Romanian Reports in Physics **72**, 705 (2020)

STATISTICAL ANALYSIS OF MINERAL ELEMENTS CONTENT IN DIFFERENT MELLIFEROUS PLANTS FROM THE DOBROGEA REGION, ROMÂNIA

L. BARBEŞ[1], A. BĂRBULESCU[2*], G. STANCIU[1*]

[1] "Ovidius" University of Constanta, Chemistry and Chemical Engineering Department
[2] "Ovidius" University of Constanta, Mathematics and Informatics Department,
124 Mamaia Bd., 90052, Constanta, Romania
E-mails: *gstanciu66@yahoo.com; lucille.barbes2009@gmail.com; alinadumitriu@yahoo.com*
[*] Corresponding authors: *Gabriela Stanciu* and *Alina Barbulescu*

Received December 5, 2019

Abstract. Different mineral elements are essential for physiological processes in the medicinal plants and food derivative products (tea, herb extracts, tinctures, honey, pollen etc.). The aim of this paper was to study the content of nine mineral elements: K, Ca, Mg, Na, Fe, Mn, Cu, Zn, and Cd in different parts (leaves, flowers and steam or bark) of three medicinal plant with highest melliferous potential: *Sambacus nigra L.*, *Hypericum perforatum* and *Tilia tomentosa* species, collected in May and June 2019 from wild flora of Dobrudja area. The quantitative determination of mineral elements in the components of these medicinal herbs was done by FAAS (flame atomic absorption spectrometry). Absorption of different metallic ions in melliferous plants is governed by soil characteristics such as pH, salinity, conductivity and organic matter content. In this respect, Zn average concentration ranged between 6.893 mg/kg in *Sambacus nigra L.* stems and 101.46 mg/kg in *Tilia tomentosa* bark collected from touristic camping area. The physiological activities of melliferous plants influence zinc absorption and the interactions with many elements such as Fe and Mn. The analysis of Cd in the melliferous plants indicated that Cd concentration ranged between 0.001 mg/kg in *Sambacus nigra L.* stems to 5.64 mg/kg in *Tilia tomentosa* leaves on the same polluted area by the intense road traffic, respectively.

Key words: FAAS; *Sambacus L.*; *Hypericum perforatum*; *Tilia tomentosa*; statistical analysis.

1. INTRODUCTION

The mineral composition of different food products with vegetable origin is correlated with the botanical plants provenience. In addition, the evaluation the different implications on the human health of the essential and non-essential mineral elements present in the medicinal plants and their derivatives have been carried out by different researchers [1, 2]. The last two decades, a high interest was shown to the apiculture development in the in the northern Dobrujda area and the quality, safety or the nutritive values of the different beekeeping products (multifloral honey, pollen, food supplies or cosmetics). The honey quality is dependent on the flower pollen and nectar that are collected and ripened by bees. The macro-elements, minor or trace and ultra-trace elements, in relationship with the geology and climate area, are very good indicators for the identification of the special chemical characteristics of the mentioned products.

The contaminants are transferred from the working bees to formed and ripened honey and can alter the high quality and chemical composition of the honey [3].

The level of metallic species in the plants can be affected by the air or water pollution and the geochemical parameters of soil, on one hand, and by the ability of cumulative selection of plants for some elements, on the other hand. Some mineral elements (such as Cu, Zn, Fe, Mn, etc.) are essentials nutrients for plants and these may also be related to the geographical origin. At high concentration in different plants' components and soil, they become phytotoxic. The heavy metals, especially Pb, Cd, Ni or Hg and As can contaminate the bee harvest area, and can be found in pollen, propolis and honey [4, 5].

Different mineral elements, major or macroelements (*e.g.* K, Ca, Mg, Na), minor or trace elements (such as Cu, Zn, Fe, Mn) and heavy metals (Pb, Cd, Ni) are released into the air from diversified sources. Then, they can be easily transport long distances away, depending on the meteorological conditions and may be absorbed by aerial parts of plants (leaves, flowers, fruits or stems) after dry or wet deposition of atmospheric fallouts on plant canopy [6].

It is highly necessary to assess health risks associated with the aerial plants uptake of many toxic elements near the vicinity of the traffic roads, industrialized and urban or rural areas [7].

Calcium is present mainly in the cell wall and the vacuole of the plants, where it is involved in cell wall stabilization and/or secretor processes. Another function of Ca is its role as second messenger in signal transduction and it is accumulated in the older plant parts (content range is from 0.1 to more than 5% in the plant dry matter). Generally, the fruits contain lower quantities of Ca than the plants' leaves [8]. Potassium may be redistributed within the plant because it is easily mobile in the phloem transport [9]. Magnesium is a constituent of chlorophyll and it is involved in the activation of many ATP dependent enzymes and carbohydrate partitioning. In the medicinal plants, the usual range of Mg content in the green tissues is about 0.15 to 0.35 % in the dry matter. Another mineral element is sodium, which is available in plants, especially absorbed from the soil. It is highly mobile and readily transferred to the principal components of plants. The range of Na content in different medicinal herbs is from 37–5584 mg/kg [8, 9]. Iron (Fe) is considered an essential element necessary for the physiological status. It is involved in chloroplast development and photosynthesis of plants, being present in the redox systems.

