

SOIL-TO-PLANT TRANSFER FACTORS FOR STABLE ELEMENTS IN LEMON BALM (*Melissa officinalis* L.) COMMONLY USED AS A MEDICINAL PLANT

Fábio V. Sussa¹; Marcos R. Furlan²; Marcos Victorino³; Paulo S. C. Silva¹

¹ Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP, Brazil
fvsussa@gmail.com
pscilva@ipen.br

² Universidade de Taubaté (UNITAU – SP)
R. Quatro de Março, 432
12020-270 São Paulo, SP, Brazil
furlanagro@gmail.com

³ Faculdade Cantareira (FIC)
R. Marcos Arruda, 729
03020-000, São Paulo, SP, Brazil
marcosvictorino@uol.com.br

ABSTRACT

This study evaluated the transfer factors (TF) of stable elements (essential and non-essential) As, Ba, Br, Ca, Cd, Co, Cr, Cu, Fe, Hf, K, La, Mg, Mn, Na, Ni, Pb, Rb, Sb, Sc, Sm, Th, Ti, V and Zn from soil to *Melissa officinalis* L. (Lemon balm). The transfer factors were calculated by dividing the metal concentration in Lemon balm leaf by the total metal concentrations in the soil. The experiments were conducted from January 2013 to January 2014 at the Municipal Gardening School area, in Ibirapuera Park, *São Paulo*, Brazil. The experimental design was completely randomized (CRD), with three treatment: control (site soil), organic (2 t ha⁻¹ poultry manure) and conventional (30 t ha⁻¹ of NPK, 6:14:8), and four harvest (April, July, October and January) with four replications. The soil samples collected (0 – 20 cm) were dried at 100 °C and grounded to 100 mesh size particles and homogenized. Lemon balm samples were collected and 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 Lemon balm leaves were dried at 100 °C and grounded to 100 mesh size particles. Instrumental neutron activation analysis (INAA), graphite furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma optical emission spectrometry (ICP-OES) were applied to determine the elemental concentration in the samples. The results showed that the metals accumulate in species with different intensity. Our findings indicated that Ca, Cd, K, Ni and Rb accumulated the most with the transfer factor of 1.0 – 12, followed by Ba, Br, Co, Cr, Cu, Mg, Mn, Na, Pb, Sb and Zn with TF of 0.1 – 1.0, while As, Fe, Hf, La, Sm, Th, Ti and V had the lowest accumulation with TF usually < 0.1.

1. INTRODUCTION

Lemon balm (*Melissa officinalis* L.) originates from Europe and is now grown all over the world. It is a medicinal and aromatic species popularly used to make tea, which serves as an antioxidant, antiviral, tranquilizer, promote sleep, reduce stress and anxiety [1-3]. Furthermore, Lemon balm is used to treat Graves', Alzheimer's and thyroid diseases [4,5]. Although many of these medicinal plants appear to be safe, the possible toxicity of these products, and toxicity itself depends on the level of product contamination [6]. Medicinal plants may be contaminated

during growth, development and processing stages. The soil geochemical characteristics, atmospheric deposition, harvesting and the ability of each plant species to selectively accumulate metals are some of the factors, which could play important roles leading to the contamination of medicinal plants by metals [7]. The determination of major, minor and trace elements in medicinal plants is of great importance due to the growth of environmental pollution that directly affects the plants and, therefore, their phytotherapies. Besides, being essential in the living system, the elements can be at the same time toxic, when at concentrations beyond those necessary for metabolic functions [8-10]. So, contamination of plant preparation with high concentration of metals can cause health problems [11].

A soil-to-plant transfer factor (FT) is an important parameter that can be used to estimate metal level in medicinal plants as a fraction of soil total concentration, and the FT assessment model is a tool to simplify the transfer estimation of these elements through the food chain [12,13]. Very limited information regarding of metal transfer from soil to medicinal plants are available. Therefore, the aim of this paper was to evaluate the TF of As, Ba, Br, Ca, Cd, Co, Cr, Cu, Fe, Hf, K, La, Mg, Mn, Na, Ni, Pb, Rb, Sb, Sc, Sm, Th, Ti, V and Zn from soil to Lemon balm species (*Melissa officinalis* L.) commonly used in folk medicine. The results were used to show that elements accumulate in this species with different intensity and provided information on the heavy metals accumulation in this plant.

