005**, *16*, 723.

5. Owour, P. O.; Obanda, M.; Nvirenda, H. E.; Mandala, M.; *Food Chem.* **2008**, *108*, 263.

6. Mossion, A.; Potin-Gautier, M.; Delerue, S.; Le Hecho, I.; Behra, P.; *Food Chem.* **2008**, *106*, 1467.

7. Tavares, E. S.; Julião, L.S.; Lopes, D.; Bizzo, H. R.; Lage, C. L. S.; Leitão, S. G.; *Braz. J. Pharmacogn.* **2005**, *15*, 1.

8. Zoghbi, M. D. G. B.; Andrade, E. H. A.; Santos, A. S.; Silva, M. H. L.; Maia, J. G. S.; *Flavour Fragr. J.* **1998**, *13*, 47.

9. Siani, A. C.; Tappin, M. R. R.; Ramos, M. F. S.; Mazzei, J. L.; Ramos, M. C. K. V.; Aquino Neto, F. R.; Frighetto, N.; *J. Agric. Food Chem.* **2002**, *50*, 3518.

10. Yetimoglu, E. K.; Ercan, O.; *J. Braz. Chem. Soc.* **2008**, *19*, 1399.

11. Amorim Filho, V. R.; Polito, W. L.; Gomes Neto, J. A.; *J. Braz. Chem. Soc.* **2007,** *18*, 47.

12. Araujo, R. G. O.; Macedo, S. M.; Korn, M. D. A.; Pimentel, M. F.; Bruns, R. E.; Ferreira, S. L. C.; *J. Braz. Chem. Soc.* **2008**, *19*, 935.

13. Gratani, L.; Crescente, M. F.; Varone, L.; *Atmos. Environ.* **2008**, *42*, 8273.

14. Boselli, E.; Minardi, M.; Giomo, A.; Frega, N. G.; *Anal. Chim. Acta* **2006**, *563*, 93.

15. Camara, J. S.; Alves, M. A.; Marques, J. C.; *Talanta* **2006**, *68*, 1512.

16. Godinho, M. D.; Pereira, R. O.; Ribeiro, K. D.; Schimidt, F.; de Oliveira, A. E.; de Oliveira, S. B.; *Quim. Nova* **2008**, *31*, 1485.

17. dos Santos, J. S.; dos Santos, N. S.; dos Santos, M. L. P.; dos Santos, S. N.; Lacerda, J. J. D.; *J. Braz. Chem. Soc.* **2008**, *19*, 502.

18. Sousa, E. T.; Rodrigues, F. D.; Martins, C. C.; de Oliveira, F. S.; Pereira, P. A. D.; de Andrade, J. B.; *Microchem. J.* **2006**, *82*, 142.

19. Silva, F. L. N.; dos Santos, J. R.; Moita Neto, J. M.; da Silva, R. L. G. D.; Flumignan, D. L.; de Oliveira, J. E.; *Quim. Nova* **2009**, *32*, 56.

20. Kara, D.; *Food Chem.* **2009**, *347*, 314.

21. Fernández-Cáceres, P. L.; Martín, M. J.; Pablos, F.; González, A. G.; *J. Agric. Food Chem.* **2001**, *49*, 4775.

22. Moreda-Piñeiro, A.; Fisher, A.; Hill, S. J.; *J. Food Comp. Anal.* **2003***, 16*, 195.

23. Riedel, G.; *Controle Sanitário dos Alimentos,* 2ª ed., Editora Atheneu: São Paulo, Brasil, 1996.

24. AOAC, *Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Washington, DC, USA, 1990, v.1.

25. Malik, J.; Szakova, J.; Drabek, O.; Balik, J.; Kokoska, L.; *Food Chem.* **2008**, *111*, 520.

Mineral Composition of *Lippia alba* (Mill.) N.E. Brown Leaves

*Pedro S. dos Reis,[a,b] Idalia Helena S. Estevam,[a] Wagna P. C. dos Santos,[c]
Maria Graças A. Korn,[b] Jorge M. David,[b] Juceni P. David,[d] Rennan G. O. Araújo,[b]
Maria Fernanda Pimentel[e] and Sérgio L. C. Ferreira*[b]*

