BIOCHEMICAL DIVERSITY ANALYSIS BASED ON MINERAL CONTENT IN THYME (*THYMUS* L.) POPULATIONS

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Abstract. *It is well known the importance of studying the mineral content of plant species, as essential and indispensable nutritional components for their good physiological functioning and going along the trophic chain, of animals and humans, based on the content of mineral elements. As a result of the demographic growth, in the last period of time, the concerns regarding the identification of new food resources, on the one hand, and the establishment of sustainable principles for the management of the existing ones, on the other hand, have increased. Thyme, like many other genera belonging to the Lamiaceae family, has economic and pharmaceutical importance, being intensively studied from a chemical point of view. In the paper, the results regarding the content in microelements (Fe, Zn, Mn, Cr, Cu, Ni) are analyzed in 17 wild populations of thyme, compared with a cultivated population (Th. vulgaris). The samples were analyzed using the atomic absorption spectroscopy method (AAS). The iron content varied widely, depending on the place of origin and the analyzed species, reaching maximum values in the cultivated population Th. vulgaris (486 mg/kg), exceeding the wild populations, with only one exception, Th dacicus harvested from the Lescovita area (564 mg/kg). Likewise, the chromium content for the cultivated population, reached high values (0.849 mg/kg), compared to the wild populations, being surpassed only by Th. pulegioides (1.250 mg/kg), collected from the Pojejena area. For all the other microelements analyzed, the values recorded for the cultivated population, did not exceed the values of the wild populations.*

Key words: *microelements, atomic absorbion spectroscopy (AAS), medicinal plants*

INTRODUCTION

Mineral elements are inorganic compounds necessary for the optimal functioning of plants, animals and humans. They are needed in small quantities, which differ both according to the nutrient and according to the species (PRODAN *et al*., 2010; TOMESCU *et al*., 2015). Essential micronutrients were found as constituents in over 1500 proteins where they fulfill catalytic, activating, coactivating and structural functions (HÄNSCH&MENDEL, 2009).

Among microelements, iron (Fe), is known to be of great structural and functional importance. In plants it is involved in photosynthesis, being present in chloroplasts but also in mitochondria, the respiratory centers of the cell (SOETAN *et al*., 2010; TRIPATHI *et al*., 2015).

In the human body, it is indispensable for red blood cells, being a component of hemoglobin, with a role in transport. Iron is necessary for the production of red blood cells, but it is also part of hemoglobin being bound to oxygen and thus facilitating the transport of oxygen from the lungs through the arteries to all cells throughout the body (MAGGINI *et al*., 2018; CIOBOTARU *et al*., 2017).

Copper (Cu) is one of the most important elements for the proper functioning of organisms, along with iron. Copper fulfills a multitude of cellular roles, of which we mention only a few: it is essential for photosynthesis and mitochondrial respiration, for carbon and nitrogen metabolism, for oxidative stress protection, and is necessary for the synthesis of the cell wall. Copper has an essential role in photosynthesis, being present in chloroplasts in a high percentage (over 70%). It is an activator and cofactor for numerous enzymes, it is also involved in protein synthesis. Copper also plays a role in mitochondrial respiration, a role in the metabolism of carbon and nitrogen, it intervenes in the synthesis of the cell wall, etc. (HÄNSCH&MENDEL, 2009; CRISTA&RADULOV, 2009)

Manganese (Mn) is found in plants, especially in chloroplasts among the organelles, being involved in photosynthesis but also in ensuring the structure and functionality of chloroplast membranes. It also intervenes in the activation of certain enzymes involved in the Krebs cycle, the absorption of potassium, copper, iron, etc. (JAVED *et al*., 2013; GÜRGAN-ESER&ADILOĞLU, 2022). In the human body, manganese fulfills numerous tasks, of which we mention a few: it is involved in the metabolism of proteins, lipids and carbohydrates, it favors liver and kidney functions; accelerates burning, helps to fix calcium, iron, as well as vitamins; has a role in bone formation; activates a large number of enzymes (TRIPATHI *et al*., 2015; FIGAS *et al*., 2021).

