



Determination of the content of micro and macro elements in laurel samples from the region of Herzegovina

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KEY CONTRIBUTION

Laurel is one of the most commonly used spices. It is a good source of macronutrients.

The use of the ICP-OES method is a rapid method for the determination of micro- and macro-elements in laurel and soil samples.

ABSTRACT

Laurel (*Laurus nobilis* L.) is an evergreen shrub or tree from the *Lauraceae* family. It is most commonly used as a spice. Laurel leaves contain essential oil, bitter substances and tannins. It has a very aromatic, slightly spicy, astringent and bitter taste. It is also often used as a preservative. However, in addition to having numerous advantages, the presence of heavy metals, even in low concentrations, can be toxic and cause numerous diseases. The aim of this work was to determine the content of micro- and macro- elements in laurel samples from the Herzegovina region. The content of metals in soil samples from which laurel was collected was also determined. The samples were prepared by wet digestion. The content of micro- and macro- elements was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). According to the results, calcium exhibited the highest concentration among the macroelements (9055.59±24.71 mg/kg), whereas sodium exhibited the lowest (259.96±1.25 mg/kg). The measured content of heavy metals is below the detection limit in laurel, and in soil samples it does not exceed the maximum allowed values.



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Introduction

Laurel (*Laurus nobilis* L.) is an evergreen tree or shrub belonging to the *Lauraceae* family. Since ancient times, its leaves and fruits have been utilized as a spice, and it has also been employed in horticulture as a tree (Abu-Dahab et al., 2014; Khodja et al., 2023). The leaves of laurel contain essential oil, primarily composed of pinene and cineole, along with bitter compounds and tannin. They possess a highly aromatic, mildly spicy, pungent, and bitter flavour. The essential oil extracted from the leaves is utilized in the perfume industry. The fruits, which contain essential oil, fatty oil, sugar, and starch are also used as a spice. Dried fruits exhibit varying percentages of essential oil (up to 10%), influenced by the location of harvesting and the method of storage (Ahmad et al., 2022). Regarding laurel leaves as a spice, both fresh and dried leaves are used which must remain whole and green. The greener the leaves, the higher the quality, whereas dry and brown leaves indicate inferior quality spices. In the marketplace, ground laurel can be found both as a standalone spice and as part of various spice blends (AL-Samarrai et al., 2017; Al Chalabi et al., 2020). It is utilized in baking, sauces, sauerkraut dishes, fish marinades, sour winter soups, and vinegar flavouring. Additionally, laurel is frequently employed as a preservative. Due to the presence of essential and fatty oils in the leaves and fruits, they exhibit potent analgesic and fungicidal properties. Laurel oil is regarded as one of the most effective oils for strengthening and prevention, with notable expectorant and mucolytic properties. Furthermore, it demonstrates a significant antiviral effect, making it advisable for use during viral infections. It also aids in digestion, inhibits intestinal fermentation, and assists in the expulsion of trapped gasses (Bendjersi et al., 2016; Alejo-Armijo et al., 2017).

Despite the many benefits that laurel leaves provide, they can also contain some toxic substances that arise from environmental influences, as well as from the methods of their cultivation, processing, and storage (Chahal et al., 2017; Bourebaba et al., 2021). The contamination of spices, such as laurel, with heavy metals can occur during their growth, affected by soil quality, nutrient availability, and the application of synthetic fertilizers, in addition to happening during the production and packaging phases (Łozak et al., 2002; Arjomandi and Shirkhanloo, 2019).

Heavy metals are a significant source of environmental pollution and have a substantial effect on the ecosystem. The introduction of heavy metals into the human body occurs through the ingestion of contaminated food and the inhalation of polluted air. Plants that develop in soil contaminated with heavy metals experience stunted growth due to changes in their physiological and biochemical functions (Samreen et al., 2021; Das et al., 2023). Human exposure to heavy metals generally arises from the consumption of affected plants. The heavy metals that present the highest risk to human health include lead, cadmium, mercury, and arsenic. Exposure to cadmium leads to kidney damage. Prolonged exposure to cadmium can also result in skeletal damage. Cadmium is rapidly transferred from the soil to the plant. Its uptake by the plant is largely influenced by the pH level and the presence of cations (Mahmood-ul-Hassan et al., 2020; Sangsuwan et al., 2021). Cadmium that is transported from the soil is primarily accumulated in the roots. The levels of cadmium found in the leaves and other aerial parts of the plant are roughly equivalent, yet significantly lower than those present in the roots. Prolonged exposure to inorganic mercury can lead to lung damage. Diagnosis of poisoning is based on neurological and psychological indicators, including mood fluctuations, fatigue, sleep disturbances, and depression. These symptoms are reversible and tend to resolve once the underlying cause is addressed. In contrast, symptoms associated with organic mercury poisoning may manifest as paraesthesia and numbness in the extremities, and can also lead to heart and coronary diseases (Wise, 2016; Posin et al., 2023). Acute lead poisoning is characterized by symptoms such as headaches, abdominal discomfort, and various

