2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 978-85-99141-05-2

PRELIMINARY ASSESSMENT OF TRACE ELEMENTS EFFECTS ON ESSENTIAL OIL PRODUCTION OF *MELISSA OFFICINALIS* L. (LEMON BALM)

Fábio V. Sussa[1], Celina L. Duarte[1], Luana S. Andrade[1], Marcos R. Furlan[2] and Paulo S. C. Silva[1]

[1] Instituto de Pesquisas Energéticas e Nucleares
(IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
pscsilva@ipen.br
clduarte@ipen.br
fabiosussa@uol.com.br

[2] Departamento de Ciências Agrárias
Universidade de Taubaté
Estrada Municipal Dr. José Luiz Cembranelli 5000
12081-010, Taubaté, SP
furlanagro@gmail.com

ABSTRACT

Melissa officinalis L., Lemon balm, (Lamiaceae) is an herb used as medicine, condiment and in the cosmetic and perfumery industry due to its essential oil. In this study a preliminary assessment of trace elements effects on essential oil production was performed in order to verify an improvement in its quality. The *Melissa officinalis* samples were harvested from three different soil sites localized in *Ibirapuera Park, São Paulo*, Brazil. Elemental concentration for the elements As, Ba, Br, Ce, Cl, Co, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Mg, Mn, Na, Nd, Rb, Sb, Sc, Se, Sm, Ta, Tb, Ti, Th, U, V, Yb, Zn and Zr was determined by Instrumental Neutron Activation Analysis (INAA) in *Melissa officinalis* leaves and surrounding soil. The essential oil was extracted from its leaves by hydrodistillation process in Clevenger apparatus and analyzed by gas chromatography coupled to a mass spectrometer (GC-MS). Certified reference materials NIST SRM-1515 Apple Leaves, NIST SRM-1547 Peach Leaves and NIST SRM-1573a Tomato Leaves were analyzed for quality control. Our results showed Geranial and Neral were identified as the major compounds in the essential oil extracted from *Melissa officinalis* L. for all collected sites. However, the relative proportion of some chemical constituents was altered according to the site collected. The preliminary results showed that the production of essential oil by *Melissa officinalis* must be positively correlated with the concentrations of Rb, Zn and negatively correlated with Sc, Mn, La, K, Fe, and Cr.

1. INTRODUCTION

Melissa officinalis L., Lemon balm, originates from Europe and is now grown all over the world [1]. It is a medicinal and aromatic species popularly used to make tea, which serves as a tranquilizer, promote sleep, reduce stress and anxiety [2,3].

Stress, anxiety, and sleep disorders increased considerably in recent years, becoming prevalent diseases affecting a high percentage of the population, [4] which has increasingly resorted to herbal medicine sedative products widely available that can perform the same therapeutic action with fewer side effects, dependence or tolerance than synthetic medicine [5].

The medicinal properties of *Melissa officinalis* are related to the wide variety of their chemical composition essential oil, such as terpenoids [6]. These are a large group of secondary metabolites, which can vary qualitatively and quantitatively, depending on various factors such as season, plant age, circulating water amount, geographical factors, climate, stress and nutrient availability in the soil [7,8].

Lemon balm essential oil is mainly produced in secretion structures known as glandular trachoma of leaves being Geranial and Neral (α-citral and β-citral) the major compounds, and also the ones of great interest for the pharmaceutical industry due to its antioxidative, antimycotic, antiviral and sedative activities [9,10,11]. Other compounds are also found in smaller quantities such as citronellal, citronellol, geraniol and nerol.

Mineral elements are involved in the structure of essential oil, yet also having undesirable effects on their regulation [12]. Chromium (Cr) stress has been found to induce the production of eugenol, a major component of *Ocimum tenuiflorum* essential oil, Holy Basil, (Lamiaceae) [13]. Nevertheless, metal effects on secondary metabolites and its chemical composition concerning the species are largely unknown. Providing information on essential oil production is fundamental to obtain the highest essential oil production by the plant to verify the economic viability of its cultivation [14].

The objective of this study was to evaluate the effect of trace elements on *Melissa officinalis* essential oil production. The *Melissa officinalis* samples were harvested from three different soil sites localized in *Ibirapuera Park, São Paulo,* Brazil.

2. EXPERIMENTAL

2.1. Sample

The *Melissa officinalis* samples were collected from three different soil sites: two in an experimental area at *Escola Municipal de Jardinagem* (P1 and P2) and the third at *Viveiro Manequinho Lopes* (P3), both localized in Ibirapuera Park*, São Paulo,* Brazil.

2.1.1. Sample preparation

The Lemon balm samples were washed with ultrapure water to remove impurities and soil particles present in all plant structures, air-dried and the leaves were separated. After these processes, the leaves samples and soil were dried at 40 °C for 12 h in an oven. The dried samples were grounded into powder at 100 mesh (150 μm diameter) size particles and homogenized.

2.2. Neutron Irradiations and counting

The element concentrations As, Ba, Br, Ce, Cl, Co, Cu, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Mg, Mn, Na, Nd, Rb, Sb, Sc, Se, Sm, Ta, Tb, Ti, Th, U, V, Yb, Zn and Zr was determined by Instrumental Neutron Activation Analysis (INAA) in *Melissa officinalis* leaves and surrounding soil. INAA, a sensitive, fast, nondestructive and multielemental technique has been frequently used to evaluate inorganic contents of medicinal plants [15,16].

The samples were irradiated at the IEA-R1 Nuclear Reactor, IPEN, together with the certified reference materials Rhyolite, Glass Mountain RGM and Syenite, Table Mountain STM from United States Geological Survey (USGS), Estuarine Sediment (1646a) from National Institute of Standard and Technology (NIST) and standard solutions pipeted in paper sheets (P.P).

