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# Elemental Analysis of *Onobrychis buhseana* Bunge Ex Boiss and *Onobrychis bobrovi* Grossh Species by ICP-MS

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**Abstract:** The element composition of the underground and surface parts of Onobrychis buhseana and Onobrychis bobrovi, which are distributed in the flora of Azerbaijan, was studied by an ICP-MS device, and the amount of 26 elements (Li, Be, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, Sn, Ba, Ce, Tl, and Pb) in the raw material was determined in ppm. The composition of raw materials is dominated by some macroelements (calcium, potassium, and magnesium), microelements (iron and zinc), and ultramicroelements (selenium, cobalt, and chromium). Some of the most frequent harmful trace elements, such as As and Cd, were discovered at extremely low concentrations.

# *Onobrychis buhseana* Bunge Ex Boiss ve *Onobrychis bobrovi* Grossh'nin ICP-MS ile Elementel Analizi

**Anahtar Kelimeler:**
Elementel analiz,
ICP-MS,
Mikrodalga sindirimi,
*Onobrychis bobrovi*,
*Onobrychis buhseana*

**Özet:** Azerbaycan florasında yayılış gösteren Onobrychis buhseana ve Onobrychis bobrovi'nin toprak altı ve toprak üstü kısımlarının element kompozisyonu ICP-MS cihazı ile incelenmiş ve 26 elementin (Li, Be, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, Sn, Ba, Ce, Tl ve Pb) miktarları hammaddelerinde ppm ile tespit edilmiştir. Hammaddelerin bileşiminde bazı makro elementler (kalsiyum, potasyum ve magnezyum), mikro elementler (demir ve çinko) ve ultramikro elementler (selenyum, kobalt ve krom) baskındır. As ve Cd gibi en yaygın toksik eser elementlerden bazıları oldukça düşük seviyelerde bulundu.

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## 1. INTRODUCTION

Traditional medicine (TM) forms the basis of healthcare. Medicinal plants containing diverse elements can be used to treat certain disorders. (WHO 2013). Plants play a crucial role in the nutrition of humans and animals, as well as in their medicinal treatment. They provide macro- and microelements that are essential for various biochemical and physiological processes.

However, some plants can absorb toxic elements from the air or soil due to anthropogenic factors, posing a danger to human life. Therefore, it is necessary to control toxic compounds and heavy metals in plants (Gill, 2014; Proshad et al., 2020).

Various methods are used to assess the quality of plant elements: inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-EOS), instrumental neutron activation analysis (INAA), atomic absorption spectrometry (Chevidaev et al., 2023; İslam et al., 2023; Bekoe et al., 2024).

The amount of 20 chemical elements in the medicinal plants Acanthus ilicifolius, Avicennia ofcinalis, and Xylocarpus mekongensis was determined by instrumental neutron activation analysis (INAA) and atomic absorption spectrometry. The study also evaluated the potential health risks associated with the use of these plants. (İslam et al., 2023).

The elemental analysis of Polyalthia longifolia (Sonn.) Thwaites revealed that the levels of toxic heavy metals (arsenic, lead, cadmium, and mercury) in the plant exceed recommended standards (Bekoe et al., 2024).

Breast collection No. 2 was analyzed using the ICP-MS method, which showed that the complex has a rich mineral composition. Additionally, the levels of heavy metals and arsenic were found to be within permissible norms (Chevidaev et al., 2023)

The nutritional value of six plant species from Bajaur province of Pakistan was evaluated through elemental analysis. Different values of elemental abundances were found depending on the phenological stage (Haq et al., 2024).

The concentrations of five macro elements (potassium, calcium, magnesium, sodium, and manganese) in cereals sold at Ajaka market in Igalamela, Nigeria were determined using titration and UV-visible spectrophotometry. The analysis results were found to be below the standard permissible limits set by the World Health Organization (WHO)/Food and Agricultural Organization (FAO) (Ojattah et al., 2023).

In this study, we aimed to investigate the elemental composition of Onobrychis buhseana and Onobrychis bobrovi species, which are widely distributed in Azerbaijan. The elemental composition was determined by digesting the study samples with concentrated nitric acid and analyzing them using inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS is a cornerstone method in analytical chemistry. It uses a high-temperature plasma source to atomize and ionize samples, followed by mass spectrometric detection to precisely quantify the elemental composition. It is renowned for its sensitivity, selectivity, and wide dynamic range, and finds extensive application in fields ranging from environmental science to bioanalysis, contributing significantly to the advancement of scientific research and industrial analysis (Ruffolo et al., 2022). The microwave digestion method was used before analysis. This method is the most useful sample preparation method required for trace metal analysis (Zhao et al., 2011).