Due to the iron association with the photosynthesis, the plants' leaves display higher Fe contents than other plant components. Manganase (Mn) plays an important role in the redox processes. The oxygen evolution in photosynthesis is Mn – dependent.

In different medicinal herbs, the level of Mn in leaves range is from 20 to 300 mg/kg. Copper (Cu) is also essential for photosynthesis and others important processes, such as pollen formation, lignifications and fertilization of plants. The normal range of copper in medicinal plants is 5 to 20 mg/kg which is required for growth. If the Cu concentration is exceeded to 30 mg/kg the copper toxicity symptoms may be arise. Zinc (Zn) is essential for the structural integrity of the plant ribosomes, is also a constituent or activator of a large number of enzymes and is required for the membrane integrity. The following values of zinc content have been found in medicinal herbs: 3.5–205 mg/kg [8]. Nickel (Ni) is usually involved in the nitrogen metabolism of plants and this element may accumulate in the seeds of different medicinal plants. The normal Ni concentrations in leaves are 0.1 to 5 mg/kg. The major focus of most researches about toxic heavy metals in medicinal plants was on cadmium (Cd) and lead (Pb). For instance, the analysis of different medicinal plants samples collected from the South – Western of Romania (Aninei Mountains area) revealed the average cadmium content of 0.5 mg/kg [7]. This value exceeds the limit of 0.30 mg Cd/kg dry plant established by the World Health Organization (WHO) [10]. Cd is regarded an easily mobile ionic species in the soil.

Herbal extracts and some phytopharmaceuticals derivatives of *Hypericum perforatum* are now successfully competing for status as a standard antidepressant therapy in the most European countries [11].

The seasonal mineral elements accumulation has been observed for the Fe, Ni and Cd during the vegetation period and the concentrations of these chemical species were more evident in leaves of *Tilia spp*. Since Cd and Pb are not involved in the metabolic activity of plants, the presence of these elements can be considered as an indicator of the environmental contamination of herbs. The measured concentrations of both Cd and Pb still secure over use of toxic metals with vegetable remedies or derivative products. Some studies pointed that for the lead content in *Tilia spp.* from Serbia were found at 17.5 mg/kg during the vegetation period, which is in accordance with a hypothesis that the passive sequestration of this toxic metal was attained in the foliage as a detoxification process [12,13].

However, the limits of tolerable daily intakes of human body are not so far from the used doses which can be accumulated during at the very intensive consumption of these remedies [14, 15].

Several studies have been carried out to determine the concentrations of the mineral element present in medicinal plants such as *Hypericum perforatum* and *Sambacus nigra L.*, melifferous tree *Tilia tomentosa* and others herbs by using the following principal techniques: atomic and mass spectrometry methods including FAAS (flame atomic absorption spectrometry) and ETAAS (electrothermal atomic absorption spectrometry) [9, 11, 15, 17–19], ICP-MS (inductively coupled plasma-mass spectrometry) [7, 12, 20], ICP-AES (inductively coupled plasma-atomic emission spectrometry) [12, 21], ICP-OES (inductively coupled plasma-optical emission spectrometry) [13], XRF (X-ray fluorescence) [22] PIXE (particle induced X-ray emission) [23] and NAA (neutron activation analysis) [24, 25]. In addition, for interpretation of the recorded data by different analytical techniques, basic statistical and multivariate procedures should be used. For instance, PCA (principal components analysis) and CA (cluster analysis) and ANOVA (analysis of variance) were used from different statistical packages [15, 16].

This paper reports the results of the study on the metal trace accumulation in the leaves and flowers of three species by the higher melliferous potential (*Tilia tomentosa* – silver linder, *Hypericum perforatum* – rattle plant and *Sambacus nigra L.* – elder) from two sites situated on the Northern Dobrudja zone. The main objectives are: (1) estimation of the mineral elements from flowers, leaves, steams, bark and soil by FAAS analysis; (2) study of the correlation between the mean concentration of K, Ca, Mg, Na, Fe, Mn, Cu, Zn, Cd, Ni in soil and their uptake in leaves and flowers collected from non-polluted and polluted sites and (3) computation of the pollution index calculation for the analysed area.

2. MATERIALS AND METHODS

The monitored sites known for their environmental favourable conditions necessary to practice beekeeping are situated near Ciucurova village, in the central part of the Tulcea County, on the Dobrudja region, România. These sites were: **site A** – "Dealul Bujorului" Natural Reservation ($44^0$57'35''N and 28°29'00''E) and **site B** – "Izvorul Cerbului" camping (44°55'35''N and 28°26'50''E) at crossing roads DN22A and DN22D (Fig. 1). The distance between them is of 5 km.

All samples were collected from non-polluted and polluted sites, in order to evaluate the natural and toxic level of inorganics from herbs. This procedure is essential in order to understand the relevance of plants' minerals content for human health. Taking into account that the pollution levels in Ciucurova area was not previously investigated, the pollution index was calculated relative to the one area considered unpolluted (zone A). The metals selected for these determinations were chosen considering their nutritional interest.

The area was chosen for this study because an increasing number of inhabitants was registered, due to the beekeeping development the last ten years, the touristic attractions and the massive forest exploitation. There are many old vehicles and trucks on the streets and sometimes leaded gasoline is still widely used. The total number of the registered vehicles increased from April to the end of October from 100 and 300 vehicles per day to 500–1000 vehicles per day.