About 150mg of fine powder of each sample (duplicate), Certified Reference Materials (CRMs) and pipetted standards was packed in polyethylene bags for irradiation.

Samples were irradiated in a neutron flux of approximately 10^{12} $n.cm^{-2}.s^{-1}$ for 20 s to determine the elements Cl, Mg, Mn, Ti and V and 8 h to determine the elements As, Ba, Br, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Na, Nd, Rb, Sb, Sc, Se, Sm, Ta, Tb, Th, U, Yb and Zn.

The gamma-ray spectra were obtained using an EG&G ORTEC counting system (high resolution solid state Ge detector, type POP TOP, Model 20190) with a resolution of 1.9keV for the 1,332keV peak of ^{60}Co. This detector was coupled to an EG&G ORTEC ACE8K card and associated electronics. Spectrum analysis was performed using the VISPECT2 software in TURBOBASIC language.

For quality control of the obtained results in elemental analysis, the certified reference materials SRM-1515 Apple Leaves, SRM-1547 Peach Leaves and SRM-1573a Tomato Leaves provided by NIST were analyzed under the same experimental conditions.

2.3. Essential oil hydrodistillation

Essential oil was extracted from Lemon balm leaves by hidrodistillation method in a Clevenger apparatus at the CRPq/IPEN (Centro do Reator de Pesquisa) Laboratory and analyzed by gas chromatography/mass spectrometry (GC/MS) at the CTR/IPEN (Centro de Tecnologia das Radiações) Laboratory.

From the fresh leaves of each plot, 30 g of sample was separated for essential oil extraction and chemical analysis. The essential oil was extract during 4 hours [17]. The essential oil was stored in amber bottles and maintained in freezer until chemical composition analysis.

2.3.1. Qualitative Analysis

The qualitative analysis of essential oil compounds was performed on a gas chromatography coupled to a mass spectrometer (Shimadzu® Co., model QP-5000) using a DB-5 ms (30 m x 0.25 mm x 0.25 µm) capillary column, injection temperature of 220 °C, interface temperature of 230 °C and helium carrier gas (flow: 1.2 mL/min). Initially, those samples were diluted in hexane (1:10 v/v), and 1 µL was injected in the column, using splitless mode. Operating conditions were undertaken at oven temperature from 60 to 120 °C at 3 °C/min. ratio (20 min.), 120 to 300 °C at 15 °C/min. ratio (12 min.). The compounds were identified by the comparative analysis of the acquired mass spectra with NIST62 database library coupled to GC/MS spectrometer, literature McLafferty & Stauffer [18] and Kováts Retention Index (K_i).

2.3.2. Determination of Kováts Retention Index

A Standard mixture of *n*-alkanes (C_7H_{16}-$C_{40}H_{82}$ Sigma Aldrish, 99 %) was used to verify GC/MS system performance and to calculate the Kováts Index of each compound in the sample [19]. The default (1 µL) of the alkanes mixture was injected into the GC/MS system operating under the same conditions described above, and their retention times (minutes) were used as an external reference standard to calculate the K_i along with the retention times of each compound of interest. In the Kováts Index or Kováts Retention Index used in gas chromatography [20], *n*-alkanes serve as the standards and logarithmic interpolation is utilized according to Equation 1:

$$K_i = 100 * \left(\frac{\log t_c - \log t_n}{\log t_{n+1} - \log t_n} + n\right) \tag{1}$$

where: k_i – Kováts Index; n - number of carbon atoms of the *n*-alkane eluting before the compound of interest; t_c - retention time of the peak of interest; t_n - retention time of the hydrocarbon standard eluting before the peak compound of interest; t_{n+1}- reference hydrocarbon standard eluting after the peak of the compound of interest.

2.4. Statistics

Statistical significance was determined by analysis of variance (ANOVA). In addition, the Tukey's test ($P \leq 0.05$) was used to find significant differences among the means. Pearson correlation coefficients were calculated with all data. Hierarchical cluster analysis (HCA) and Factor analysis, multivariate statistical methods, were used for grouping accessions according to their similarity. The proximity of the groups in the obtained dendrogram reflects the similarity of determined parameters [21]. The analyses were performed with STATISTIC software.

3. RESULTS AND DISCUSSION

Instrumental Neutron Activation Analysis (INAA) was applied to determine elemental concentration in Lemon balm leaves and surrounding soil samples.

To assess the accuracy and precision of the methodology three certified reference materials NIST-1515, NIST-1547 and NIST-1573a were analyzed. Most of results are in good agreement with the certified values for determined elements, except for some elements whose values were higher than presented in the certificate (FIG. 1).