2. MATERIALS AND METHODS

The experiments were carried out in the Municipal Gardening School area, in Ibirapuera Park, São Paulo, Brazil, latitude 23° 35' S, longitude 46° 39' O, with the following chemical properties of the 0-0.2 m topsoil layer: pH (CaCl₂) = 6.9; organic matter (OM) = 15 g dm⁻³, P = 1 mg cm⁻³; K⁺ = 3.5 mmol_c dm⁻³; Ca²⁺ = 64 mmol_c dm⁻³; Mg²⁺ = 8 mmol_c dm⁻³; H + Al = 15 mmol_c dm⁻³; cation exchange capacity (CEC) = 91 mmol_c dm⁻³ and sulfur (S) = 30 mmol_c dm⁻³ of soil; and base saturation (V%) = 83%. The studies were conducted from January 2013 to January 2014.

The experimental design was completely randomized (CRD), with three treatment: control (site soil), organic (2 t ha⁻¹ poultry manure) and conventional (30 t ha⁻¹ of NPK, 6:14:8), and four harvest (April, July, October and January) with four replications.

Soils were collected from the surface (0-0.2 m depth). The soil samples were dried at 100 °C for 12 h in an oven. The dried samples were grounded into powder at 100 mesh (150 µm diameter) size particles and homogenized.

The Lemon balm samples collected 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 leaf samples were dried at 100 °C for 12 h in an oven. The dried samples were grounded into powder at 100 mesh (150 µm diameter) size particles and homogenized.

The As, Ba, Br, Ca, Co, Cr, Fe, Hf, K, La, Mg, Mn, Na, Rb, Sb, Sc, Sm, Th, Ti, V and Zn concentrations were determined by Instrumental Neutron Activation Analysis (INAA) in soil and Lemon balm samples. INAA, a sensitive, fast, nondestructive and multielemental technique, has been frequently used to evaluate inorganic contents in soil and medicinal plants [14,15]. These samples were irradiated at the nuclear research reactor IEA-R1 in IPEN

(*Instituto de Pesquisas Energéticas e Nucleares*, Brazil), under two irradiations schemes, short (20 seconds) and long irradiations (8 hours). For the determination of As, Ba, Br, Ca, Co, Cr, Fe, Hf, K, La, Na, Rb, Sb, Sc, Sm, Th and Zn, about 150 mg of the powdered leaf samples, 100 mg of powdered soil and 100 mg of the powdered reference materials (IAEA-336 Lichen, NIST 1573a Tomato Leaves, NIST 1547 Peach Leaves, NIST 1646a Estuarine Sediment and USGS STM-2 Table Mountain Syenite) were weighed in previously cleaned polyethylene bags. These samples were carried out under a thermal neutron flux of $10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ for 8 hours. For determination of Mg, Mn, Ti and V, approximately 70 mg of the powdered leaf samples, 30 mg of powdered soil and 50 mg of the powdered reference materials (NIST 1573a Tomato Leaves, NIST 1547 Peach Leaves, NIST 1646a Estuarine Sediment) were carried out at the pneumatic facility with a thermal neutron flux of approximately $10^{11} \text{ n cm}^{-2} \text{ s}^{-1}$ for 20 seconds. 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 1.9 keV for the 1,332 keV 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 the determination of Cd, Cu, Ni and Pb, about 300 mg each of the powdered soil and leaf samples was digested in 22.5 ml of mixture of concentrated acid solution (HNO_3 , HCl , HClO_4 , H_2O_2 in ratio of 5:15:0.5:2). The corresponding solution was heated until a clear solution appeared. The clear solution was diluted up to 50 ml with distilled water and filtered with Watt man filter paper no.1.

A Perkin Elmer AAnalyst800 Graphite Furnace Atomic Absorption Spectrometer (GF AAS) was used for the determination of the elements Cd, Cu and Pb. This method provides both sensitivity and selectivity since other elements in the sample will not generally absorb the chosen wavelength and thus, will not interfere with the measurement [16]. Cathode lamps were used as radiation source. Argon gas was used for all the experiments as carrier and for cleaning the graphite furnace. The standard working solutions of interest elements were prepared to make the standard calibration curve.

