UDK 635.71:661.482]:543.554 **Original Scientific Paper**
Doi 10.7251/SANUS2503351G

ANALYSIS OF MICRO AND MACRO ELEMENTS IN ROSEMARY SAMPLES BY ICP-OES

***Vesna Gojković Cvjetković*[1], *Željka Marjanović-Balaban*[2], *Vesna Antunović*[3], *Dijana Mihajlović*[4], *Marko Ivanović*[1], *Jelena Vuković*[1], *Milomirka Obrenović*[1], *Kristina Đerić*[1], *Milenko Smiljanić*[1]**

[1]University of East Sarajevo, Faculty of Technology Zvornik, Republic of Srpska, Bosnia and Herzegovina

[2]University of Banja Luka, Faculty of Forestry, Banja Luka, Republic of Srpska, Bosnia and Hergegovina

[3]University of Banja Luka, Faculty of Medicine, Banja Luka, Department of pharmacy, Republic of Srpska, Bosnia and Herzegovina

[4]University of Banja Luka, Faculty of Agriculture, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

***Abstract**. Rosemary (Rosmarinus officinalis) is a Mediterranean plant that is used not only as a decoration, but also as a spice and has medicinal properties. This plant contains a lot of medicinal substances, such as vegetable fats, plant hormones, vitamin C, enzymes, essential oils, tannins, heterosides, choline, and cineole. However, the presence of some heavy metals, even in small concentrations, can be toxic and lead to serious diseases. In this paper, the determination of micro and macro elements in rosemary samples from Herzegovina region was carried out. Also, the content of elements in soil samples from which rosemary was taken for analysis was determined. Sample preparation was carried out by wet digestion. The concentration of selected metals in solutions after digestion was determined using inductively coupled plasma optical emission spectrometry (ICP-OES).*

Based on the obtained results, the highest concentration of macro elements was measured for Ca and is 11508.74±37.33 mg/kg, and the lowest concentration of macro elements was measured for Na and is 387.77±1.64 mg/kg. The measured concentrations of heavy metals (As, Cd, Pb) are below the detection limit. The concentration of micro and macro elements in soil samples is below the maximum permitted concentrations.

***Key words:** macro and micro elements, rosemary, ICP-OES*

Introduction

Rosemary (*Rosmarinus officinalis*) is a plant that originated in the Mediterranean, but is now cultivated worldwide. This aromatic plant has dark green needle-like leaves and blue flowers. It is used in human nutrition as a spice [1, 2]. Spices are dried parts of plants that are used in a certain form to achieve a better aroma and improve the acceptability of food [3].

In addition to its culinary uses, rosemary has a long history of medicinal importance in traditional medicine due to its many medicinal properties. It is used to improve memory, relieve pain, stimulate hair growth, and improve circulation. Rosemary is

rich in antioxidants that help neutralize free radicals and reduce oxidative stress in the body. This supports overall cellular health and helps prevent chronic diseases [4, 5].

The active ingredients of rosemary also have anti-inflammatory properties that help reduce the risk of chronic inflammation. It improves blood circulation, supports heart health and contributes to the general well-being of blood vessels. Rosemary also stimulates bile secretion, facilitates the digestion of fats and improves digestion, thereby supporting the health of the gastrointestinal tract. It also has a positive effect on the immune system, helping the body fight infections and maintain general resistance [6, 7, 8].

Rosemary has properties that allow it to fight against certain bacteria, supporting hygiene and health, and has a beneficial effect on regulating blood sugar levels, which is useful for people with diabetes. It is used in various ways, such as tea, tincture and oil. In addition to having many benefits, spices can also contain some toxic substances that come from the environment, their production, processing and storage conditions [9, 10]. Contamination of spices with heavy metals can occur during the cultivation of plant species, as well as during the production and packaging processes [11].

Essential metals (Cu, Zn, Cr, Fe, and Co) are required in a small amounts for the proper functioning of enzymatic system, the formation of hemoglobin and the synthesis of vitamins, as well as for growth, development and photosynthesis in plants. On the other hand, toxic metals and metalloids (Pb, Cd, As, Hg) that are not needed by the human body can cause harmful effects on human health [12, 13].