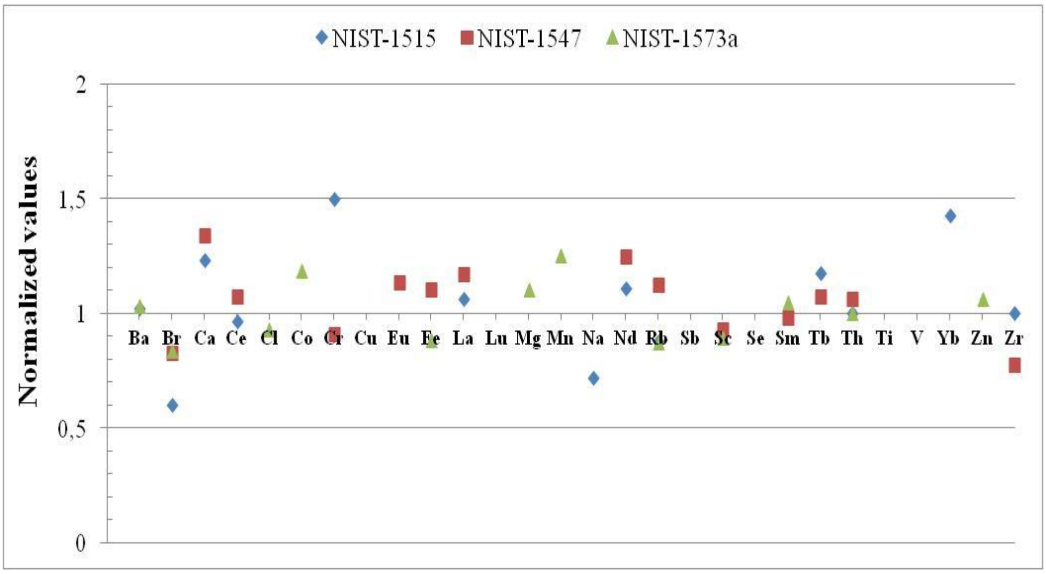

Figure 1: Concentration values measured for reference materials normalized by the respective certified concentration.

The *Z*-score values [22] calculated for the elements determined in the reference materials are shown in Figure 2. For most elements the *Z*-score values were |Z| < 3, which means that the obtained results are in the 99 % confidence interval of the certified values. For Cr and Co, the *Z*-score were high due to the low concentration of these elements in the samples.

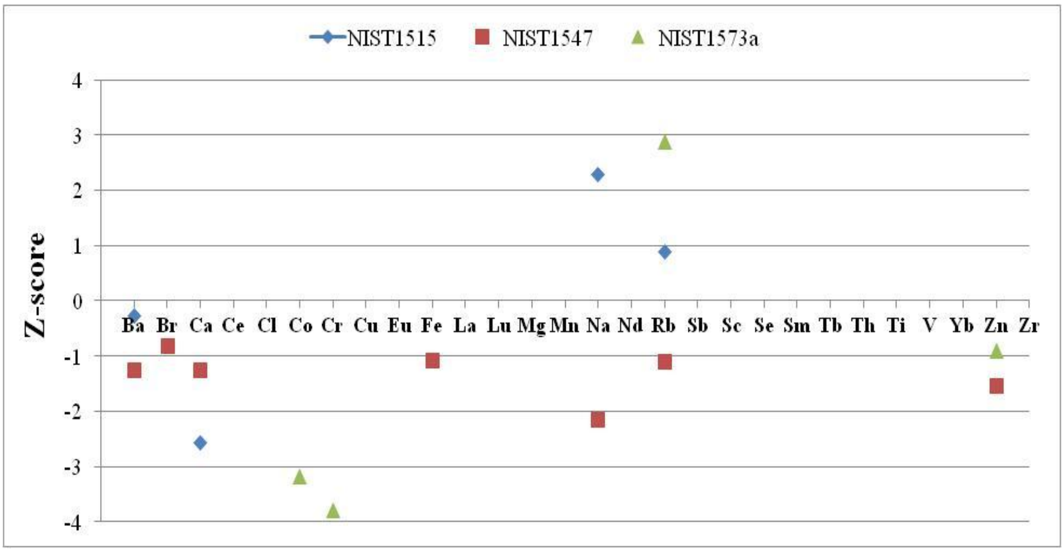

Figure 2: *Z*-score values obtained for the elements determined in reference materials NIST-1515, NIST-1547 and NIST-1573a.

The elemental concentration was determined on Lemon balm leaves and surrounding soil and the results are given in Table 1.

Table 1: Elemental concentration (μg g^{-1}) in soil and leaves at three different sites.