The Onobrychis genus is a group of medicinal plants belonging to the leguminous family. It is primarily found in temperate zones of South Asia, the Mediterranean region, Europe, and Asia. According to The Plant List website, the genus has 528 distinct species, only 171 of which have been recognized (http://www.theplantlist.org). Azerbaijan's flora includes 22 species of the genus Onobrychis. Onobrychis heterophylla is native to Azerbaijan and grows in the Lerik region, while Onobrychis bakuensis is native to the Caucasus and is prevalent in northern Iran (Флора Азербайджана, 1954; Ranjbar et al., 2010).

Onobrychis species have been reported to possess antioxidant, antibacterial, anti-inflammatory, and anti-carcinogenic properties (Isam et al., 2020; Altn et al., 2023). Onobrychis is a plant that provides biological nitrogen fixation, which helps to maintain soil fertility and vitality. It is also cultivated as a valuable fodder plant for farm animals and is suitable for the production of high-quality honey due to its rich supply of nectar. The plant is abundant in proteins, carbohydrates, phytic acid, microelements, and vitamins A, D, E, K, rutin, and isoquercetin (Kozuharova, 2018; Beyaz R., 2019; Emre et al., 2019; Huyen et al., 2020; Craine et al., 2023).

The plant contains various chemical elements, classified into three categories based on their abundance: macro-, micro-, and ultramicroelements. These elements are crucial to the human body as they influence essential physiological processes such as growth, development, fertilization, respiration, hematopoiesis, and immunogenesis.

The objective of this study is to quantify the essential and toxic elements present in these medicinal plants and to investigate the potential health risks associated with their use.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials

During the flowering period of the plant, O. buhseana samples were gathered from Badamli village in the Shahbuz district of Nakhchivan MR, and O.bobrovi samples were collected from Jengi village in the Gobustan district in May 2022. Above-ground and underground parts of both species were sampled separately. The above-ground part of O. buhseana species was marked as Sample 1, the underground part as Sample 2, the above-ground part of O. bobrovi species as Sample 3, and the underground part as Sample 4.

### 2.2. Chemicals and Reagents

The reagents used during the sample preparation process are 65% (v/v) nitric acid ($HNO_3$) and 30% (v/v) hydrogen peroxide ($H_2O_2$) products of Carlo Erba and Merck brands, respectively. The ultrapure water was obtained by a water purification system (Millipore Direct-Q® 3 UV).

### 2.3. Microwave Digestion and Sample Preparation

The microwave digestion method was used before analysis. The process uses microwaves to heat a combined sample with concentrated acid and dissolve the solids for analysis. Microwave and acid digestion are known to be a suitable method for the degradation of many kinds of samples such as soils, organic samples, and heat-resistant alloys. At the same time, it reduces the risk of contamination from the external environment and allows more reliable results to be obtained. The first step of the method is to weigh the sample into a container and add concentrated acid. Microwave energy causes rapid heating of the sample and concentrated acid. Because this mixture is in a closed container, it can reach very high temperatures. In this case, the oxidative potential of the acid increases. In this way, concentrated acid can separate the sample better. The device used for the microwave digestion method consists of Milestone Ethos, Start laboratory station with built-in temperature sensor, 640-260 terminal with easy CONTROL software, and HPR1000/10S high-pressure split rotor. The samples weigh approximately 0.5 g and the weighing results are given in Table 1.

**Reagents:** 6 ml of $HNO_3$, 2 ml of $H_2O_2$

The sample was weighed, and the sample and reagents were taken into the TMF vessel and closed using a torque wrench. The sample was burned by the "application note" procedure and the program below (Table 2). After degradation, the samples filtered through filter paper were made up to 50 ml with ultrapure water.

### 2.4. Element Determination with ICP-MS

The instrument used in the study was Thermo Scientific – X Series 2 ICP-MS. Fluid flow rates were controlled with a peristaltic pump, sample flow was pumped at 0.8 ml/min, the wash station at 4 ml/min, and the wash station overflow at 8 ml/min. All samples were measured three times. In addition, the operating conditions of the system are given in Table 3.

## 3. RESULTS AND DISCUSSION

The element abbreviation, isotope masses, regression coefficient, and calibration range of the analytes are given in Table 4. Quantitative results and %RSD values of analytes are given in Table 5. Results are given in ppm for all elements.