The aim of this work was to analyze micro and macro elements in rosemary samples collected from Herzegovina and also to analyze the presence of micro and macro elements in soil samples from which rosemary was collected. The determination of micro and macro elements was performed using inductively coupled plasma optical emission spectrometry (ICP-OES).

Material and method

Sample preparation

Sample of spices (rosemary) were obtained from the area of Herzegovina (Gacko). Also, the soil on which rosemary grows was sampled from this area. The samples were prepared by first weighing 1.000 g of the sample. Then, wet digestion was performed with 30 ml of 65% HNO_3 in vessels by heating for 12 hours at a temperature of 90 °C. After cooling to room temperature, the samples were diluted with redistilled water, and then filtered through filter paper (syringe filters, Filtratech, France) in a 50 ml volumetric flask and made up to the mark with redistilled water [14]. Three repetitions were performed for each analysis. A blank sample was prepared in the same way for both.

Analysis of micro and macro elements in rosemary samples

The quantitative evaluation of all samples was carried out using ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry, Agilent Technologies 5100). The settings of the instrument and the parameters measured are as follows: plasma power

(1400 W), gas flow: coolant (13 L/min), auxilary (0.80 L/min), type of nebulizer (cross flow), nebulizer flow rate (0.95 L/min), pump speed: 30 and plasma observation: axial. Three replicates were performed to ensure the results were as accurate and precise as possible. The limit of detection for: Ag (4 μg/kg), Al (6 μg/kg), B (5 μg/kg), Cd (0.2 μg/kg), Co (3 μg/kg), Cr (1 μg/kg), Fe (2 μg/kg), Li (6 μg/kg), Mn (0.4 μg/kg), Ni (2 μg/kg), Pb (5 μg/kg), Zn (1 μg/kg), As (14 μg/kg), Cu (3 μg/kg), P (50 μg/kg), Sr (0.6 μg/kg), Ca (3 μg/kg), K (20 μg/kg), Mg (1 μg/kg), Na (20 μg/kg), Be (0.1 μg/kg) and B (5 μg/kg).

Results and discussion

The concentrations of Ag, Al, B, Cd, Co, Cr, Fe, Li, Mn, Ni, Pb, Zn, As, Cu, P, Sr, Ca, K, Mg, Na, Be, and B were analyzed in rosemary samples collected from the Herzegovina region.

Table 1 presents the detected metal concentrations in these rosemary samples. The observed concentrations were evaluated against the allowable limits.

Table 1. Concentration of micro and macro elements in rosemary samples

Elements	Concentration (mg/kg)	Literary values (mg/kg)
Ag	<LD	NA
Al	34.28 ±1.38	NA
B	<LD	< 20 mg [15]
Cd	<LD	NA
Co	<LD	NA
Cr	1.86 ±0.40	6.0 [16]
Fe	59.88±0.96	10-30 mg/day [15]
Li	<LD	NA
Mn	14.41±0.06	8.3-38.9 [16]
Ni	<LD	4.80 [16]
Pb	<LD	1.00 [17]
Zn	10.15±0.29	15 mg/day [15]
As	<LD	NA
Cu	8.42 ±1.25	3.1-9.2 [16]
P	1781.59±10.12	900 mg/day [15]
Sr	23.47±0.11	NA
Ca	11508.74 ±37.33	1200 mg/day [15]
K	8164.44±2.09	3100 mg/day [15]
Mg	2172.84±10.86	350 mg/day [15]
Na	387.77±1.64	500 mg/day [15]
Be	<LD	NA
Ba	8.93±0.08	NA

NA – not available

Calcium, potassium, magnesium, and sodium are classified as macroelements and are readily absorbed by the body. The concentration of calcium in the rosemary samples was the highest, measuring 11508.74±37.33 mg/kg. The recommended daily intake of Ca is 1200 mg/day [15].