Element	P1†a		P2†a		P3†a	
	Soil	Leaves	Soil	Leaves	Soil	Leaves
As	15 ± 1	0.37 ± 0.04	11 ± 1	nd	24 ± 3	nd
Ba	338 ± 21	68 ± 3	282 ± 17	35 ± 3	345 ± 23	106 ± 5
Br	30.6 ± 0.4	23.9 ± 0.1	15,3 ± 0.2	26.3 ± 0.1	38.7 ± 0.3	12.6 ± 0.1
Ca	13172 ± 958	15019 ± 1013	nd	10528 ± 628	14304 ± 1218	14690 ± 1430
Ce	78 ± 2	1.8 ± 0.1	60 ± 2	0.21 ± 0.04	37 ± 1	0.28 ± 0.03
Cl	nd	nd	nd	5012 ± 326	nd	5835 ± 83
Co	2.97 ± 0.14	0.20 ± 0.01	3.9 ± 0.2	0.13 ± 0.01	2.43 ± 0.06	0.087 ± 0.002
Cr	67 ± 5	2.6 ± 0.2	127 ± 21	1.5 ± 0.3	81 ± 2	0.9 ± 0.1
Cs	1.7 ± 0.1	0.08 ± 0.01	2.3 ± 0.2	0.09 ± 0.01	2.1 ± 0.1	0.11 ± 0.01
Eu	0.63 ± 0.02	0.026 ± 0.001	0.66 ± 0.02	nd	0.44 ± 0.02	0.006 ± 0.001
Fe	36624 ± 312	900 ± 9	24604 ± 209	209 ± 5	53058 ± 493	254 ± 3
Hf	12.0 ± 0.3	0.56 ± 0.01	9.1 ± 0.2	0.054 ± 0.005	16 ± 0.3	0.052 ± 0.004
K	7799 ± 164	29825 ± 368	10956 ± 221	26816 ± 341	3282 ± 91	24334 ± 300
La	36.9 ± 0.9	0.996 ± 0.025	31.5 ± 0.8	0.18 ± 0.01	14.1 ± 0.4	0.145 ± 0.005
Lu	0.55 ± 0.01	0.018 ± 0.001	0.28 ± 0.01	nd	0.94 ± 0.03	nd
Mg	10291 ± 429	5387 ± 593	10601 ± 526	4901 ± 106	16380 ± 777	4954 ± 99
Mn	208 ± 1	16.6 ± 0.2	318 ± 2	14.1 ± 0.2	146 ± 1	11.5 ± 0.1
Na	1414 ± 26	91 ± 2	1612 ± 26	56 ± 1	507 ± 9	54 ± 1
Nd	28 ± 2	nd	21 ± 2	nd	11 ± 1	nd
Rb	38 ± 2	22 ± 1	48 ± 2	41 ± 1	19 ± 1	41 ± 1
Sb	0.40 ± 0.04	0.08 ± 0.01	0.36 ± 0.04	0.21 ± 0.04	1.5 ± 0.1	0.055 ± 0.005
Sc	10.4 ± 0.2	0.26 ± 0.01	7.4 ± 0.2	0.043 ± 0.001	14.2 ± 0.4	0.046 ± 0.001
Se	5.4 ± 0.6	0.23 ± 0.07	1.7 ± 0.4	nd	2.1 ± 0.5	nd
Sm	5.74 ± 0.13	0.126 ± 0.003	4.30 ± 0.09	0.019 ± 0.001	2.08 ± 0.05	0.018 ± 0.001
Ta	3.5 ± 0.1	0.041 ± 0.005	1.5 ± 0.1	nd	2.9 ± 0.2	nd
Tb	0.79 ± 0.06	0.015 ± 0.003	0.77 ± 0.09	nd	0.69 ± 0.06	nd
Th	27 ± 1	0.52 ± 0.02	16 ± 1	0.06 ± 0.01	25 ± 1	0.074 ± 0.004
Ti	8236 ± 521	155 ± 29	3759 ± 263	171 ± 21	11187 ± 717	29 ± 13
U	5.3 ± 0.2	nd	2.9 ± 0.1	nd	4.6 ± 0.2	nd
V	148 ± 15	nd	66 ± 4	2.0 ± 0.1	251 ± 42	0.5 ± 0.1
Yb	3.5 ± 0.2	0.34 ± 0.02	2.0 ± 0.1	nd	4.9 ± 0.3	nd
Zn	154 ± 5	73 ± 2	126 ± 8	88 ± 4	78 ± 3	102 ± 3
Zr	413 ± 40	16 ± 3	293 ± 28	nd	527 ± 51	nd

† Mean of two replicates; [a] mean and Uncertainties; nd – not determined.

As can be seen in Table 1, Ca, Fe, K, Mg and Ti are the most abundant elements in soil. Among the elements determined in Lemon balm leaves the higher concentrations were observed for Ca, Cl, K and Mg. The concentration of Ca in these herbs ranged from 14690 to 15019 µg g^{-1}., with maximum value at P1 site. The concentration of Cl ranged from 5012 to 5835 µg g^{-1}. The K concentration ranged from 24334 and 29825 µg g^{-1} and Mg concentration ranged from 4901 to 5387 µg g^{-1}. The Th concentrations are fairly low and the U concentrations were not determined in the samples. Potentially toxic elements As and Sb were found at high concentrations in soil but at very low concentrations in the leaves samples.

Samples of *Melissa officinalis* were rich in essential macrominerals, especially Ca, K and Mg. Mineral content on this species is in agreement with those obtained by other authors in similar studies [4]. The results clearly showed that elemental content in the medicinal plants varies according to its origin.

In this study, Neral, Geranial, Citronellal, Nerol, Geraniol and Citronellol were selected as the components of interest since in commercial essential oil it is recommended that Neral, Geranial, and Citronellal must present as major chemical compounds, and preferentially Nerol, Geraniol and Citronellol should be absent [8].

The constituents of lemon balm essential oil are given in Table 2 and the chromatograms of lemon balm essential oil are presented in Figure 3.

Table 2: Major constituents of Lemon balm essential oil distilled from fresh leaves harvested at three different sites.

Compounds	%			KI^a	$KI^{lit.}$
	P1	P2	P3		
Neral	42.7^a	44.2^b	44.2^b	1250	1240
Geranial	50.9^b	53.0^a	52.5^a	1282	1270
Citronellal	0.26^b	0.40^b	0.95^a	1154	1153
Nerol	-	-	-	-	-
Geraniol	6.1^a	2.4^b	2.3^b	1261	1255
Citronellol	-	-	-	-	-

† Within line, mean followed by different letters are significantly different at $P \leq 0.05$ (Tukey's test); KI^a - Kovats Index in this work, $KI^{lit.}$ in literature.

The observed values are shown by the percentage of constituents. The major components of the essential oil were Neral and Geranial in all sites. It was observed the minor percentage of Citronellal and Geraniol.