[a]*Departamento de Ciências Exatas e da Terra, Universidade do Estado da Bahia, 41195-001 Salvador-BA, Brazil*

[b]*Grupo de Pesquisa em Química e Quimiometria, Universidade Federal da Bahia, 40170-290 Salvador-BA, Brazil*

[c]*Departamento de Química, Centro Federal de Educação Tecnológica da Bahia, 40301-015 Salvador-BA, Brazil*

[d]*Departamento do Medicamento, Faculdade de Farmácia, Universidade Federal da Bahia,
40170-290 Salvador-BA, Brazil*

[e]*Departamento de Engenharia Química, Universidade Federal de Pernambuco, Av. Arthur de Sá s/n, Cidade
Universitária, 50740-521 Recife-PE, Brazil*

Table S1. Phosphorus and mineral contents of *Lippia alba* leaves

Sample	Ba	Zn	Cu	Fe	Mn	Ni	Ca	Mg	P
AMR	1.08 ± 0.03	2.37 ± 0.17	0.82 ± 0.01	3.89 ± 0.28	0.82 ± 0.01	0.06 ± 0.01	1174 ± 18	715 ± 11	247 ± 25
CAM1	1.76 ± 0.39	2.83 ± 0.28	1.16 ± 0.11	12.29 ± 0.75	6.04 ± 0.01	0.06 ± 0.01	1711 ± 22	8591 ± 22	7204 ± 64
CAM2	0.96 ± 0.25	2.45 ± 0.42	0.53 ± 0.02	5.82 ± 0.42	2.74 ± 0.01	0.05 ± 0.01	1630 ± 28	6268 ± 41	4379 ± 40
CAM3	0.72 ± 0.15	4.02 ± 0.33	0.97 ± 0.05	4.47 ± 0.55	6.54 ± 0.43	0.03 ± 0.02	1047 ± 17	869 ± 28	278 ± 18
CZA	3.70 ± 0.28	6.31 ± 0.28	1.29 ± 0.11	9.7 ± 1.6	2.09 ± 0.11	*	3887 ± 15	529 ± 18	500 ± 22
EUC1	2.30 ± 0.08	1.43 ± 0.11	0.72 ± 0.02	18.19 ± 0.54	3.19 ± 0.01	0.09 ± 0.01	1161 ± 14	1071 ± 26	210 ± 12
EUC2	4.87 ± 0.08	4.07 ± 0.09	1.59 ± 0.04	28.5 ± 1.9	5.43 ± 0.11	0.15 ± 0.03	3139 ± 58	449 ± 5	355 ± 7
FSA	0.79 ± 0.07	2.95 ± 0.03	0.97 ± 0.06	4.51 ± 0.36	0.83 ± 0.13	*	1711 ± 43	381 ± 6	377 ± 13
JEQ	2.13 ± 0.69	4.33 ± 0.69	0.66 ± 0.07	8.1 ± 1.3	2.97 ± 0.01	0.15 ± 0.01	2895 ± 56	6440 ± 20	9214 ± 11
LAF	0.66 ± 0.16	1.78 ± 0.28	0.92 ± 0.04	12.9 ± 1.9	2.74 ± 0.01	0.05 ± 0.01	1475 ± 11	684 ± 11	309 ± 41
RIP	5.32 ± 0.18	2.57 ± 0.03	0.52 ± 0.02	16.53 ± 0.72	2.26 ± 0.07	0.12 ± 0.01	2820 ± 67	626 ± 7	500 ± 7