An Inductively Coupled Plasma-Optical Emission Spectrometry (ICP OES) was used to determination of Ni concentrations. The detailed methodology for ICP OES measurements, as well as the calibration curves used for the concentrations in all the samples are presented in the literature [17].

Transfer factor (TF) was calculated for each metal according to the following equation 1:

$$TF = \frac{C_{plant}}{C_{soil}} \quad (1)$$

Where, C_{plant} and C_{soil} represents the metal content (mg kg^{-1}) in plant and soil on dry weight basis, respectively [18].

3. RESULTS AND DISCUSSION

In order to evaluate the precision and accuracy of the results, the Standard Reference Material (SRM) from the IAEA-336 Lichen, NIST 1573a Tomato Leaves, NIST 1547 Peach Leaves, NIST 1646a Estuarine Sediment and USGS STM-2 Table Mountain were analyzed. As recommended in its certificate, elemental concentrations in the reference material were calculated on a dry weight basis. A subsample of the reference material was separated to determine the moisture content by drying at 85 °C for 24 hours. The weight loss obtained was used to correct the results.

In Figure 1 are shown the Z-score values calculated for the elements determined in the SRM. For all elements the Z-score values were $|Z| < 3$, which means that the results obtained are in the 99% confidence interval of the certified values [19].

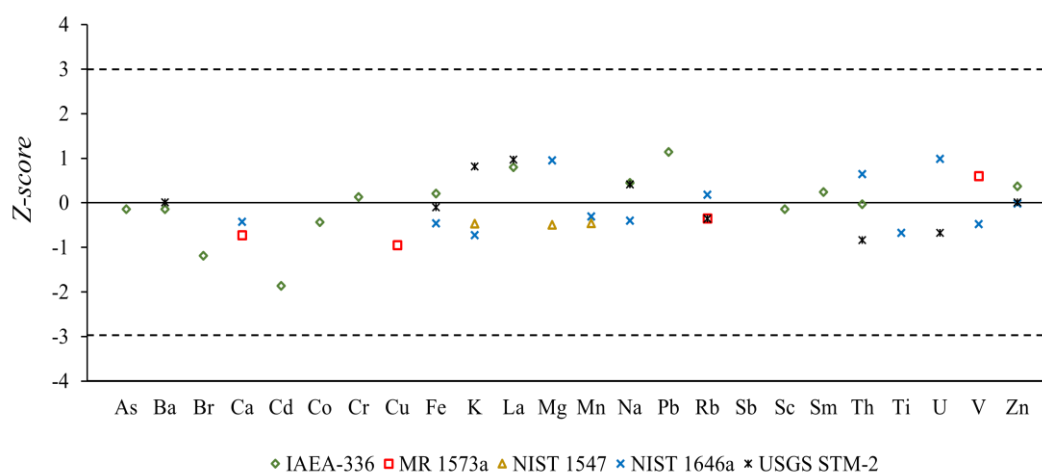


Figure 1: Z-score values obtained for the elements determined in geological and biological references materials.

Summarized elemental concentrations in all the soils and the Lemon balm leaf samples (on the dry weight basis) are shown in Table 1 and 2, respectively. The number of samples (N^a) consider only the results above the detection limit. The tables also list median, arithmetic mean, and geometric mean.

As can be seen in the Table 1, Ca, Fe, K, Mg and Ti are the most abundant elements in soil. The concentration ranges for these major elements in the surface soil were found 5640-12346, 57799-72201, 1445-3244, 13086-34477 and 3727-13594 mg kg^{-1} , respectively. Whereas the concentration ranges of essential and non-essential like As, Cd, Co, Cr, Cu, Mn, Ni, Pb, V and Zn are found 27-40, 0.03-0.07, 2.9-3.7, 84-133, 1-28, 109-223, 7-15, 10-34, 37-246 and 53-332 mg kg^{-1} , respectively. The mean concentrations for Hf, La, Rb, Sb, Sc, Sm and Th are 17, 23, 17, 1.9, 1.8, 3.2 and 31 mg kg^{-1} .

The maximum/minimum ratios were less than 10 for 24 elements and > 10 for 1 element. According to [20], the observed variation is related to agricultural management, control, organic and conventional, as shown in Figure 2. The fertilized systems showed higher

concentrations of major and minor nutrients, and trace elements. It has been reported that fertilizer systems are sources of these elements and contribute to soil improvement [21]. The mean concentrations of potentially toxic elements like As, Cd, Cr, Cu, Pb and Zn did not exceed the World Average value, according to [22].