Forests and steppe prevails in the Ciucurova zone. The forests from this area are located at altitudes of 150–250 m and contain a variety of hardwood trees (linden tree such as silver linden – Tilia tomentosa, acacia, oak, poplar, hornbeam etc). The honeybee melliferous is based to the forest area which includes the Tilia spp. (*e.g.* silver lime – Tilia tomentosa). The pastoral beekeeping is advisable to begin on April and continue until the end of July, which the period when Tilia spp. is finally blooming [27].

There are a biggest variety of medicinal herbs (Hippericum spp., Adoxaceae spp.) in closed to the forest and steppe in the studied area.

Tilia tomentosa (*Tt*) is a deciduous tree, growing up to 20–35 m tall, with a trunk up to 0.5–1 m in diameter. The leaves of this tree are alternately arranged, green and mostly hairless above and with a coarsely toothed margins. The flowers are pale yellow, have a strong scent and are pollinated by honeybees. This specie is widely grown as an ornamental tree throughout Europe. It is very tolerant of urban pollution, soil compaction, heat, and drought, and would be a good street tree in urban areas. For a given soil moisture Tilia tomentosa seedlings grow higher (40–47%) in height on leached chernozem and dark grey soils than on carbonate chernozem. The use of silver lime for intensive timber production plantations on sites with calcium carbonate concentrations in surface horizon higher than 1.5% is not advisable [27].

Hypericum perforatum (*Hp*) is a flowering plant from the Hypericaceae family. The reddish stems of this plant are branched in the upper section, and can grow up to 1 m high. The stems are woody near their base and may appear jointed from leaf scars. The leaves are yellow-green in color, with scattered translucent dots of glandular tissue. The flowers measure up to 2.5 cm across, and are colored bright yellow with conspicuous black dots. The flowers appear at the ends of the upper branches, between late spring and early to mid summer.

Sambucus nigra L. (*SnL* – known as elderberry or elder) is a complex specie belonging to Caprifoliaceae family to most of Europe. It grows in a variety of conditions including both wet and dry fertile soils, primarily in sunny locations.

Elderberry is a small tree, growing up to 6 m tall, rarely reaching 10 m tall. The bark is light grey when young, changes to a coarse grey outer bark. The leaves are arranged in opposite pairs with 10–30 cm long. Its flowers have five stamens, which are borne in large, flat corymbs 10–25 cm diameter in late spring to mid-summer, the individual flowers are ivory white, 5–6 mm diameter, with five petals; they are pollinated by bees.

The study was carried out in the period May 2019 – June 2019, when the analysed plants are in the best blooming period. In order to be statistically representative for the populations (of flowers, leaves, stems or bark and soil), the samples were collected in conformity to the statistical rules [28], as follows. All samples were collected as mixture leaves and flowers (n = 10), in the period May–June, 2019. Leaf and flower samples of Tilia tomentosa were collected from 30 trees (15 trees per site) with the age of about 20–30 years, 25–35 m tall, with big branches, thick grey-brown barks and small leaves, without mechanical or biological damages. The trees were located approximately 15 m from one other on zone A and 15–20 m from one other on zone B. At least 300 g fresh flowers and leaves without tails were collected from each tree, at a height of 2.5–3 m above the ground by evenly including all directions and cutting off at least four branches around the crown periphery of each tree. Bark samples were collected at a height of 1 m, from the trees' external surface, from four different directions, using a stainless-steel knife. All samples were washed with bidistilled water. Samples of plant tissues were dried in oven at 70°C for 24 hours. A mixer grinder was used to powder the samples while preventing overheating.

For metals concentration determination by FAAS, 0.5 of powder of dry leaves of medicinal plant has been mineralized with 5 mL nitric acid and 40 mL deionized water to 120°C for 130 minutes. The clear solutions were filtered and stored in 50 mL volumetric bottles with distilled deionized water. Metals concentration determinations by FAAS were done using a ContrAA® 700 spectrometer.

The instrument setting and operational conditions were conducted in accordance with the manufacturers' specifications. Therefore, a Certipure multi-element standard solution from Merck (1 mg/mL of each metal) was used for calibration. The following performance parameters have been determined: concentration domain (µg/L) and correlation coefficients of the calibration curve (R^2), limits of detection (LOD), limits of quantitation (LOQ) (Table 1).

Table 1

Performance parameters for FAAS measurements

Element	**Concentration domain (mg/L)**	R^2	**LOD (mg/L)**	**LOQ (mg/L)**
Potassium (K)	1.000–5.000	0.9971	3.339	29.35
Calcium (Ca)	40.000–200.000	0.9995	4.740	20.14
Magnesium (Mg)	1.000–5.000	0.9978	2.104	15.13
Sodium (Na)	5.000–25.000	0.9966	1.241	4.712
Iron (Fe)	0.050–2.000	0.9929	0.5084	0.1183
Manganese (Mn)	0.050–2.000	0.9939	0.4704	0.0597
Copper (Cu)	0.050–2.000	0.9994	0.0426	0.0586
Zinc (Zn)	0.050–1.000	0.9941	0.0423	0.1076
Cadmium (Cd)	0.050–1.000	0.9955	0.0610	0.0693

Similar procedures were applied for the component parts of *Sambacus nigra* and *Hypericum perforatum*. Determination of elemental concentrations in the samples of leaves, flowers, stems and bark were performed using the method of curve calibration according to the absorber concentration. Several solutions of different known concentrations were prepared and the elemental concentration in unknown samples was determined by extrapolation from the calibration curve. All samples' concentrations were reported as mg/kg dry weight of material.