neurological issues. The presence of lead in the body can result in insomnia and restlessness, with children potentially experiencing challenges in concentration and learning. The highest levels of lead in plants are typically found in the roots. In its inorganic state, plants exhibit poor absorption and transport of lead to their above-ground structures, except in acidic soil conditions (Sachdeva et al., 2018).

Organic lead compounds are readily absorbed and distributed throughout various parts of the plant. High concentrations of lead hinder the growth of leaves and roots, disrupt the photosynthesis process, and impact the morphology and anatomy of plants. Inorganic arsenic is highly toxic and can lead to gastrointestinal issues, cardiovascular and nervous system disorders, and in extreme cases, death. Additionally, there is a heightened risk of developing kidney, bladder, and skin cancers. Individuals who are exposed to arsenic through inhalation may be at risk of developing lung cancer. The likelihood of arsenic poisoning escalates with prolonged exposure to the source contamination (Wani et al., 2015; Collin et al., 2022).

The aim of this paper was to assess the levels of micro- and macro- elements in laurel samples obtained from the Herzegovina region. Additionally, the elemental content in the soil samples where laurel is cultivated was evaluated. The analysis of micro- and macro- element content was conducted using inductively coupled plasma optical emission spectrometry ICP-OES method. Following the analysis, the resulting values were compared against the recommended and maximum permissible limits.

Materials and methods

Sample preparation

Laurel samples were collected from the Herzegovina region, specifically in Gacko, located near the mine and thermal power plant. Additionally, the soil in which the laurel thrives was also sampled from this location. The preparation of the samples began with weighing approximately 1 g of each sample. Subsequently, wet digestion was conducted using 30 ml of 65% HNO₃ in vessels, heated for 12 hours at a temperature of 90 °C. Once cooled to room temperature, the samples were diluted with redistilled water, then filtered through filter paper into a 50 ml volumetric flask, and brought up to the mark with redistilled water (Huremović et al., 2014). Each analysis was performed in triplicate. A blank sample was prepared using the same method.

Determination of micro and macro elements in laurel samples

The quantitative assessment of all samples was conducted utilizing ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry, Agilent Technologies 5100, USA). The instrument settings and the parameters measured are detailed as follows: plasma power (1400 W), gas flow: coolant (13 L/min), auxiliary (0.80 L/min), type of nebulizer (cross flow), nebulizer flow rate (0.95 L/min), pump speed: 30, and plasma observation: axial. The calibration standards were created in order to quantify the concentrations of macro and micro elements. The calibration solutions were prepared using a multistandard IV standard solution (Merck, LOT Number 023J5431) containing Ag, Al, B, Cd, Co, Cr, Fe, Li, Mn, Ni, Pb, Zn, As, Cu, P, Sr, Ca, K, Mg, Na, and Be, each at a concentration of 1000 mg/kg. Both the standard solution and the samples were diluted using distilled water that had been purified by HPLC grade. To ensure that the concentrations of calibration standards fell within the expected range of test element concentrations, the standard solution was appropriately diluted. Argon served as the carrier gas. Table 1 shows the wavelength for each element, as well as the detection limit.