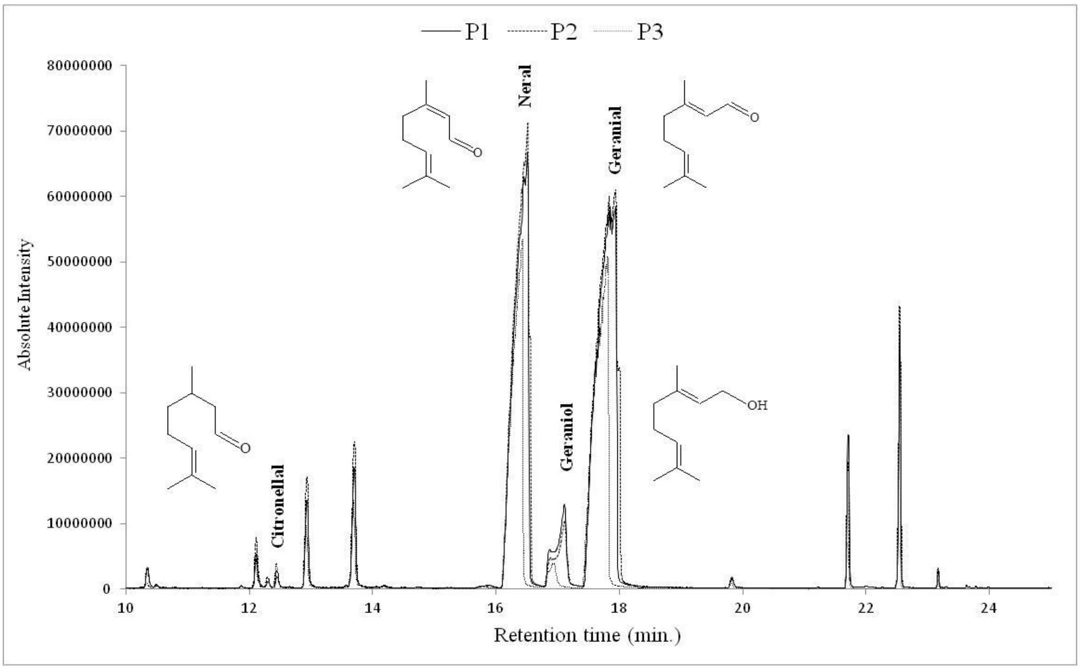

Figure 3: Chromatogram of Lemon balm essential oil extracted at three sites.

Significant differences in essential oil chemical composition were observed in P1 site for the compounds Neral, Geranial and Geraniol. For Citronellal, significant difference was observed in P3 site. Nerol and Citronellol were not identified.

There are many studies related to the medicinal effect of Lemon balm essential oil and the factors influencing essential oil production, such as drying temperature, harvest time, seasonality, light intensity, leaves production, fertilization effect, but the trace elements effect on essential oil production in this species are not known.

Pearson correlation coefficients were applied to examine the relationship between trace elements present in soil, leaves, and chemical constituents of essential oil (Table 3, Table 4 and Table 5). Hierarchical cluster analysis was used to examine the similarities between trace elements in Lemon balm leaves and their essential oil constituents (FIG. 4).

In Table 3, it was observed, for soil samples, that the concentration of Fe negatively correlated with the concentration of Co, Eu, K, Mn and Na, and positively correlated with concentration of As, Br, Hf, Lu, Yb and Sc. Mn concentration was negatively correlated with V. Kabata-Pendias reported negative correlations (antagonist effects) between Fe/Co, Fe/Mn and V/Mn [23]. Concentration of Ti positively correlated with the concentration of Br, V and Yb, and negatively correlated with the concentration of Co and Mn. Concentration of Mg negatively correlated with the concentration of Na, Ce, La, Sm and Zn, and positively correlated with the concentration of As and Lu.

In Table 4 are shown the correlations between elements present in Lemon leaves and soil. At present, 12 of the elements (Br, Ca, Cl, Co, Fe, K, Mn, Na, Rb, Ti, V, and Zn) determined in this study are known for being essential for plants, several are known for being necessary for only for few species, and others are known for stimulating effects on plant growth, but their functions are not yet recognized [23].

For the major elements (Ca, K and Na), it was observed that the Ca concentration in leaves was positively correlated with the elements Ba, Br, Ta and U in soil; and negatively correlated with the elements Co, Cr and Mn also in soil. The concentration of K was negatively correlated with the Sb soil elements.

For trace elements (Cl, Fe, Mn and Zn), it was observed that the Cl concentration in leaves was positively correlated with the elements As, Br, Lu, Mg, U and Yb in soil; and negatively correlated with Ce, Cr, La, Sm and Zn also in soil. Iron concentration in leaves was negatively correlated with Cs in soil. Antimony concentration in soil was negatively correlated with K and Mn leave concentrations and positively correlated with Zn.

2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
***ISBN:* 978-85-99141-05-2**

Table 3: Pearson correlation coefficients obtained for soil components.

	As	Br	Ca	Ce	Co	Cr	Eu	Fe	Hf	K	La	Lu	Mg	Mn	Na	Nd	Sb	Sc	Rb	Sm	Ta	Th	Ti	V	Zr
As																		0.95							
Br	0.93[a]																	0.95					1.00		
Ca																-0.96				-0.96	-1.00				
Ce			-0.97																	1.00					
Co	-0.92	-0.99																-0.97					-1.00		
Cr		-0.83																			-0.85				
Cs						0.83																			
Eu	-0.83																	-0.84							
Fe	0.95	0.95			-0.97		-0.85											1.00							
Hf	0.95	0.94			-0.95			0.98										0.98							
K	-0.97	-0.95			0.95			-0.98	-0.99									-0.98							
La				0.97																0.98					
Lu	0.98	0.95			-0.94			0.96	0.96	-0.99								0.96	-0.93						
Mg	0.91			-0.83							-0.91	0.86								-0.86					
Mn	-0.91	-0.99			0.98	0.82		-0.95	-0.96	0.97		-0.95						-0.95					-1.00		
Na	-0.97	-0.86			0.87		0.88	-0.95	-0.96	0.96		-0.95	-0.90	0.86			-0.89	-0.95							
Nd				0.99							0.97									0.99					
Th																					0.93				0.88
U			-0.97			-0.89															0.99	0.92			
V	0.98	0.98			-0.97				0.96	-0.98		0.99		-0.95									1.00		
Yb	0.97	0.97			-0.95			0.94	0.96	-0.98		0.98		-0.96	-0.92			0.94					1.00	1.00	
Zn				0.89							0.92		-0.95			0.85				0.91					

[a] Only values significant at $p \leq 0.05$ level are shown.