Table 1. Concentration of 25 elements for three cultivation systems soil samples.

	As	Ba	Br	Ca	Cd	Co	Cr	Cu	Fe	Hf	K	La	Mg
-----mg kg ⁻¹ -----													
N ^a	24	8	24	23	23	24	24	24	24	24	24	24	24
Minimum	27	68	20	5640	0.03	2.9	84	1	57799	12	1445	18	13086
Maximum	40	196	26	12346	0.07	3.7	133	28	72201	20	3244	31	34477
Max./Min.	1	3	1	2	2	1.3	2	24	1	2	2	2	3
Mean	33	110	23	8500	0.05	3.2	100	17	65637	17	2460	23	24195
Median	32	92	23	8205	0.05	3.2	99	17	66134	17	2447	22	23600
Geometric Mean	33	104	23	8256	0.05	3.2	99	15	65557	17	2418	23	23772
	Mn	Na	Ni	Pb	Rb	Sb	Sc	Sm	Th	Ti	V	Zn	
-----mg kg ⁻¹ -----													
N ^a	24	23	24	22	10	18	24	22	23	17	24	7	
Minimum	109	175	7	10	5	0.7	16	2.3	27	3727	37	53	
Maximum	223	538	15	34	30	3.9	19	5.2	35	13594	246	332	
Max./Min.	2	3	2	3	6	5	1	2.3	1	4	7	6	
Mean	160	391	11	14	17	1.9	18	3.2	31	8842	176	111	
Median	160	388	11	12	17	1.4	18	3.1	30	8814	178	78	
Geometric Mean	157	384	11	13	15	1.7	18	3.1	31	8566	168	91	

* Numbers of samples for which concentration were determined.

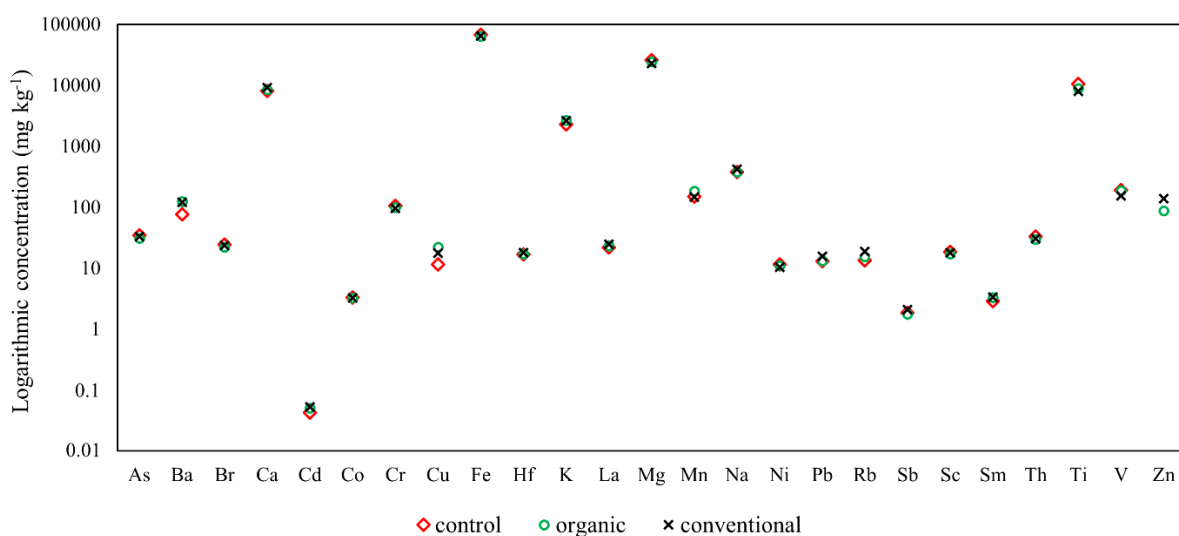


Figure 2: Median value of logarithmic concentration in soil from control, organic and conventional cultivation systems for Lemon balm.