Table 1 Calibration parameters: detection wavelength (nm) and limit detection ($\mu\text{g}/\text{kg}$)

Elements	Detection Wavelength (nm)	Limit of detection ($\mu\text{g}/\text{kg}$)
Ag	328.068	4
Al	396.152	6
Cd	226.502	0.2
Co	238.892	3
Cr	205.560	1
Fe	259.940	2
Li	670.783	6
Mn	257.610	0.4
Ni	231.604	2
Pb	220.353	5
Zn	213.857	1
As	188.980	14
Cu	327.395	3
P	213.618	50
Sr	407.771	0.6
Ca	396.847	3
K	766.491	20
Mg	279.553	1
Na	589.592	20
Be	313.042	0.1
B	455.403	5

Results and discussion

Table 2 shows concentrations of micro and macro elements in samples of laurel.

It is evident from the results (Table 2) that the highest concentration of Ca in the laurel samples was 9055.59 ± 24.71 mg/kg. The most prevalent mineral in the human body is calcium, which is a macroelement. About 98 percent of calcium is located in bones, 1 percent is found in teeth, and the remaining 1 percent is found in bodily fluids and tissues (Heaney, 2010). For adults, 1200 mg per day is the recommended daily intake (Institute of Medicine, 2011). After Ca, K is the element with the highest concentration recorded. The value that was observed is 6414.20 ± 26.32 mg/kg. Only a minor portion (2%) of potassium is present in intercellular fluids; the majority (8%) is located in cells (Cheng et al., 2013). For K, a daily dose of 3100 mg is advised. One of the fundamental macroelements is magnesium. Laurel samples from the Herzegovina region have a determined magnesium value of 1143.75 ± 1.18 mg/kg. A daily dosage of 350 mg is advised. Na concentration was found to be 259.96 ± 1.25 mg/kg. Saliva, digestive fluids, blood plasma, and extracellular fluid are all sources of sodium in the human body. Body fluids contain around 60% of sodium, cells contain 10%, and bones contain 30%. In bodily fluids, sodium ions control osmotic pressure and the acid-base balance. Adults are advised to consume 500 mg of sodium each day (Cao et al., 2019). P is also a macroelement. Laurel samples have a measured P concentration of 1116.21 ± 8.50 mg/kg. More than 85% of the phosphorus in the human body is bound in the teeth and bones. Other tissues contain the remaining portion of phosphorus. ATP, creatine phosphate, and other high-energy phosphorus molecules are involved in the metabolism of carbohydrates and supply energy for biochemical reactions in the brain, liver, muscles, and other organs. Complex proteins and phospholipids also include phosphorus. It contributes to the regular maintenance of the concentration of hydrogen ions. Adults are advised to consume 900 mg per day (Goretti Penido

and Alon, 2012). Fe is one of the microelements. Laurel samples have a measured Fe content of 40.00 ± 0.25 mg/kg. All of the body's cells depend on iron to function. Haemoglobin, myoglobin, and some enzymes (cytochromes, catalases, and peroxidases) all contain iron. Oxygen delivery to the body would be impossible without iron. The body requires 10-30 milligrams of iron per day (Allen et al., 2006; Quintero-Gutiérrez et al., 2008). Another microelement is zinc. It is a necessary component of food. It is a part of more than 100 enzymes. Zinc is required for the production of DNA and RNA, is a part of the insulin molecule, and has a role in the metabolism of carbohydrates (Costa et al., 2023). In this investigation, the Zn content was determined as 12.84 ± 0.21 mg/kg. Zinc intake should not exceed 15 milligrams per day (Grujić and Miletić, 2006). Copper is another essential microelement. The body and food both contain trace amounts of it. When it comes to haemoglobin, erythrocytes, cell respiration, and energy production in cells, copper is a potent catalyst. It is a component of muscles, connective tissue, and bones (Tapiero et al., 2003). The laurel samples have a measured Cu content of 4.81 ± 0.12 mg/kg. For copper, a daily dose of 2.0-3.0 mg is advised. Cobalt (Co) is another trace element found in both the body and food. Its primary physiological role is to assist some bacteria in producing vitamin B12. It is a component of blood plasma and red blood cells. The pancreas, liver, kidneys, and spleen all contain large amounts of it (Mehri, 2020). The Co concentration found in the study's samples is less than the $3 \mu\text{g/kg}$ detection limit. $0.2 \mu\text{g}$ of cobalt every day is what the organism needs (Grujić and Miletić, 2006).