Following calcium, potassium exhibited the next highest concentration at 8164.44±2.09 mg/kg. The recommended daily intake of K is 3100 mg/day [15]. Potassium plays a crucial role in maintaining the acid-base balance within the body, as well as regulating osmotic pressure. Additionally, it influences carbohydrate metabolism. Magnesium plays a crucial role in regulating protein synthesis, blood pressure, as well as muscle and nerve functions. Additionally, it is essential for active transport across the cell membrane. The magnesium concentration in rosemary samples was measured at 2172.84±10.86 mg/kg. The advised daily intake of magnesium for adults is approximately 350 mg/day. In the human body, sodium contributes to water metabolism, muscle contraction, and facilitates the transport of carbon dioxide to the lungs. The sodium concentration was recorded at 387.77±1.64 mg/kg.

Zinc is a trace element that is necessary for both the treatment of mental illnesses and normal brain function. Prostate function, protein and glucose metabolism, and many other biological processes also depend on it. Alkaline phosphatase, alcohol dehydrogenase, carbonic anhydrates, lactate dehydrogenase, superoxide bismutase, RNA, and DNA polymerase are among the metalloenzymes whose depends on zinc. In this investigation, the zinc content was 10.15±0.29 mg/kg. Adults are advised to consume 15 milligrams of zinc each day.

Iron plays a crucial role in the function of certain enzymes that produce energy. In this study, the iron concentration was measured at 59.89±0.96 mg/kg. The recommended daily intake of Fe is 10-30 mg/day.

Phosphorus plays a role in maintaining acid-base balance in the body. Phosphoric acid residues are also part of nucleotides, i.e. DNA and RNA. The measured phosphorus concentration in rosemary samples is 1781.59±10.12 mg/kg. The recommended daily intake of P is 900 mg/day.

Copper, in conjunction with zinc, plays a crucial role in preserving the elasticity of fibers, thereby enhancing the support for the skin’s structure. A deficiency in copper can lead to anemia, hair depigmentation, and bone deformities. Furthermore, a diet lacking in specific essential elements, including zinc, iron, and copper, may result in the increased accumulation of certain toxic metals such as cadmium or lead.

Manganese is important for bone formation, lipid metabolism, energy production and nucleotide synthesis. The measured concentration in rosemary samples is 14.41±0.06 mg/kg.

Aluminium serves as a cofactor in the attachment of guanine nucleotides, which is crucial for protein metabolism. Elevated concentrations of this element in the kidneys of patients harm the skeletal system by adversely influencing the process of bone formation, resulting in osteomalacia. The measured concentration of this element in rosemary samples is 34.28±1.38 mg/kg.

Heavy metals are categorized as a distinct group of elements recognized as some of the most hazardous inorganic pollutants in the environment. They are non-biodegradable and have toxic effects even at minimal concentration. Certain heavy metals are classified as trace microelements, as they can also be toxic when present in elevated concentrations. The accumulation of heavy metals in plants is influenced by their overall content in the soil, their affinity, and the interactive effects of various soil characteristics. According to the measured concentrations (As, Cd, Pb), the detected levels for these elements are below the detection limit.

Huremović et al. [14] determined the content of heavy metals in spices from the market in Sarajevo, Bosnia and Herzegovina. Based on the obtained results, the measured concentrations of Cr, Cu, Mn, Ni, Fe, and Zn were within the range of normal values for the mentioned metals.

Savić et al. [18] determined the mineral content of spice samples using the ICP-OES method. Based on the obtained results, it was shown that the analyzed spice samples (curcuma, anise, cinnamon, ginger, coriander, sesame, chili, curry) are a good source of calcium, sodium, potassium and magnesium and represent an important source of nutrients for humans.

Based on the obtained results in this paper, it can be seen that they are in agreement with Huremović et al. [14] and Savić et al. [18].

Table 2 shows the measured concentration of micro and macro elements (mg/kg) in soil samples (pH=7.14, temperature 24 °C) from which rosemary from the Herzegovina area was samples.

Table 2. Concentration of micro and macro elements in soil samples

Elements	Concentration (mg/kg)	Maximum allowed concentrations (mg/kg soil) [19]
Cu	88.66±1.21	to 100
Mn	288.52±2.25	NA
Zn	164.52±0.59	to 300
Cd	2.41±0.10	to 3
Pb	68.03±1.06	to 100

NA – not available

Based on the obtained results (Table 2), it can be seen that the measured concentration of micro and macro elements (Cu, Mn, Zn, Cd, Pb) from soil samples from the Herzegovina area does not exceed the maximum permitted concentrations, according to the Regulation on permissible quantities of hazardous and harmful substances in soil and irrigation water and method of their testing [19].