Zinc in the plant body is an activator of numerous enzymes, it plays an important role in the biosynthesis of chlorophyll, starch, proteins, certain vitamins, but also in the development of seeds. In the human body, it ensures the proper functioning of the central nervous system (CRISTA&RADULOV, 2009; VASIL'EVA *et al*., 2022). Over 1200 proteins are formed by zinc-proteins , with transcription factors as major subgroup (HÄNSCH&MENDEL, 2009).

MATERIAL AND METHODS

Biological material

The analyzed populations were harvested during the flowering period (May-June, 2019), as presented in previous studies (IMBREA *et al*., 2012; BEICU *et al*., 2023). The taxonomic determination was made on the basis of plant identification books (*Flora of Romania vol. VIII*, GUȘULEAC,1961; *Illustrated Flora of Romania*, CICÂRLAN, 2009; *Vascular plants from Romania*, SÂRBU *et al*., 2013).

Samples voucher for each population were stored after identification in the herbarium of the Botany Department within the University of Life Sciences "King Mihai I" from Timișoara.

In the present paper, seventeen wild populations of thyme and one cultivated population, were analyzed. The collection areas of the analyzed samples mostly came from Caraș-Severin county. Only the samples harvested from Silagiu and the cultivated sample originating from Lovrin, are found in Timiș county, as shown in table 1.

Determination of total mineral content

Samples of dry biological material were weighed for each studied population (500mg), placed in numbered ceramic vessels. The ceramic vessels were placed in a calciner (Nabertherm LE2/11) at 600ºC for 10 hours to obtain ash. The resulting ash should have a uniform color (white or gray) and should not contain black coal dots (BEICU *et al*., 2023).

Determination of mineral elements by atomic absorption spectroscopy (SAA)

The microelements were determined by atomic absorption spectrophotometry (Varian 220 FAA spectroscopic equipment) through an official method certified by the AOAC (Association of Official Analytical Chemists) at the wavelength specific to each element, according to the specific methodology, as presented in previous studies (BEICU *et al*., 2023).

RESULTS AND DISCUSSIONS

For each plant sample collected, the calcined mass was calculated. The percentage of total mineral substances, relative to the dry plant sample, was determined. The average concentration for the eighteen populations studied was 6.33±2.24% (table 1).

Table 1

Content of macroelements in the analyzed *Thymus* populations

Sample	Population	Harvesting place	Fe (mg/kg)	Zn (µg/kg)	Mn (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)
P1	*Th. comosus*	Dobraia	117.892	0.025	36.884	0.062	5.153	0.207
P2	*Th. dacicus*	Coronini	367.528	0.040	19.814	0.229	6.257	0.816
P3	*Th. dacicus*	Lescovița	564.238	0.028	24.258	-	6.613	0.824
P4	*Th. glabrescens*	Silagiu	8.721	0.041	20.185	0.044	7.695	0.821
P5	*Th. dacicus*	Ostrov	154.004	0.019	40.395	0.547	2.415	-
P6	*Th. pannonicus* ssp. *auctus*	Silagiu	423.854	0.079	144.901	1.547	10.678	1.856
P7	*Th. praecox* ssp. *janke*	Domogled	158.360	0.043	12.727	0.572	4.481	-
P8	*Th. praecox* ssp. *janke*	Coronini	275.951	0.029	8.798	0.773	5.010	0.567
P9	*Th. praecox* ssp. *polytrichus*	Semenic	146.914	0.048	220.038	0.651	7.156	2.836
P10	*Th. praecox* ssp. *polytrichus*	Gozna	166.113	0.047	203.516	0.527	10.258	2.291
P11	*Th. pulegioides* ssp. *chamaedrys*	Pojejena	48.734	0.020	86.257	0.380	0.809	-
P12	*Th. pulegioides* ssp. *chamaedrys*	Carașova	114.405	0.031	105.112	0.502	3.028	-
P13	*Th. pulegioides* ssp. *montanus*	Pojejena	340.897	0.039	188.561	1.250	2.892	1.437
P14	*Th. pulegioides* ssp. *montanus*	Carașova	281.738	0.038	34.043	0.445	18.965	0.195
P15	*Th. pulegioides* ssp. *pulegioides*	Carașova	87.667	0.025	94.087	0.049	2.955	-
P16	*Th. pulegioides* ssp. *pulegioides*	Nermet	66.092	0.043	15.632	0.431	6.408	-
P17	*Th. pulegioides* ssp. *pulegioides*	Văliug	65.952	0.049	50.067	0.040	8.383	0.760
P18	*Th. vulgaris*	Lovrin	486.103	0.027	32.349	0.849	5.544	-