Table 2 Micro and microelement concentrations in samples of laurel

Elements	Concentration (mg/kg)	Literary values (mg/kg)
Ag	<LD	NA
Al	27.23 ± 2.14	NA
B	<LD	<20 mg
Cd	<LD	0.05-2.00 (Codex Alimentarius, 2024)
Co	<LD	0.2 μg
Cr	0.73 ± 0.17	0.05-2.0 mg/day (Grujić and Miletić, 2006)
Fe	40.00 ± 0.25	10-30 mg/day (Grujić and Miletić, 2006)
Li	<LD	NA
Mn	34.67 ± 0.11	3-9 mg/day (recommended daily intake)
Ni	1.60 ± 0.19	900 mg/day (recommended daily intake)
Pb	<LD	0.01-3.00
Zn	12.84 ± 0.21	15 mg/day
As	<LD	0.1-0.2
Cu	4.81 ± 0.12	2.0-3.0
P	1116.21 ± 8.50	900 mg/day
Sr	19.05 ± 0.06	NA
Ca	9055.59 ± 24.71	1200 mg/day (recommended daily intake)
K	6414.20 ± 26.32	3100 mg/day
Mg	1143.75 ± 1.18	350 mg/day
Na	259.96 ± 1.25	500 mg/day (recommended daily intake, Grujić and Miletić, 2006)
Be	<LD	NA
Ba	3.10 ± 0.04	NA

All cells and blood plasma contain the metal manganese. Due to the fact that it is a component of enzymes and contributes to the production of energy in cells, proteins, cholesterol, bones, and mucopolysaccharides, it falls under the category of vital metals (Li and Yang, 2018). The concentration of Mn detected is 34.67 ± 0.11 mg/kg. Adults need three to nine milligrams of this element per day. HDL cholesterol in the blood can be slightly decreased by chromium. The Cr concentration was found to be 0.73 ± 0.17 mg/kg. Heavy metals can interfere with essential physiological processes and are associated with a variety of health problems. Despite its effects on chromosomes and its role in the development of cancer, arsenic (As) is not as hazardous as other minerals in this category. It accumulates in the body, particularly in the skin, hair, and certain internal organs. Faeces are the body's way of getting rid of arsenic (Vargas-Rondón et al., 2018). Cadmium (Cd) is present in food from natural sources. It can cause disruptions in organ function by displacing zinc from certain metabolic activities. Zinc shields tissues against cadmium-induced harm. Cadmium accumulation and toxicity are more likely when zinc intake is insufficient. Deformation of bones results from cadmium's effects on their structure. Common conditions include cancer, heart and kidney problems, and anemia. Among heavy metals, lead (Pb) contains the highest proportion of harmful constituents. Excessive levels of lead can be harmful to one's health (Chunhabundit, 2016; Pokharel and Wu, 2023). The heavy metal (As, Cd, and Pb) concentrations measured in this paper are below the detection limit.

Table 3. shows the measured concentration of microelements (mg/kg) in soil samples from which laurel from the Herzegovina area was samples.

Table 3 Concentration of microelements in soil samples

Elements	Concentration (mg/kg)	Maximum allowed concentrations (mg/kg soil)
Cu	74.04 ± 0.79	to 100
Mn	222.61 ± 1.14	NA
Zn	93.55 ± 0.55	to 300
Cd	1.75 ± 0.06	to 3

The measured concentration of micro elements (Cu, Mn, Zn, Cd) in soil samples from the Herzegovina region does not surpass the maximum allowed concentrations, as per the Regulation on Permissible Quantities of hazardous and harmful substances in Soil and irrigation water and method of their testing (Official Gazette of the Republic of Serbia, 23/94), as can be observed from the results obtained (Table 3).

Conclusion

The following results were drawn from the examination of the micro and macro components in the laurel samples and micro components in soil samples from the Herzegovina region. A quick, easy, and effective way for identifying the micro and macro elements in extracts is the ICP-OES method. As laurel is rich in both macro- and microelements, its inclusion in human nutrition is recommended owing to its nutritional composition. Among the macroelements, calcium exhibited the highest concentration (9055.59 ± 24.71 mg/kg), whereas sodium exhibited the lowest (259.96 ± 1.25 mg/kg). The detected concentration of heavy metals is below the limit of detection. The concentration of microelements in the soil samples were below the maximum permissible levels.

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Conflicts of Interest: The authors declare no conflict of interest.

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