Conclusion

Based on the analysis of micro and macro elements in rosemary samples using the ICP-OES method, the following conclusions were reached. Rosemary samples contain micro and macro elements in different concentrations, which in most cases do not exceed the permissible concentration. Of the macro elements, the highest

concentration was measured for Ca and is 11508.74±37.33 mg/kg, and the lowest for Na and is 387.77±1.64 mg/kg. The obtained results showed that rosemary samples represent a good source of Ca, K, Mg, and Na. The measured concentration of heavy metals (As, Cd, Pb) is below the detection limit. As for the measured concentration of micro and macro elements in the soil samples from which rosemary was collected, they do not exceed the maximum permissible concentration.

Funding. This work is a part of the research project "Analysis of the presence of heavy metals in spices and medicinal herbs from the area of Herzegovina", financed by the Ministry of Science and Technology Development and Higher Education of the Republic of Srpska.

References

[1] Andrade JA, Faustino C, Garcia C, Ladeiras D, Reis CP, Rijo P. *Rosmarinus officinalis* L.: an update review of its phytochemistry and biological activity. Future Sci OA. 2018;4(4):FSO283. https//doi.org/10.4155/fsoa-2017-0124.

[2] Jardak M, Elloumi-Mseddi J, Aifa S, Mnif S. Chemical composition, anti-biofilm activity and potential cytotoxic effect on cancer cells of *Rosmarinus officinalis* L. essential oil from Tunisia. Lipids Health Dis. 2017;16(1):190. https//doi.org/ 10.1186/s12944-017-0580-9.

[3] De La Torre JE, Gassara F, Kouassi AP, Brar SK, Belkacemi K. Spice use in food: Properties and benefits. Crit Rev Food Sci Nutr. 2017;57(6):1078-1088. https//doi.org/10.1080/10408398.2013.858235.

[4] Rašković A, Milanović I, Pavlović N, Ćebović T, Vukmirović S, Mikov M. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC Complement. Altern. Med. 2014;14:225. https//doi.org/10.1186/1472-6882-14-225.

[5] Nieto G, Ros G, Castillo J. Antioxidant and Antimicrobial Properties of Rosemary (*Rosmarinus officinalis* L.): A review. Medicines (Basel). 2018;5(3):98. https://doi.org/10.3390/medicines5030098.

[6] Habtemariam S. The therapeutic potential of rosemary (*Rosmarinus officinalis*) diterpenes for Alzheimer's disease. Evid. Based Complement. Altern. Med. 2016;2016:2680409. https//doi.org/10.1155/2016/2680409.

[7] Nieto G, Bañon S, Garrido MD. Incorporation of thyme leaves in the diet of pregnant and lactating ewes: Effect on the fatty acid profile of lamb. Small Rumin. Res. 2012;105:140–147. https//doi.org/10.1016/j.smallrumres.2011.11.016.

[8] Nieto G, Estrada M, Jordán MJ, Garrido MD, Bañon S. Effects in ewe diet of rosemary by-product on lipid oxidation and the eating of cooked lamb under retail display conditions. Food Chem. 2011;124:1423–1429. https//doi.org/ 10.1016/j.foodchem.2010.07.102.

[9] Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Razis AFA, Modu B, Calina D, Sharifi-Rad J. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front Chem. 2023;11:1158198. https//doi.org// 10.3389/fchem.2023.1158198.

[10] Chibuye B, Singh IS, Ramasamy S, Maseka KK. Natural antioxidants: A comprehensive elucidation of their sources, mechanisms, and applications in health. Next Research. 2024;1(2):100086. https://doi.org/10.1016/j.nexres.2024.100086.

[11] Kowalska G. The Safety Assessment of Toxic Metals in Commonly Used Herbs, Spices, Tea, and Coffee in Poland. Int J Environ Res Public Health. 2021;18(11):5779. https//doi.org/10.3390/ijerph18115779.