2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-05-2**

Table 4. Pearson correlation coefficients obtained for the elements determined in soil and Melissa leaves.

	Ba*	Br*	Ca*	Cl*	Co*	Cr*	Cs*	Eu*	Fe*	K*	La*	Mn*	Na*	Rb*	Sb*	Sc*	Ti*	V*	Zn*
As	0.97[a]	-0.90		0.98															
Ba			0.82																
Br	0.96		0.84	0.96															
Ce				-0.99			-0.92												
Co	-0.96	0.85	-0.86																
Cr			-0.86	-0.99				-1.0											
Cs									-0.87		-0.83		-0.97	0.84		-0.86			
Eu		0.85			0.95												0.99		
Fe	0.97	-0.95																	
Hf	0.98	-0.94																	
K	-0.99	0.93						1.0											
La				-0.96			-0.94											0.92	
Lu	0.99	-0.89		0.97															
Mg				1.0			0.89											-0.96	
Mn	-0.98	0.84	-0.84					1.0											
Na	-0.93	0.96						1.0											
Nd							-0.90												
Rb	-0.91																		
Sb										-0.88		-0.96							0.91
Sc	0.97	-0.95																	
Sm				-0.99			-0.94												
Ta			0.89																
Ti															-1.0				
U			0.86	0.96															
V	0.98														-1.0				
Yb	0.98	-0.84		0.99															
Zn				-0.99	0.89	0.81	-0.96											0.93	

[*] Elemental concentration in leaves of *Melissa officinalis* L.
[a] Only values significant at $p \leq 0.05$ level are shown.

In Table 5, it can be observed that Neral and Geranial were negatively correlated with Ce, Co, Cr, Eu, Fe, Hf, La, Na, Sc, Sm and Th and positively correlated with Rb. Citronellal concentration was negatively correlated with the concentration of Br, Co, Cr, Eu, Mn. Geraniol concentration was positively correlated with Ce, Co, Cr, Eu, Fe, Hf, La, Na, Sc, Sm and Th and negatively correlated with the concentration of Rb.

The dendrogram presented in Figure 4 was obtained from hierarchical cluster analysis (HCA) applied to the results of elemental concentration in Lemon balm leaves and essential oil constituents.

This graph reveals two main groups: group I includes the monotherpenes aldehydes, Neral, Geranial and Citronellal, alkaline-earth metals Ca and Ba; alkaline-metals Cs and Rb and the elements Cl and Zn. Group II includes the monotherpene alcohol, Geraniol, Rare Earth Elements La, Ce, Sm and Eu, alkaline-metal Na and K, the transition metals Fe, Mn, Hf, Co, Cu, Sc, V and Ti.

Table 5: Pearson correlation coefficient obtained for Melissa leaves elements and essential oil constituents.

	Neral	Geranial	Citronellal	Geraniol
Ba*	0.02	-0.17	0.75	-0.05
Br*	-0.31	-0.14	-0.92**	0.35
Ca*	-0.51	-0.69	0.29	0.50
Ce*	-0.98**	-0.97**	-0.75	0.98**
Cl*	0.04	-0.88	0.86	-0.10
Co*	-0.93**	-0.91**	-0.93**	0.96**
Cr*	-0.93**	-0.80**	-0.86**	0.93**
Cs*	0.60	0.54	0.76	-0.65
Eu*	-1.00**	-0.99**	-0.99**	0.99**
Fe*	-0.99**	-0.98**	-0.61	1.00**
Hf*	-0.99**	-0.98**	-0.80	0.99**
K*	-0.72	-0.60	-0.80	0.73
La*	-0.98**	-0.95**	-0.67	0.99**
Mg*	-0.47	-0.47	-0.40	0.49
Mn*	-0.78	-0.65	-0.89**	0.80
Na*	-0.98**	-0.97**	-0.62	1.00**
Rb*	0.99**	0.97**	0.65	-1.00**
Sb*	0.25	0.32	-0.32	-0.19
Sc*	-0.99**	-0.97**	-0.64**	1.00**
Sm*	-0.97**	-0.95**	-0.77	0.97**
Th*	-0.98**	-0.96**	-0.76	0.98**
Ti*	-0.26	-0.04	-0.77	0.25
V*	-0.60	-0.51	-0.92**	0.65
Zn*	0.81	0.64	0.79	-0.78
Neral	1.00	0.94**	0.64	-0.99**
Geranial	0.94**	1.00	0.46	-0.96**
Citronellal	0.64	0.46	1.00	-0.66
Geraniol	-0.99**	-0.96**	-0.66	1.00

* Elemental concentration in leaves of *Melissa officinalis* L.
** Significant at $p \leq 0.05$ level.