For monitoring the atmospheric deposition of Fe, Mn, Cu, Zn and Cd and the concentration of other elements (K, Ca, Mg and Na), soil samples from the Ciucurova village area were analysed. The procedure is described in [17, 28]. Flame Atomic

Absorption Spectrometry (FAAS) was employed to determine the trace metals concentrations in the final solutions. To check the analytical precision, randomly chosen samples (about 20% of the total numbers) were measured in triplicate according to International Standard Reference Material: NIST SRM 2709, 2710 and 2711 for soil [17, 28]. Measurements of pH and conductivity were carried out in the similar procedure described in [28].

In order to evaluate the general degree of plant pollution in the studied areas, a pollution index was used. This index defines the plant contamination with heavy metals based on the sum of the ratios of the actual concentration over the alert threshold as defined by Romanian legislation for the heavy metals considered. Equation (1) shows the formula used to calculate the soil pollution index (SPI)

$$\mathrm{SPI} = \left(\frac{1}{n}\right)\sum\nolimits_{1=1}^{n} \frac{c_i}{c_{(mad)i}} \times 100 \tag{1}$$

where: n is the number of elements, C_i is the concentration of the element in soil (mg/kg d.m.), C_{mad} is the maximum concentration of an element in soil (mg/kg d.m.) [29].

To assess the overall metal accumulation by the plants, a metal accumulation index (MAI) was computed according to the formula:

$$\mathrm{MAI} = \left(\frac{1}{N}\right)\sum\nolimits_{1=1}^{n} lj \tag{2}$$

where N is the total number of analysed metals, lj is the sub-index for the variable j, obtained by dividing the mean value by its standard deviation [31].

Bioaccumulation factor (BAF) indicating the leaves/soil metal content ratio was also computed for assessing the metal uptake from soil.

3. RESULTS AND DISCUSSION

The macronutrients and minor or trace elements content found in the samples under investigation is shown in Table 2 and Table 3, respectively.

Potassium content of the studied plants is ranged between 0.438 g/kg in *Hp* flowers (area B) and 138 g/kg in *SnL* stems (area A). Similar research on the content of potassium in *Sambacus nigra L.* indicates the maximum amount of this macronutrient of 61.5 g/kg in the village Caraşova, which is situated in the south western of Romania [18]. For elderberry (*Sambacus nigra L.*), calcium content in leaves (10.463 g/kg) were close to the values determined for the same species (8.016 mg/kg) by Imbrea *et al.* [18].

Table 2

Macronutrients mean concentration in melliferous plants

Element/ Site	*Sambacus nigra*			*Hypericum perforatum*		
	Flowers	Leaves	Stems	Flowers	Leaves	Stems
	Concentration (g/kg dry plant)					
K – (A)	45.52 ± 0.41	24.81 ± 0.18	138.0 ± 1.01	0.772 ± 0.03	0.847 ± 0.2	0.678 ± 0.02
K – (B)	40.14 ± 0.62	18.64 ± 0.14	98.45 ± 1.05	0.438 ± 0.01	0.671 ± 0.01	0.836 ± 0.01
Ca – (A)	4.473 ± 0.02	10.44 ± 0.15	5.748 ± 0.16	1.262 ± 0.02	2.934 ± 0.25	3.329 ± 1.01
Ca – (B)	3.896 ± 0.01	8.57 ± 0.04	3.562 ± 0.15	0.827 ± 0.01	1.863 ± 0.51	2.874 ± 0.62
Mg – (A)	14.61 ± 0.15	8.488 ± 0.06	11.55 ± 0.21	0.235 ± 0.02	0.286 ± 0.01	0.246 ± 0.01
Mg – (B)	11.44 ± 0.18	6.463 ± 0.15	9.254 ± 0.25	0.187 ± 0.01	0.165 ± 0.02	0.268 ± 0.01
Na – (A)	0.098 ± 0.01	0.126 ± 0.01	0.103 ± 0.01	0.121 ± 0.03	0.148 ± 0.01	0.242 ± 0.02
Na – (B)	0.072 ± 0.01	0.097 ± 0.01	0.1 ± 0.01	0.094 ± 0.01	0.152 ± 0.01	0.173 ± 0.01

Element/ Site	*Tilia tomentosa*			Soil
	Flowers	Leaves	Bark	
	Concentration (g/kg dry plant)			
K – (A)	0.840 ± 0.03	2.536 ± 0.25	3.582 ± 0.46	4.05 ± 0.15
K – (B)	0.638 ± 0.01	1.042 ± 0.56	9.050 ± 0.82	3.12 ± 0.02
Ca – (A)	4.832 ± 0.82	3.764 ± 0.64	10.463 ± 0.14	71.37 ± 2.01
Ca – (B)	2.564 ± 0.41	5.652 ± 0.58	8.672 ± 0.36	20.54 ± 1.01
Mg – (A)	0.176 ± 0.01	0.544 ± 0.02	9.362 ± 0.74	15.71 ± 0.58
Mg – (B)	0.124 ± 0.01	0.358 ± 0.01	14.686 ± 0.95	14.86 ± 0.34
Na – (A)	0.145 ± 0.02	0.376 ± 0.01	0.091 ± 0.01	0.196 ± 0.01
Na – (B)	0.086 ± 0.01	0.262 ± 0.01	0.786 ± 0.01	0.201 ± 0.01