[12] Jomova K, Makova M, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Rhodes CJ, Valko M. Essential metals in health and disease. Chem Biol Interact. 2022;1:367:110173. https//doi.org/10.1016/j.cbi.2022.110173.

[13] Naveed S, Oladoye PO, Alli YA. Toxic heavy metals: A bibliographic review of risk assessment, toxicity, and phytoremediation technology. SCENV. 2023;2:100018. https://doi.org/10.1016/j.scenv.2023.100018.
[14] Huremović J, Badema B, Muhić-Šarac T, Selović A, Memić M. Sadržaj teških metala u začinskom bilju s tržišta u Sarajevu, Bosna i Hercegovina. Hem. Ind.2014;63(3-4):77-81.
[15] FAO/WHO, Contaminants. In Codex Alimentarius, vol. XVII, Edition 1. FAO/WHO, Codex Alimentarius Commision, Rome, 1984.
[16] Divirikii U, Horzum N, Soylak M, Elci L. Trace heavy metal contents of some spices and herbal plants from western Anatolia, Turkey. Int. J. Food Sci. Tech. 2006;41(6):712-716.
[17] Regulation on maximum allowed quantities of certain contaminants in food, Food Safety Agency of Bosnia and Herzegovina, Official Gazette 2023.
[18] Savić S, Petrović S, Petronijević M, Cvetanović A, Petronijević Ž. Determination of the mineral content of spices by ICP-OES. Advanced technologies. 2019;8(1):27-32.
[19] Regulation on permissible quantities of hazardous and harmful substances in soil and irrigation water and methods of their testing, Official Gazette of the Republic of Serbia, 23/94.

ANALIZA MIKRO I MAKRO ELEMENATA U UZORCIMA RUZMARINA ICP-OES METODOM

***Vesna Gojković Cvjetković*[1], *Željka Marjanović-Balaban*[2], *Vesna Antunović*[3], *Dijana Mihajlović*[4], *Marko Ivanović*[1], *Jelena Vuković*[1], *Milomirka Obrenović*[1], *Kristina Đerić*[1], *Milenko Smiljanić*[1]**

[1]Univerzitet u Istočnom Sarajevu, Tehnološki fakultet Zvornik, Republika Srpska, Bosna i Hercegovina

[2]Univerzitet u Banjoj Luci, Šumarski fakultet, Banja Luka, Republika Srpska, Bosna i Hercegovina

[3]Univerzitet u Banjoj Luci, Medicinski fakultet, Katedra za farmaciju, Republika Srpska, Bosna i Hercegovina

[4]Univerzitet u Banjoj Luci, Poljoprivredni fakultet, Banja Luka, Republika Srpska, Bosna i Hercegovina

Sažetak. *Ruzmarin (Rosmarinus officinalis) je mediteranska biljka koja se koristi, ne samo kao ukras, već i kao začin, a ima i ljekovita svojstva. Ova biljka sadrži mnogo ljekovitih supstanci, kao što su biljne masti, biljni hormoni, vitamin C, enzimi, eterična ulja, tanini, heterozidi, holin, cineol. Međutim, prisustvo teških metala, čak i u malim koncentracijama, može biti toksično i dovesti do ozbiljnih poremećaja i bolesti. U ovom radu vršena je analiza mikro i makro elemenata u uzorcima ruzmarina sa područja Hercegovine. Takođe, određen je i sadržaj mikro i makro elemenata u uzorcima zemljišta sa kojeg je ruzmarin uzet za analizu. Priprema uzoraka je vršena mokrom digestijom. Određivanje koncentracije mikro i makro elemenata vršeno je primjenom optičke emisione spektrometrije sa induktivno spregnutom plazmom (ICP-OES). Na osnovu dobijenih rezultata, najveća koncentracija makroelemenata izmjerena je za Ca i iznosi 11508,74±37,33 mg/kg, a najniža koncentracija makroelemenata izmjerena je za Na i iznosi 387,77±1,64 mg/kg. Izmjerene koncentracije teških metala (As, Cd, Pb) su ispod granice detekcije. Koncentracija mikro i makro elemenata u uzorcima zemljišta je ispod maksimalno dozvoljenih koncentracija.*

Ključne riječi: *makro i mikro elementi, ruzmarin, ICP-OES*