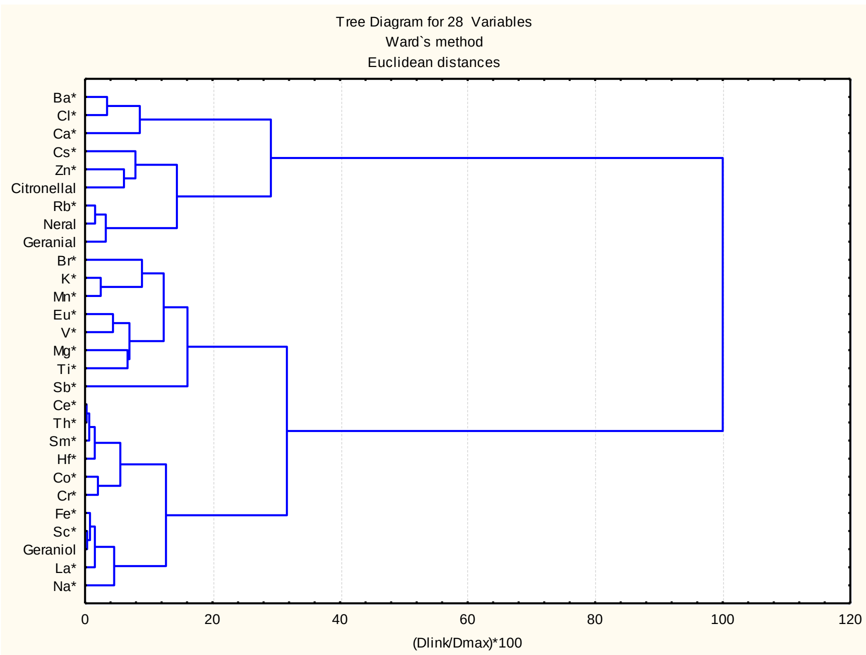

Figure 4: Cluster analysis dendrogram of trace elements and essential oil constituents.

Factor analysis was applied to the elemental concentrations in Lemon balm leaves and essential oil constituents. This analysis showed the occurrence of three main factors with eigenvalues greater than one, accounting for 95 % of the total variance. The main contribution (67.3 % of variance) comes from variables included in Factor I: Cr, Cs, Fe, K, La, Mn, Rb, Sc, Zn, Neral, Geranial, Citronellal and Geraniol. Factor II (21 % of variance) is composed of Ba, Br and Ca; and Factor III (6.6 % of variance) of Mg (FIG.5).

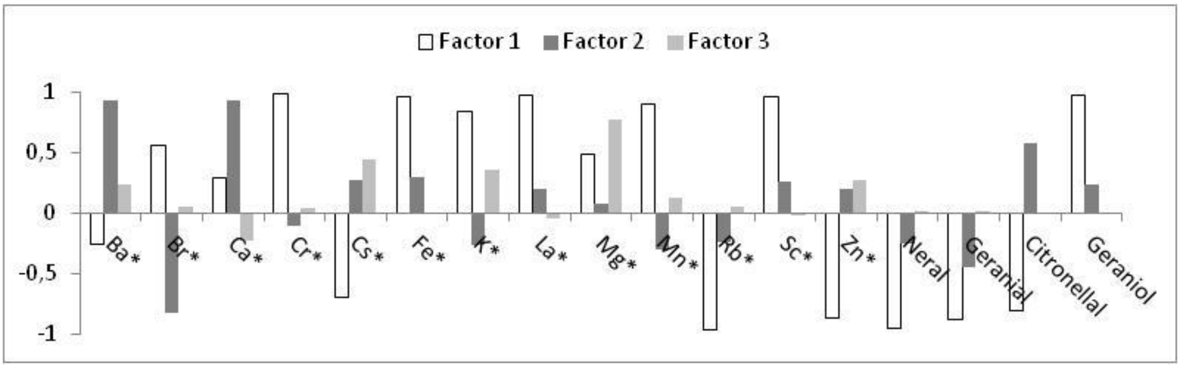

Figure 5: Factor loadings of variables.

4. CONCLUSIONS

In this study, the element concentrations of As, Ba, Br, Ce, Cl, Co, Cu, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Mg, Mn, Na, Nd, Rb, Sb, Sc, Se, Sm, Ta, Tb, Ti, Th, U, V, Yb, Zn and Zr was determined by Instrumental Neutron Activation Analysis (INAA) in soil and *Melissa officinalis* leaves in order to evaluate its essential oil production. The elements Ca, Fe, K, Mg and Ti were the most abundant found in soil samples. Among the elements determined in Lemon balm leaves the higher concentrations were observed for Ca, Cl, K and Mg. Potentially toxic elements As and Sb were found at high concentrations in soil but at very low concentrations in leave samples. The major components of the essential oil were Neral and Geranial.

Correlation coefficient analysis showed good correlations between Ca in leaves and the elements Ba, Br, Ta and U, positively correlated and with Co, Cr and Mn, negatively correlated, in soil. For the trace elements Cl, Fe, Mn and Zn, It was observed that the Cl concentration in leaves was positively correlated with As, Br, Mg, U and Yb in soil and negatively correlated with Ce, Cr, La, Sm and Zn. Fe concentration was negatively correlated with Cs in soil. Sb concentration in soil was negatively correlated with K and Mn and positively correlated with Zn in soil.

Neral e Geranial showed good correlations with the elements Ce, Co, Cr, Eu, Fe, Hf, La, Na, Sc, Sm and Th (negatively correlated) and Rb (positively correlated). Citronellal concentration was negatively correlated with the concentration of Br, Co, Cr, Eu, Mn. Geraniol concentration was positively correlated with Ce, Co, Cr, Eu, Fe, Hf, La, Na, Sc, Sm and Th and negatively correlated with the concentration of Rb.

From the hierarchical cluster analysis (HCA) three main groups were obtained indicating that the major constituents of essential oil, Neral and Geranial were well correlated with Rb and with higher dissimilarity with the elements Br, Cr, K, La, Mg and Mn. From the factor analysis results it was possible to verify that the elements that share the same variability with the main essential oil constituents, Neral, Geranial and Citronellal, are Rb, Zn with positive correlation.