A heterogeneous distribution of mineral elements was observed in the populations studied, the distribution of microelements by species corresponds to the data from the literature (KADIFKOVA-PANOVSKA *et al.*, 1997; KUCUKBAY& KUYUMCU, 2010; TOMESCU *et al.*, 2015; VASIL'EVA *et al.*, 2022).

At the individual level, the maximum amount of iron (Fe), was detected in the population of *Th. dacicus* from the Lescovita area (564.238 mg/kg), and the minimum amount was detected in the population of *Th. glabrescens* harvested from the Silagiu area (8.721mg/kg) (figure 1).

The maximum amount of zinc (Zn), at the individual level, was detected in the population of *Th. pannonicus ssp. auctus* from the Silagiu area (0.079 µg/kg), and the minimum amount was detected in the population of *Th. dacicus* from the Ostrov area (0.019µg/kg) (figure 2).

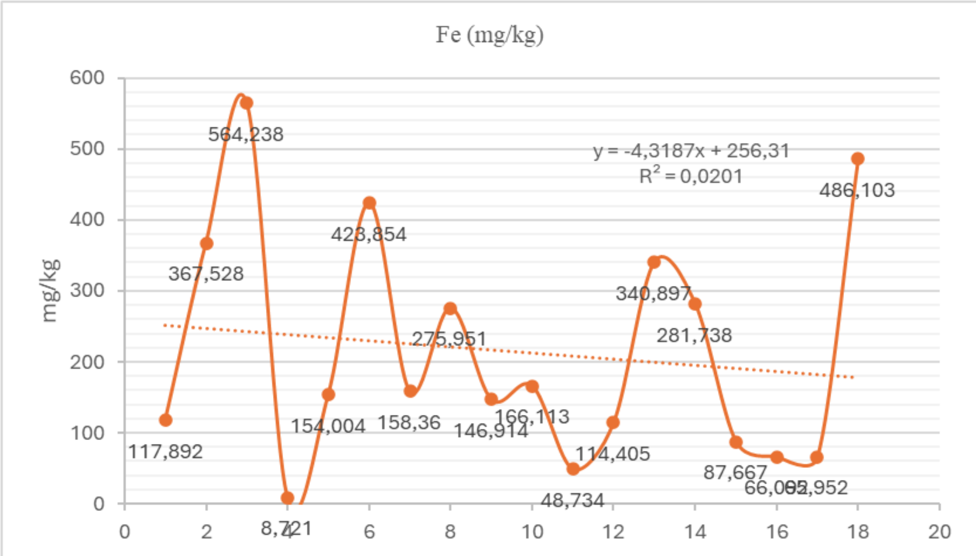

Figure 1. Distribution of iron (Fe) in the analyzed *Thymus* populations

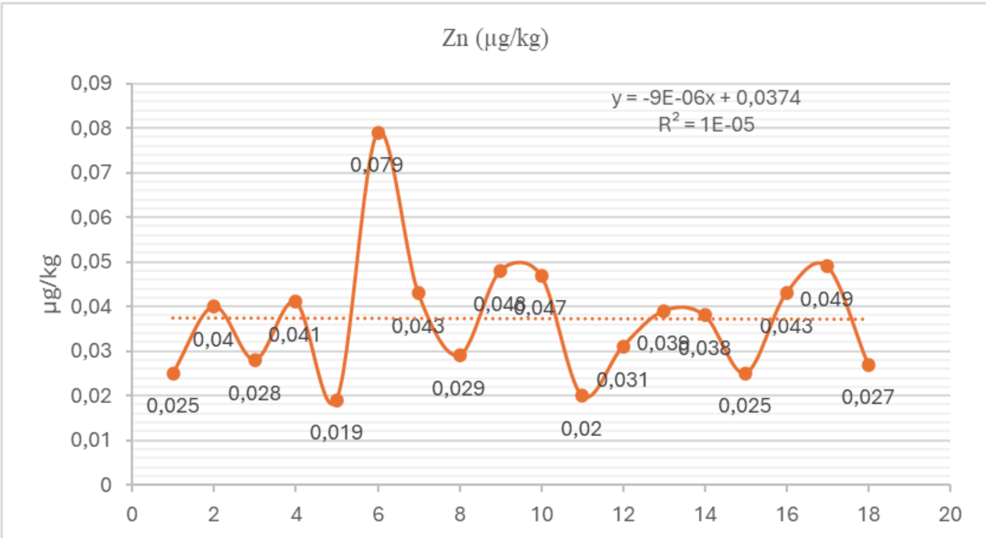

Figure 2. Distribution of zinc (Zn) in the analyzed *Thymus* populations

For manganese (Mn), the maximum amount was detected in the population of *Th. praecox ssp. polytrichus*, from the Semenic area (220.038 mg/kg), and the minimum amount was detected in the population of *Th. praecox ssp. janke*, from the Domogled area (8.798mg/kg) respectively in the population of *Th. praecox ssp. janke* from the Coronini area (12.727 mg/kg) (figure 3). It is observed that within the same species, depending on the subspecies and the place of harvest, the values change between minimum and maximum.