As can be seen in the Table 2, Ca, Fe, K and Mg are the most abundant elements in the Lemon balm leaf samples. Calcium is an essential element for plant and its content varied from 8849 to 24628 mg kg⁻¹. Iron is an essential element for plant protein synthesis. The range of variation for this element was from 433 to 4138 mg kg⁻¹. The range of Fe concentrations are in agreement with the literature [22-24]. Potassium, an essential element that is generally supplied to plants by means of fertilization process also showed a wide variation with a higher value found in organic system (Table 3). The potassium concentrations determined ranged from 20617 to 41139 mg kg⁻¹. Magnesium, besides being a chlorophyll component is also an enzyme co-factor. Its concentration varied from 1622 to 7680 mg kg⁻¹ and these values are in good agreement with the literature, registering concentrations which varies from 3700 to 40000 mg kg⁻¹ in Lemon balm samples [23,24].

Table 2. Concentration of 25 elements in Lemon balm leaf sample.

	As	Ba	Br	Ca	Cd	Co	Cr	Cu	Fe	Hf	K	La	Mg
	-----mg kg ⁻¹ -----												
N ^a	7	22	24	24	24	23	23	24	24	17	24	20	24
Minimum	0.3	46	5	8849	0.02	0.2	5	7	433	0.1	20617	0.2	1622
Maximum	2.3	102	66	24628	0.12	2.8	283	17	4138	1.2	41139	1.9	7680
Max./Min.	8	2	12	3	6	17	55	2	10	16	2	12	5
Mean	1.2	66	24	12901	0.05	0.8	65	11	1212	0.4	29263	0.6	5428
Median	1.0	62	14	12123	0.05	0.6	50	10	852	0.2	27647	0.4	5800
Geometric Mean	0.8	64	19	12497	0.05	0.6	38	11	994	0.3	28698	0.5	5161
Ponderate values in plants	0.009-1.5 ^b	1-198 ^b	< 40 ^b	2000-35000 ^c	3-30 ^b	0.18-0.24 ^b	0.1-0.5 ^b	5-1500 ^b	1-1000 ^b	0.01-0.4 ^b	5000-60000 ^c	0.003-15 ^b	1000-8000 ^c
	Mn	Na	Ni	Pb	Rb	Sb	Sc	Sm	Th	Ti	V	Zn	
	-----mg kg ⁻¹ -----												
N ^a	24	22	22	21	24	14	24	17	17	13	21	24	
Minimum	8	18	3	1	10	0.1	0.03	0.03	0.1	68	0.4	31	
Maximum	41	266	46	7	28	0.5	1.21	0.23	2.0	494	5.2	89	
Max./Min.	5	15	15	5	3	4	35	7	18	7	15	3	
Mean	19	79	21	4	18	0.2	0.3	0.08	0.6	176	1.7	54	
Median	19	43	22	3	18	0.2	0.2	0.05	0.3	118	1.2	56	
Geometric Mean	18	55	14	3	18	0.2	0.2	0.06	0.4	142	1.3	52	
Ponderate values in plants	30-500 ^b	trace ^b	0.1-3.0 ^b	0.1-10 ^b	20-70 ^b	0.06-4.3 ^b	0.13-0.14 ^b	0.1-0.8 ^b	<5.0 ^b	15-1500 ^b	5-50 ^b	1-150 ^b	

^a Numbers of samples determined.

^b [22]

^c [25]

Essential and non-essential microminerals are present in Lemon balm leaves with significant quantities of As (0.3 to 2.3 mg kg⁻¹), Cd (0.02 to 0.12 mg kg⁻¹), Co (0.2 to 2.8 mg kg⁻¹), Cr (5 to 283 mg kg⁻¹), Cu (7 to 17 mg kg⁻¹), Mn (8 to 41 mg kg⁻¹), Ni (3 to 46 mg kg⁻¹), Pb (1 to 7 mg kg⁻¹), V (0.4 to 5.2 mg kg⁻¹) and Zn (31 to 89 mg kg⁻¹). The mean concentrations for Hf, La, Rb, Sb, Sc, Sm, Th and Ti are 0.4, 0.6, 18, 0.2, 0.3, 0.08, 0.6 and 176 mg kg⁻¹. Very few values have been reported in the literature for Hf, Rb, Sb, Sc, Ti and V in the Lemon balm. In general, most elements occurred at below levels in aboveground parts of Lemon balm, according to the

pondered values reported in literature. Excepted to Ni which presented levels that can be considered toxic, about 10 times the level for normal plants [22,25]. The critical toxicity levels on plant are $> 10 \text{ mg/kg}$ dry weight (DW) in sensitive species, $> 50 \text{ mg/kg}$ DW in moderately tolerant species, and $> 1000 \text{ mg/kg}$ DW in Ni hyperaccumulator plants [33].