An exception is magnesium amount in *SnL* flowers which was almost five times higher than the values specified by literature (14.61 g/kg compared to 3.23 g/kg). Na content ranged from 0.072 g/kg for the *SnL* flowers in B area to 0.786 g/kg in *Tt* bark în same B zone. The obtained values for Na in *Hp* species were within the range 0.094–0.242 g/kg for flowers and stems, respectively, 0.4–0.64 mg/kg in comparison with the literature data [11]. It is known that the soil with high levels of K or Ca will typically provide less Mg to the plants. Potassium is a stronger competitor with Mg than calcium. When the soil K level is higher than desired, plant Mg levels are low. Correlations between the average content of macronutrients and medicinal herb species shows that the highest rate of absorption is in potassium case, followed by calcium, and the lowest is in magnesium case. All studied medicinal herb species contain important quantities of potassium, calcium and magnesium and should be used in human nutrition.

Several studies [17–19] have demonstrated that pH, conductivity and salinity of the soil influence the absorption and migration of different metallic ions (*e.g.* copper, cadmium, zinc etc.) from soil in the herbs. In small quantities, these metals ensure a good functioning of the plant metabolism, but are toxic in high concentration. It is well known [18] that acidic soils increase the absorption of metals by plants. In our study, the analyses of soil samples collected from Ciucurova region show that the pH is moderate basic. So, we can conclude that in the monitored area is expected a lower concentration of heavy metals in the plant samples. In this respect, high levels of heavy metals in the soil do not indicate similar high concentrations in plants (Table 3). The extent of accumulation and toxic level will depend on the perennial plant and the analyzed heavy metal.

Table 3

Minor and trace elements mean concentration in melliferous plants

Element/ Site	***Sambacus nigra***			***Hypericum perforatum***		
	Flowers	**Leaves**	**Stems**	**Flowers**	**Leaves**	**Stems**
	Concentration (mg/kg dry plant)					
Fe – (A)	95.91 ± 0.22	270.3 ± 0.43	133.2 ± 0.72	99.64 ± 0.15	126.45 ± 1.02	47.71 ± 0.81
Fe – (B)	115.4 ± 0.13	280.7 ± 0.22	150.4 ± 0.54	185.83 ± 0.26	201.37 ± 3.05	83.72 ± 0.78
Mn – (A)	27.17 ± 0.01	117.4 ± 0.32	18.12 ± 0.01	11.91 ± 0.11	254.28 ± 4.07	7.25 ± 0.31
Mn – (B)	35.74 ± 0.11	231.6 ± 0.53	32.68 ± 0.01	20.67 ± 0.71	194.62 ± 1.01	15.74 ± 0.12
Cu – (A)	0.407 ± 0.01	0.14 ± 0.01	0.001 ± 0.01	22.56 ± 0.12	0.465 ± 0.01	19.85 ± 0.25
Cu – (B)	5.495 ± 0.01	15.69 ± 0.11	1.34 ± 0.01	43.62 ± 0.51	5.602 ± 0.21	17.64 ± 0.1
Zn – (A)	38.60 ± 0.12	65.375 ± 0.23	9.85 ± 0.01	23.66 ± 0.02	30.2 ± 1.01	13.16 ± 0.2
Zn – (B)	28.225 ± 0.01	45.872 ± 0.41	6.893 ± 0.01	30.64 ± 0.32	75.62 ± 2.07	24.88 ± 0.18
Cd – (A)	0.001 ± 0.001	0.188 ± 0.01	0.001 ± 0.001	0.16 ± 0.01	0.078 ± 0.01	4.65 ± 0.04
Cd – (B)	0.083 ± 0.01	0.04 ± 0.01	0.001 ± 0.001	0.68 ± 0.01	0.84 ± 0.01	0.42 ± 0.01

Element/ Site	***Tilia tomentosa***			**Soil**
	Flowers	**Leaves**	**Bark**	
	Concentration (mg/kg dry plant)			
Fe – (A)	61.39 ± 1.01	125.647 ± 4.02	146.78 ± 1.01	489.9 ± 2.03
Fe – (B)	92.83 ± 4.02	160.76 ± 3.02	167.84 ± 2.05	625.8 ± 4.02
Mn – (A)	16.67 ± 2.01	12.072 ± 0.61	89.568 ± 1.15	454.1 ± 2.31
Mn – (B)	21.32 ± 1.05	10.65 ± 0.34	102.64 ± 3.62	275.6 ± 3.15
Cu – (A)	20.56 ± 1.23	2.587 ± 0.21	26.7 ± 0.17	2.855 ± 0.01
Cu – (B)	6.07 ± 0.21	4.362 ± 0.42	35.84 ± 0.57	35.86 ± 0.14
Zn – (A)	14.91 ± 0.16	9.073 ± 0.55	71.62 ± 1.14	156.5 ± 1.48
Zn – (B)	20.56 ± 0.18	12.47 ± 0.73	101.46 ± 2.02	286.7 ± 1.35
Cd – (A)	0.08 ± 0.01	0.765 ± 0.01	0.03 ± 0.01	1.55 ± 0.02
Cd – (B)	5.07 ± 0.2	5.64 ± 0.62	0.93 ± 0.01	1.92 ± 0.01