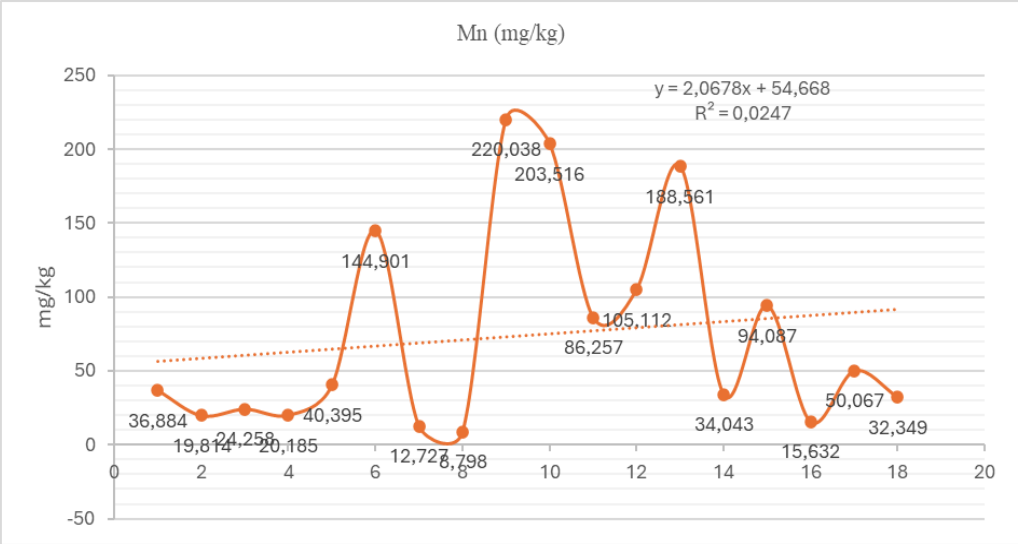

Figure 3. Distribution of manganese (Mn) in the analyzed *Thymus* populations

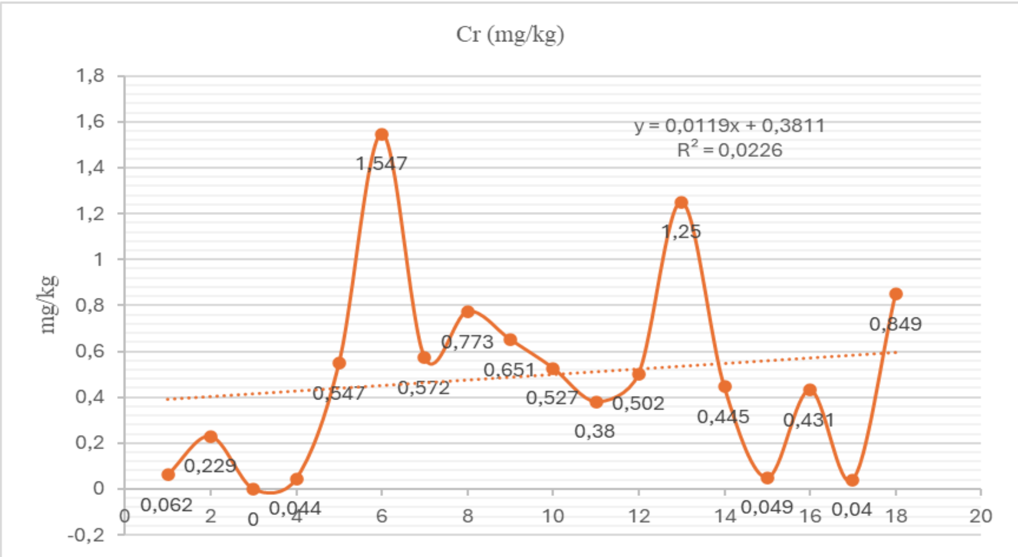

Figure 4. Distribution of chromium (Cr) in the analyzed *Thymus* populations

Like iron, the maximum amount of chromium (Cr), was detected in the population of *Th. pannonicus ssp.* auctus from the Silagiu area (1.547 mg/kg), and the minimum amount was detected in the population of *Th. pulegioides ssp. pulegioides* from the Nemet area (0.040mg/kg). In the population of *Th. dacicus* from the Lescovita area, chromium was below the detection limit and could not be determined (figure 4).

The maximum amount of copper (Cu) was detected in the population of *Th. pulegioides ssp. montanus* from the Carașova area (564.238 mg/kg), and the minimum amount was detected in the population of *Th. pulegioides ssp. chamaedrys* from the Pojejena area (0.809 mg/kg) (figure 5).