In fact, among the elements determined in this paper, only As, Cd and Pb has been limited by WHO [26] in plants used as raw material for medicinal purpose, in concentrations of 1.0, 0.3 and 10 mg kg^{-1} , respectively. Observing the data in Figure 3, it can be noted that this value was exceeded in samples grown in the conventional and control cropping systems for As and in no samples for Cd and Pb.

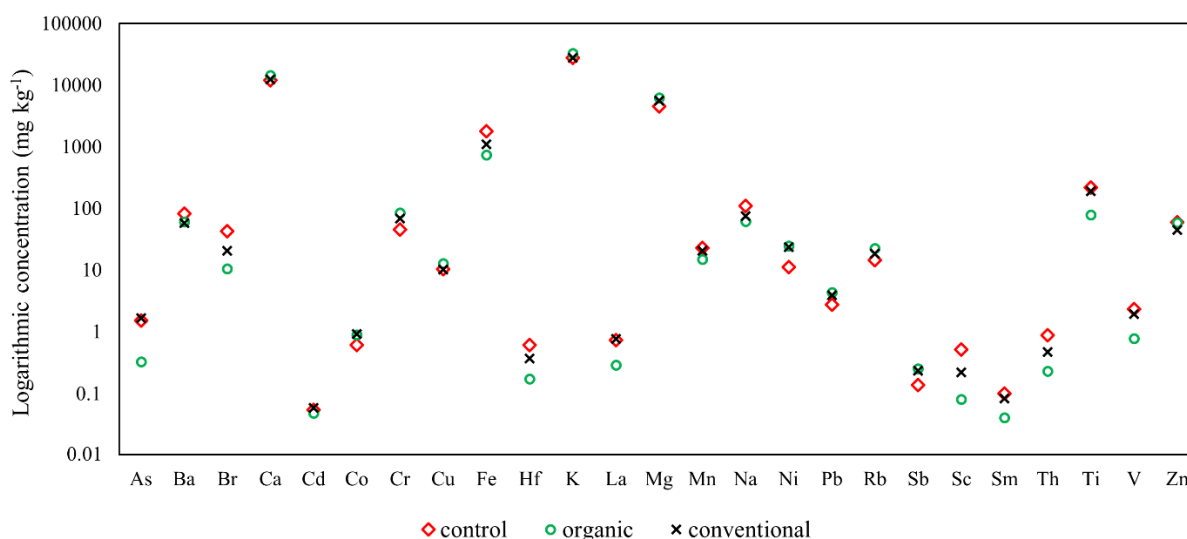


Figure 3: Median value of logarithmic concentration in leaves from control, organic and conventional cultivation systems for Lemon balm.

Soil-to-plant TF of the 25 elements (As, Ba, Br, Ca, Cd, Co, Cr, Cu, Fe, Hf, K, La, Mg, Mn, Na, Ni, Pb, Rb, Sb, Sc, Sm, Th, Ti, V and Zn) as a function of agricultural management are presented in Figure 4. The TF values for most elements varied greatly between control, organic and conventional systems. According to the agricultural management, TF values were ranked in the following order control $>$ conventional $>$ organic. The low TF value for organic system may be due to strong elemental adsorption into organic matter, which renders its less bioavailable to plants [27-29].

From Figure 4, it is observed that the elements which showed higher transfer factor were Ca (ranges from 1.44 to 1.56), Cd (1.02 to 1.30), K (9.67 to 12,78), Ni (1.07 to 2.60) and Rb (1.14 to 2.24), followed by Ba (0.48 to 1.10), Br (0.48 to 1.74), Co (0.20 to 0.30), Cr (0.45 to 0.91), Cu (0.60 to 1.70), Mg (0.18 to 0.26), Mn (0.08 to 0.16), Na (0.20 to 0.31), Pb (0.22 to 0.27), Sb (0.10 to 0.16) and Zn (0.42 to 1.70). While As (0.01 to 0.05), Fe (0.01 to 0.02), Hf (0.01 to 0.03), La (0.01 to 0.03), Sc (0.004 to 0.03), Sm (0.01 to 0.02), Th (0.006 to 0.03), Ti (0.01 to 0.03) and V (0.005 to 0.01) had the lowest TF accumulation. Among the toxic elements that showed highest TF in Lemon balm leaf are Cd and Ni. These might be due to the higher mobility of Cd of natural occurrence in soil [30] and the increased concentration of Ni from leaves as opposed to the soil may suggests the effect of anthropogenic sources [31, 32].