The high concentration obtained for Fe (280.7 mg/kg in *SnL* leaves), Mn (254.28 mg/kg in *Hp* leaves), Zn (101.46 mg/kg in *Tt* bark), Cu (43.62 mg/kg in *Hp* flowers) and Cd (5.07 mg/kg in *Tt* flowers and 5.64 mg/kg in *Tt* leaves) respectively. The recorded content of Cd in the plant is higher than the maximum level (in according by Romanian Order 756/1997) from soil (Table 4) in the analyzed touristic area. Therefore, Cd concentrations in these plants must be correlated with deposition of toxic particulate matter from the polluted air, but not with the soil concentrations. In contrast, Cu content in *Hp* flowers was correlated with the deposition and the soil content (Cu – 35.86 mg/kg).

Table 4

Romanian Order 756/1997 – level of inorganic compounds in soil (mg/kg dry matter)

Element	Method	Normal level	Maximum level	Intervention level
Fe	SR ISO 11047-99	3000	4500	7000
Mn	SR ISO 11047-99	900	2000	4000
Cu	SR ISO 11047-99	20	250	500
Zn	SR ISO 11047-99	100	700	1500
Cd	SR EN 11885-09	1	5	10

The environmental factors, including the atmospheric pollution, the plant age (depending of the season; in this case it was the top blooming period, which is the best pollination period by bees) and soil conditions (arid end of spring and summer beginning) in which plants grow influence the concentration of elements in plants. Principal sources of environmental contamination with different metals are the transport, public facilities, landfill waste and fertilizers.

This study shows that the distance from the transport routes and the traffic load intensity affects the mineral composition of the analyzed material. The melliferous plant flowers collected in the rural areas far from roads were characterized by the highest total content in macronutrients and the smallest total content of heavy metals, respectively. According to other authors, an increasing traffic flow is positively correlated with the content of Fe, Mn, Zn and Cd, and negatively correlated with Cu (Table 5).

Table 5

The Pearson correlation coefficients between the elements in melliferous plants (statistically significant values are in bold type – $\alpha < 0.05$)

zone B	K	Ca	Mg	Na	Fe	Mn	Cu	Zn	Cd
K	1.000								
Ca	0.134	1.000							
Mg	**0.799**	0.386	1.000						
Na	–0.367	0.155	–0.514	1.000					
Fe	–0.024	**0.520**	0.091	–0.047	1.000				
Mn	–0.101	0.451	0.090	–0.145	**0.809**	1.000			
Cu	–0.393	–0.315	–0.347	–0.196	0.217	–0.082	1.000		
Zn	–0.409	–0.068	–0.209	–0.089	**0.523**	**0.808**	0.097	1.000	
Cd	–0.427	0.046	–0.524	**0.547**	–0.267	–0.365	–0.268	–0.332	1.000

Our study showed a clear relationship between the content of Fe, Zn, Mn and Cd in flowers of plant and road traffic (flowers of *SnL*, *Hp* and *Tt* contained sometimes much more Fe, Mn, Zn and Cd than other analyzed elements as flowers, steam or bark of these plants). Pearson's correlation coefficients for the zone B is relatively high, some of them vary from 0.5 to 0.8. This indicates a medium correlation between pairs of the metals (Fe-Mn; Zn-Mn). However some pollution spread over much larger areas affecting almost the whole forest area near to road.

Nonparametric ANOVA for the series of elements in the plants' components are presented in Table 6. For group A, the homogeneity of the elements' distribution in the different parts of the plants can't be rejected, while for the group B it can be rejected at the significance level of 95%. So, in zone B, the pollutants' absorption in different parts of the plants is not homogenous.

Table 6

ANOVA results

Group A					
Source	**SS**	**df**	**MS**	**F**	**p-value**
Treatment	9.312	8	1.164	1.17	0.3296
Error	71.688	72	0.99		
Total	81.000	80			
Group B					
Source	**SS**	**df**	**MS**	**F**	**p-value**
Treatment	19.065	8	2.383	2.77	0.0100
Error	61.935	72	0.860		
Total	81.000	80			

To detect the inhomogeneity, the non-parametric Mann-Witney test was performed. The results show difference between the absorption of elements in the pairs (Bark3 and Stem2), (Bark3 and Flower3).

The SPI computed for the site B (24.28) is bigger than for the site A (17.62), showing a higher contamination in B. For SbL, MAI was 2.228 and 2.112, respectively. For Hp, MAI was 3.429 and 2.362, respectively. For Tt was 1.311 and 1.313, respectively. The MAI index for all the species in A zone is 1.186 and for B, 1.210, so they are not significantly different.

The highest BAF has been found for K, Na, Cu and Cd. The BAF levels calculated for Mg, Fe and Mn were above 1. Only the BAF for Zn and Ca for all analysed species were below 0.5 (Table 7).