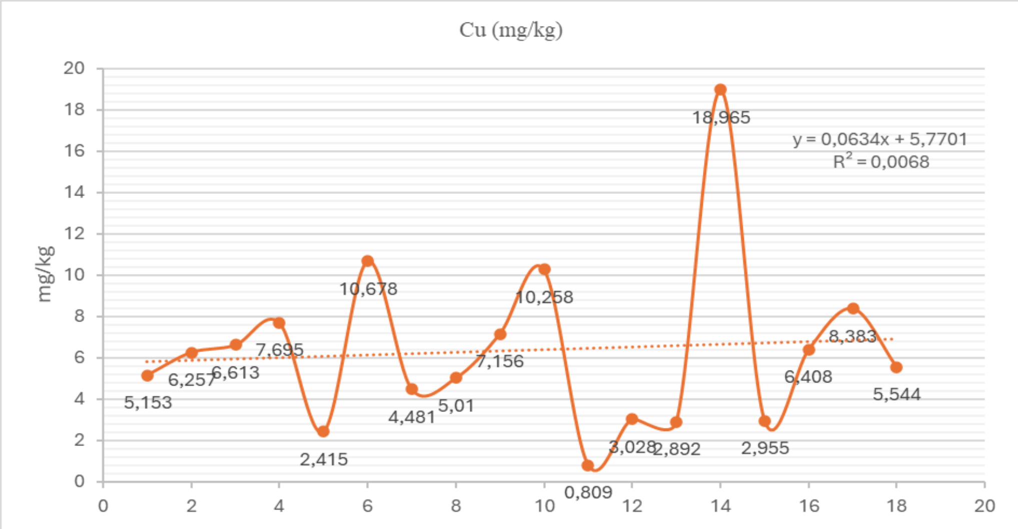

Figure 5. Distribution of copper (Cu) in the analyzed *Thymus* populations

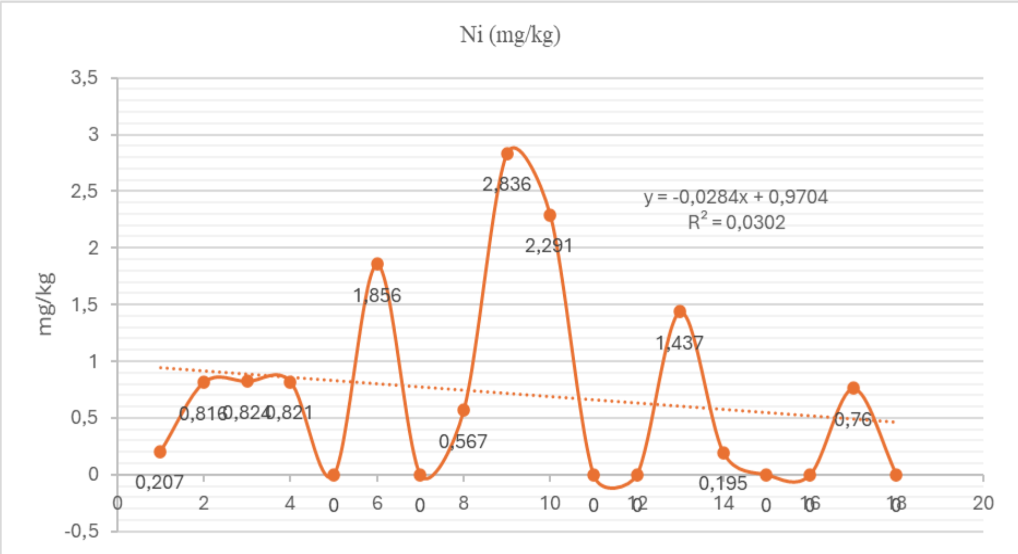

Figure 6. Distribution of nickel (Ni) in the analyzed *Thymus* populations

Regarding the maximum amount of nickel (Ni), it was detected in the population of *Th. praecox ssp. polytrichus*, from the Semenic area (2.836 mg/kg), and the minimum amount was detected in the population of *Th. pulegioides ssp. montanus* from the Carașova area (0.195mg/kg) (figure 6).

In populations of *Th. vulgaris* (Lovrin), *Th. pulegioides ssp. chamaedrys* (Carașova, Pojejena), *Th. pulegioides ssp. pulegioides* (Carașova, Nermet), *Th. dacicus* from the Ostrov area and *Th. praecox ssp. janke* from the Domogled area, nickel was below the detection limit and could not be determined.

CONCLUSIONS

The microelements identified in the eighteen *Thymus* populations studied were the following: iron (Fe), with an average value of 215.287±162.819 mg/kg; zinc (Zn), with an average value of 0.037±0.014 µg/kg; manganese (Mn), with an average value of 74.312±70.253 mg/kg; chromium (Cr), with an average value of 0.523±0.417 mg/kg; copper (Cu), with an average value of 6.372±4.115 mg/kg and nickel (Ni), with an average value of 1.146±0.857 mg/kg.

We can observe the influence of the place of harvest, the chemical composition of the soil as well as the interconnection between the mineral elements, on the content of microelements in thyme population, as mentioned in previous studies.

The research results are relevant for further studies that could develop new chemotaxonomy tools at the species level.

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