Nevertheless, Ni accumulator plants may also reflect their potential to sequester high levels of Ni in their tissue [33], under normal growing conditions, plants can potentially accumulate certain metal ions up to orders of magnitude greater than the surrounding medium [34].

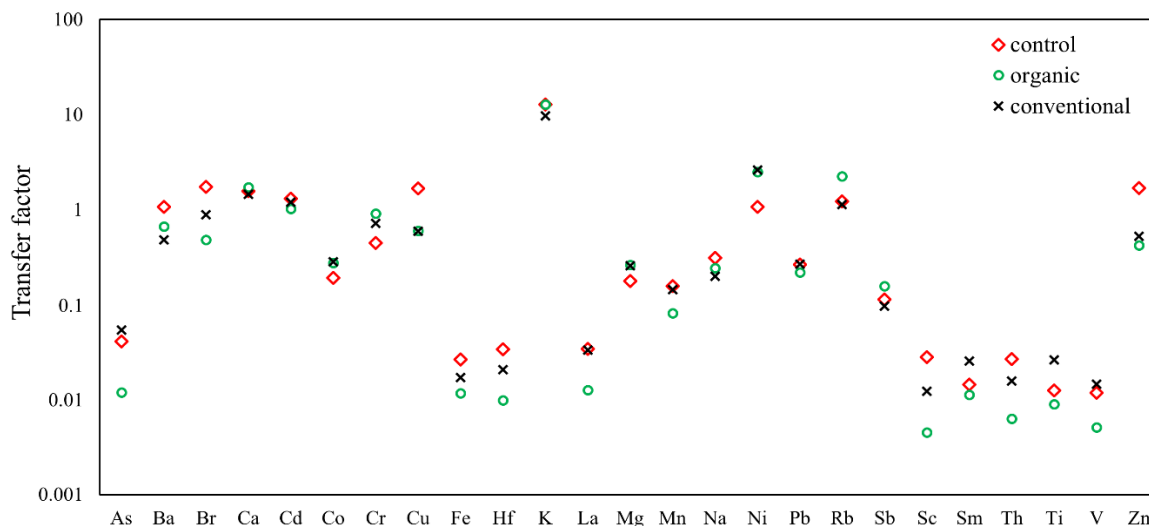


Figure 4: Transfer factor of elements from soils into Lemon balm sample.

4. CONCLUSIONS

In conclusion, the most elements occurred at low levels in Lemon balm leaves, excepted to Ni that presented toxic metal level, about 10 times the normal level for plants. Soil-to-plant TF determined allowed to evaluate the proportion of the content of the elements present in the soil that is transferred to the plants. The results of the present study revealed that Ca, Cd, K, Ni and Rb accumulated with the higher transfer factor values, between 1.0 and 12; followed by Ba, Br, Co, Cr, Cu, Mg, Mn, Na, Pb, Sb and Zn with TF in the range of 0.1 – 1.0, while As, Fe, Hf, La, Sc, Sm, Th, Ti and V had the lowest accumulation, with TF usually < 0.1. Among the potentially toxic elements that shown highest TF in Lemon balm are Cd and Ni. Although soil-to-plant TF values are low, the presence of heavy metals in *Melissa officinalis* leaves reflect the transfer of metals from the soil, their absorption and translocation. This condition indicates that a part of the total contaminant content in the soil is biologically bioavailable to the evaluated plant species and exposes the potential risk that the consumption of medicinal plants may pose.

ACKNOWLEDGEMENTS

The authors are grateful to technical staff of gardening municipal school for the support and collaboration with our study. The authors thank CNPq (The Brazilian National Research Council) for financial support (Process n° 141977/2012-4) and IPEN-CNEN/SP.

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