SSA1	0.95 ± 0.16	2.30 ± 0.06	1.43 ± 0.15	5.3 ± 1.3	2.16 ± 0.01	0.06 ± 0.01	1359 ± 17	5040 ± 28	5421 ± 54
SSA2	2.09 ± 0.34	2.88 ± 0.23	1.42 ± 0.05	15.66 ± 0.24	7.43 ± 0.80	0.02 ± 0.01	2351 ± 22	555 ± 10	254 ± 77
SSA3	1.77 ± 0.89	3.26 ± 0.22	0.85 ± 0.11	6.36 ± 0.69	3.60 ± 0.03	0.12 ± 0.03	2047 ± 33	4875 ± 28	4974 ± 38
SSA4	0.89 ± 0.03	1.74 ± 0.18	0.79 ± 0.18	4.12 ± 0.42	3.08 ± 0.06	*	1227 ± 27	2402 ± 30	3029 ± 17
SSA5	1.41 ± 0.42	1.93 ± 0.22	0.97 ± 0.07	5.58 ± 0.61	5.02 ± 0.03	0.04 ± 0.01	1070 ± 19	6407 ± 58	3763 ± 47
SSA6	1.45 ± 0.01	3.26 ± 0.27	0.79 ± 0.11	7.4 ± 0.2	2.25 ± 0.01	0.06 ± 0.01	3020 ± 20	8302 ± 11	5904 ± 42
SSA7	1.33 ± 0.03	3.54 ± 0.35	0.94 ± 0.13	11.4 ± 0.5	2.92 ± 0.03	0.06 ± 0.02	1996 ± 30	6025 ± 32	6744 ± 29
SSA8	1.52 ± 0.28	5.84 ± 0.55	0.90 ± 0.05	5.07 ± 0.14	1.20 ± 0.06	*	1887 ± 13	7090 ± 25	3563 ± 55
SSA9	0.76 ± 0.11	2.29 ± 0.06	1.72 ± 0.04	8.3 ± 0.7	0.17 ± 0.01	*	2037 ± 55	314 ± 9	389 ± 10
SSA10	1.56 ± 0.28	1.35 ± 0.18	0.33 ± 0.11	0.98 ± 0.64	3.06 ± 0.01	0.04 ± 0.01	1882 ± 37	3107 ± 38	2368 ± 39
SSA11	1.07 ± 0.22	1.53 ± 0.08	0.86 ± 0.20	7.30 ± 0.55	1.52 ± 0.00	0.03 ± 0.01	767 ± 20	5631 ± 42	3615 ± 21
SIF	0.44 ± 0.08	2.48 ± 0.11	0.60 ± 0.05	4.07 ± 0.83	1.19 ± 0.30	0.04 ± 0.01	884 ± 10	615 ± 11	290 ± 26
TUC1	7.10 ± 0.14	1.83 ± 0.02	0.55 ± 0.02	18.9 ± 1.4	9.68 ± 0.02	0.06 ± 0.02	1390 ± 27	934 ± 13	260 ± 23
TUC2	5.60 ± 0.08	2.30 ± 0.05	*	27.0 ± 0.5	4.14 ± 0.04	*	3872 ± 64	527 ± 2	404 ± 4
TUC3	4.74 ± 0.03	3.67 ± 0.19	1.14 ± 0.02	21.84 ± 0.70	1.89 ± 0.04	0.06 ± 0.01	2339 ± 52	480 ± 9	309 ± 5
VEC	0.36 ± 0.11	2.77 ± 0.55	0.49 ± 0.11	4.01 ± 0.33	0.96 ± 0.01	0.05 ± 0.01	975 ± 14	783 ± 13	247 ± 22
RIO	3.02 ± 0.06	4.55 ± 0.14	1.42 ± 0.03	34 ± 2	3.16 ± 0.21	*	2855 ± 91	268 ± 5	289 ± 5

Average of three determinations ± 95% confidence interval. * Value calculated for Cu < 0.01 and for Ni < 0.001.