Although not conclusive given the small number of samples analyzed in this study, the results indicate that the production of essential oil by *Melissa officinalis* must be positively correlated with the concentrations of Rb, Zn and negatively correlated with Sc, Mn, La, K, Fe, and Cr.

ACKNOWLEDGMENTS

The authors are grateful to Agronomist Engineer Marcos Victorino and Technical Staff of *Escola Municipal de Jardinagem* for the support and collaboration with our study. The authors thank CNPq for financial support.

REFERENCES

1. V. Schhultz, R. Hansel, V. Tyler, *Rational phytotherapy: a physician’s guide to herbal medicine*, New York: Springer-Verlag (1998).
2. A. May, O. A. Bovi, L.V. Sacconi, A. G. Samra, M. Q. Pinheiro, “Produtividade da biomassa de melissa em função de intervalo de cortes e doses de nitrogênio,”. *Horticultura Brasileira*, **26**, pp. 312-315 (2008).
3. A. C. B. Sodré, J. M. Q. Luz, L. L. Haber, M. O. M. Marques, C. R. Rodrigues, A. F. Blank, “Organic and mineral fertilization and chemical composition of lemon balm (*Melissa officinalis*) essential oil,” *Revista. Brasileira de Farmacognosia*, **22**(1) (2012).
4. M. E. Petenatti, E. M. Petenatti, L. A. Del Vitto, M. R. Téves, N. O. Caffini, E. J. Marchevsky, R. G. Pellerano, “Evaluation of macro and microminerals in crude drugs and infusions of five herbs widely used as sedatives,” *Revista. Brasileira de Farmacognosia*. **21**(6) (2011).
5. H. Cass, “Herbs for the nervous system: Ginkgo, kava, valerian, passion flower,” *Seminars in Integrative Medicine* Vol. 2, pp. 82-88 (2004).
6. T. C. Colussi, L.F. Dalmolin, M. Pachtmann, G. B. L. de Freitas, “*Melissa officinalis* L: Características gerais e biossíntese dos principais metabólitos secundários,” *Revista de Biologia e Farmácia*, **5**(2) (2011).
7. J. Sorensen, “*Melissa officinalis,” International Journal of Aromatherapy*. **10**, pp. 7-17 (2000).
8. A. F. Blank, S. M. Fontes, J. L. S. Carvalho Filho, P. B. Alves, R. Silva-Mann, M. C. Mendonça, M. F. Arrigoni-Blank, M. O. Rodrigues, “Influência do horário de colheita e secagem de folhas no óleo essencial de melissa (*Melissa officinalis* L.) cultivada em dois ambientes,” *Revista Brasileira de Plantas Medicinais*, **8**(1), pp.73-78, 2005.
9. M. Teske, A. M. M. Trenttini, *Compêndio de Fitoterapia*, Herbarium, Curitiba & Brazil (1997).

10. H. Lorenzi, F. J. A. Matos, *Plantas medicinais do Brasil: nativas e exóticas*, Nova Odesa, Plantarum, p. 544 (2002).
11. M. R. Meira, S. A. Manganotti, E. R. Martins, "Crescimento e produção de óleo essencial de *Melissa officinalis* L. nas condições climáticas de Montes Claros – MG," *Biotemas*, **24**(1), pp. 1-8 (2011).
12. R. A. Street, "Heavy metals in medicinal plants products – An African perspective", *South African Journal of Botany*, **82**, pp. 67-74 (2012).
13. V. Rai, P. Vajpayee, S. N. Singh, S. Mehrotra, "Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L," *Plant Science*, **167**, pp. 1159-1169 (2004).
14. E. M. Aoyama, A. Indirunas, M. R. Furlan, "Leaves production of *Melissa officinalis* L. (Lamiaceae) in Taubate, Sao Paulo", *Revista Biociências*, **17**(1), pp. 57-65 (2011).
15. C. I. Yamashita, M. Saiki, J. A. A. Sertié, "Elemental analysis of leaves and extracts of *Casearia* medicinal plants by Instrumental neutron activation analysis," *Journal of Radioanalytical and Nuclear Chemistry*, **270**(1), pp. 181-186 (2006).
16. F. V. Sussa, S. R. Damatto, D. I. T. Fávaro, B. P. Mazzilli, P. S. C. Silva, "Radioactive and stable elements' concentration in medicinal plants from Brazil," *Journal of Radioanalytical and Nuclear Chemistry*, **281**, pp. 165-170 (2009).
17. Brasil. Farmacopéia Brasileira, volume 2 / Agência Nacional de Vigilância Sanitária, Brasília: ANVISA 5º Ed., Vol. 2, p. 1130 (2010).
18. F. W. McLafferty, D. B. Stanffer, *Registry of Mass Spectral Data*, Wiley-Interscience, New York (1989).
19. R. P. Adams, *Identification of Essential Oil Components by Gas Cromatography / Mass Spectroscopy*, Allured Publishing Corporation, Carol Stream, USA (2001).
20. M. C. Viegas, D. G. Bassoli, "Utilização do Índice de Retenção Linear para caracterização de compostos voláteis em café solúvel utilizando GC-MS e coluna HP-INNOWAX," *Química. Nova,* **30** (8), pp. 2031-2034 (2007).
21. W. R. Atchley, E. H. Bryant, *Multivariate Statistical Methods: Among-Groups Covariation, Dowdsen Hurtchingon & Ross, Inc*., p.264 (1975).
22. P. Bode; Ph.D. Thesis, *Delft University of technology*, The Netherlands, p. 148 (1996).
23. A. Kabata-Pendias, *Trace elements in soils and plants*, 4th ed (2001).