Table 7

Bioaccumulation factor results

Element/Zone	A	B	Species
K	0.88	2.99	*Tt* bark
Na	1.92	0.46	*Tt* leaves
	1.30	3.91	*Tt* bark
Mg	0.6	0.99	*Tt* bark
Fe	0.55	0.45	*SnL* leaves
Mn	0.26	0.84	*SnL* leaves
	0.56	0.71	*Hp* leaves
Cu	7.9	1.22	*Hp* flowers
	6.95	0.49	*Hp* stems
	7.2	0.17	*Tt* flowers
	0.91	0.12	*Tt* leaves
	9.35	1.00	*Tt* bark
Cd	0.05	2.43	*Tt* flowers
	0.49	2.94	*Tt* leaves

Remark that the highest BAF values for all the elements K, Na, Cu and Cd are those for pentru *Tt* species (in flowers, leaves and bark), proving that this species is a bioaccumulator of macro and micro-elements.

4. CONCLUSIONS

The results of our study emphasized that the mean concentration of the elements in the leaves and bark of *Tilia tomentosa* didn't significantly differ. Also, no difference between the mean concentrations of the pollutants in soil, respectively the plants' tissues at the two sites was found. The linear dependence between the concentration of metal accumulation in leaves and soil, and the inexistence of such dependence between the concentration of the metal accumulation in leaves and bark suggest that the uptake mechanisms from soil to the leaves and from soil to bark of this species are different. The concentration of the analyzed elements in *Sambucus nigra, Hypericum perforatum* and *Tilia tomentosa* decreased in the following order K > Ca > Mg > Na > Fe > Mn > Zn > Cu > Cd. The results demonstrated that flowers of these species can be used as a reliable source for obtaining high quality melliferous products that complement the deficiency of major nutrients in the human diet. The analysis of the concentration factors proved that the bark acted as bioaccumulator, so it can be used for monitoring the atmospheric pollution, especially with heavy metals, in zones with intense traffic. The study demonstrate that the capacity of accumulation of *Tilia tomentosa* is superior to those of *Sambacus nigra* and *Hypericum perforatum* [20], so this tree is a good alternative to these plants and can be used for biomonitoring the pollution in urban zones. The results support the hypothesis that the traffic is one of the most important agents of pollution near the natural reservation area.

REFERENCES

1. G. Stanciu, R. Rotariu, A. Popescu, A. Tomescu, *Phenolic and mineral composition of wild chicory grown in Romania,* Rev. Chim. (Bucharest) **70**(*4*), 1173–1177 (2019).
2. A.G. Adeniyi, J.O. Ighalo, *Biosorption of pollutants by plant leaves: An empirical review*, J. Environ. Chem. Eng. **7**(*3*) 103100 (2019).
3. P. Pohl, H. Stecka, I. Sergiel, P. Jamroz, *Different aspects of the elemental analysis of honey by flame atomic absorption and emission spectrometry: A review*, Food Anal. Method **5**, 737–751 (2012).
4. S. Bogdanov, M. Haldimann, W. Luginbuhl, P. Gallmann, *Minerals in honey: environmental, geographical and botanical aspects*, J. Apicult. Research **46**(*4*), 269–275 (2007).
5. C. Cimpoiu, A. Hosu, V. Miclaus, A. Puscas, *Determination of the floral origin of some Romanian honeys on the basis of physical and biochemical properties*, Spectrochim. Acta A **100**, 149–154 (2013).
6. M. Shahid, C. Dumat, S. Khalid, E. Schreck, T. Xiong, N. K. Niazi, *Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake*, J. Hazard. Mater. **325**, 36–58 (2017).

7. D.S. Antal, D. Coricovac, C.M. Soica, F. Ardelean, I. Panzaru, C. Danciu, V. Vlaia, C. Toma, *High cadmium content in wild-growing medicinal plants from south-western Romania unexpected results of a survey on 29 species*, Rev. Chim. (Bucharest) **65**(*9*), 1122–1125 (2014).
8. U.V. Karpiuk, K.M. Al Azzam, Z.H.M. Abudayeh, V. Kislichenko, A. Naddaf, I. Cholak, O. Yemelianova, *Qualitative and Quantitative Content Determination of MacroMinor Elements in Bryonia Alba L. Roots using Flame Atomic Absorption Spectroscopy Technique*, Adv. Pharma. Bull. **6**(*2*), 285–291 (2016).
9. R. Chizzola, *Metallic mineral elements and heavy metals in medicinal plants*, Med. Aromat. Plant Sci. Biotehnol. **6**, 39–53 (2012).
10. World Health Organization (WHO), Quality control methods for medicinal plant materials, 2011; https://apps.who.int/iris/handle/10665/44479 (accessed on 5 November 2019)
11. M.R. Gomez, S. Cerutti, R.A. Olsina, M.F. Silva, L.D. Martinez, *Metal content monitoring in Hypericum perforatum pharmaceutical derivatives by atomic absorption and emission spectrometry*, J. Pharm. Biomed. Anal. **34**, 569–576 (2004).
12. M. Tomasevic, M. Anicic, *Trace element content in urban tree leaves and SEM – EDAX characterisation of deposited particles*, Facta Universitatis **8**(1), 1–13 (2010).
13. M. Anicic, T. Spasic, M. Tomasevic, S. Rajsic, M. Tasic, *Trace elements accumulation and temporal trends in leaves of urban deciduous trees (Aesculus hippocastanum and Tilia spp.*), Ecol. Indic. **11**, 824–830 (2011).
14. P. Konieczynski, A. Arceusz, M. Wesolowski, *Essential elements and their relations to phenolic compounds in infusions of medicinal plants acquired from different European regions*, Biol. Trace Elem. Res. **170**(*2*), 466–475 (2016).
15. A. Arceusz, M. Wesolowski, I. Radecka, *Macro- and micro- elements in some herbal drug raw materials and their water extracts consumed in Poland*, Cent. Eur. J. Chem. **9**(*5*), 917–924 (2011).
16. L.D. Vieira, K. Takeda Da Silva, R.S. Giarola, G.F. Inocente, H. Kushima, C.A.H. Lima, J.M. Hormaza, Peerj **6**, 2012; Doi: 10.7717/Peerj.5375
17. C. Radulescu, C. Stihi, I.V. Popescu, I. Ionita, I.D. Dulama, A. Chilian, O.R. Bancuta, E.D. Chelarescu, D. Let, *Assessment of heavy metals level in some perennial medicinal plants by flame atomic absorption spectrometry*, Rom. Rep. Phys. **65**(*1*), 246–260 (2013).
18. I.M. Imbrea, I. Radulov, A.L. Nicolin, F. Imbrea, *Analysis of macroelements content of some medicinal and aromatic plants using flame atomic absorption spectrometry (FAAS)*, Rom. Biotechnol. Lett. **21**(*4*), 11642–11649 (2016).
19. P. Konieczynski, M. Wesolowski, *Determination of zinc, iron, nitrogen and phosphorus in several botanical species of medicinal plants*, Pol. J. Environ. Stud. **16**(*5*), 785–790 (2007).
20. A.M.C. Salvador, *Chemical characterization and biological evaluation of Sambucus nigra L. berries and flowers in view of their valorization*, PhD Thesis, 2017 https://www.semanticscholar.org/paper/chemical-characterization-and-biological-evaluation-salvador/064e1827139cdbc8174da9fd7a407f318f66f449 (accesed on 5 November 2019).
21. B. Kolodziej, N. Maksymiec, K. Drojdjal, J. Antonkiewicz, *Effect of traffic pollution on chemical composition of raw elderberry (Sambucus nigra L.)*, J. Elem. S. 67–78 (2012); DOI: 10.5601/Jelem.2012.17.1.06
22. I. De La Calle, M. Costas, N. Cabaleiro, I. Lavilla, C. Bendicho, *Fast method for multielemental analysis of plants and discrimination according to the anatomical part by total reflection X-ray fluorescence spectrometry*, Food Chem. **138**(*1*), 234–241 (2013).
23. R. Venkateswara, P. Venkateswarulu, C. Kasipathi, S. Sivajyothi, *Trace elemental analysis of Indian natural moonstone gems by PIXE and XRD techniques*, Appl. Radiat. Isotopes **82**, 211–222 (2013).
24. I. Fatima, S. Waheed, J.H. Zaidi, *Elemental analysis of Anethum gravedlens*, *Sismbrium Irio Linn and Veronia Anthelmintica seeds by instrumental neutron activation analysis*, Appl. Radiat. Isotopes **71**(*1*), 57–61 (2013).
25. S. Waheed, I. Fatima, *Instrumental neutron activation analysis of Emblica officinalis*, *Terminalia belerica and Terminalia chebula for trace element efficacy and safety*, Appl. Radiat. Isotopes **77**, 139–144 (2013).

26. P. Konieczynski, M. Wesolowski, *Phytate, inorganic and total phosphorus and their relations to selected trace and major elements in herbal teas*, Acta Pol. Pharm. **71**(*1*), 85–93 (2014).
27. D. Jitariu, M. Popescu, I. Moise, C. Urdea, *Rural sustainable development opportunity by exploiting the melliferous resources – Case study: the Ciucurova village, Tulcea county, Romania,* J. Environ. Prot. Ecol. **15**(*3*), 1074–1085 (2014).
28. L. Barbeş, A. Bărbulescu, *Monitoring and statistical assessement of heavy metals in soil and leaves of Populus Nigra L.*, Environ. Eng. Manag. J. **16**(*1*), 187–196 (2017).
29. S. Lupşor, G. Stanciu, D. Epure, E. Chirila, *Heavy metals and pesticides analysis from Black Sea algae*, in: L.I. Simeonov, M. A. Hassanien (eds.), *Exposure and Risk Assessment of Chemical Pollution – Contemporary Methodology*, Book Series: Nato Science for Peace and Security Series C-Environmental Security, 2009, pp. 431–437.
30. G. Stanciu, S. Lupşor, *Detection of heavy metals and organic pollutants from Black Sea marine organisms*, in: L. Simeonov, E. Chirila (eds.), *Chemicals as Intentional and Accidental Global Environmental Threats*, Book Series: Nato Science for Peace and Security Series C-Environmental Security, 2006, pp. 451–454.
31. A. Nadgórska-Socha, M. Kandziora-Ciupa, M. Trzęsicki, G. Barczyk, *Air pollution tolerance index and heavy metal bioaccumulation in selected plant species from urban biotopes*, Chemosphere **183**, 471–482 (2017).