**Sample-1**

Ca >K >Mg >Na >B >Sr >Al >Fe >Ba >Mn >Zn >Sn >Ni >Li >Cu >Se >V >Co >Cr >As >Ce >Cd >Pb >Ag >Be >Tl

**Sample-2**

Ca >K >Mg >Sr >Fe >Al >Na >Ba >Mn >B >Zn >Sn >Ni >Cu >V >Cr >Li >Co >Ce >As >Se >Cd >Ag >Be >Tl>Pb

**Table 1.** Weight of Sample

| Sample | Weight (g) |
|---|---|
| Sample-1 | 0.5104 |
| Sample-2 | 0.5016 |
| Sample-3 | 0.5039 |
| Sample-4 | 0.5122 |

**Table 2.** Microwave program

| Step | Time | T1 | T2 | P | Power |
|---|---|---|---|---|---|
| 1 | 00:15:00 | 200ºC | 110ºC | 45 bar | Max Power* |
| 2 | 00:15:00 | 200ºC | 110ºC | 45 bar | Max Power* |

*Max Power: 1500W for Ethos and 1200W Start units.

**Table 3.** Operating conditions of the system

| Description | Extraction | Lens 1 | Lens 2 | Lens 3 | Sampling Depth | Cool | Auxiliary | Nebuliser | Forward power | D1 | CCT Gas 1 | CCT Gas 2 | D2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XSII Xt Redoks | -96 | -990 | -75.3 | -143.5 | 150 | 13 | 0.7 | 0.97 | 1400 | -43.9 | 0 | 0 | -151 |

**Sample-3**

K >Ca >Mg >Na >Fe >Al >Sr >B >Mn >Zn >Sn >Ba >Li >Cu >Ni >Pb >V >Cr >Ce >Se >Co >Cd >As >Ag >Be >Tl

**Sample-4**

Ca >K >Al >Mg >Fe >Sr >Li >Mn >Ba >B >Na >Zn >Sn >Ni >Cu >V >Cr >Ce >Co >As >Pb >Cd >Be >Se >Ag >Tl

ICP-MS was used to perform elemental analysis on O. buhseana and O. bobrovi species collected from various regions of Azerbaijan. The data from four samples, examining twenty-six elements, is summarised in Table 5 according to their amount (mg/kg) in fresh weight. The following elements were determined in ppm concentrations: Li, Be, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, Sn, Ba, Ce, Tl, and Pb. No traces of Au and Hg were found in any of the samples.

Elements can generally be categorized into two types: essential and toxic (Mohamed et al., 2003).

Potassium (Mg) ions are essential for protein and carbohydrate production, while magnesium ions are involved in over 300 intracellular enzymatic processes. Calcium (Ca) ions are rigid constants in the body that are crucial for bone tissue growth and, along with magnesium ions, help to maintain neuromuscular conduction in the body. Sodium (Na) is essential for maintaining acid-base and fluid balance (homeostasis) in the body, as well as for normal nerve and muscle function (Брин et al., 2016).

In the body, iron (Fe) participates in energy release mechanisms, enzymatic reactions, immunological functions, and cholesterol metabolism. The transferrin protein is responsible for supplying iron to cells and tissues. Selenium's (Se) biological purpose in the body is related to its antioxidant activity. Additionally, Se acts as an antagonist to mercury and arsenic, boosts the body's immune function, and minimizes the risk of producing new derivatives (Радыш et al., 2017).

Zinc (Zn) is a cofactor for many enzymes involved in biochemical processes. It is necessary for protein synthesis, including collagen, and contributes to cell division and differentiation as well as T-cell immunity formation (Roohani et al., 2013).

**Table 4.** Calibration ranges and regression coefficients for the element analysis

| No | Elements | Isotope | $R^2$ | Calibration Range (ppb) |
|---|---|---|---|---|
| **1** | Li | 7 | 0.999971 | 1-100 |
| **2** | Be | 9 | 0.999281 | 1-100 |
| **3** | B | 11 | 0.999724 | 1-100 |
| **4** | Na | 23 | 0.999354 | 100-1000 |
| **5** | Mg | 24 | 0.998140 | 100-1000 |
| **6** | Al | 27 | 0.999488 | 1-100 |
| **7** | K | 39 | 0.992875 | 100-1000 |
| **8** | Ca | 44 | 0.998674 | 100-1000 |
| **9** | V | 51 | 0.998134 | 1-100 |
| **10** | Cr | 52 | 0.998429 | 1-100 |
| **11** | Mn | 55 | 0.998513 | 1-100 |
| **12** | Fe | 56 | 0.998960 | 100-1000 |
| **13** | Co | 59 | 0.999620 | 1-100 |
| **14** | Ni | 60 | 0.999194 | 1-100 |
| **15** | Cu | 65 | 0.9996550 | 1-100 |
| **16** | Zn | 66 | 0.999040 | 1-100 |
| **17** | As | 75 | 0.999275 | 1-100 |
| **18** | Se | 82 | 0.999949 | 1-100 |
| **19** | Sr | 88 | 0.999717 | 1-100 |
| **20** | Ag | 107 | 0.999665 | 1-100 |
| **21** | Cd | 111 | 0.999133 | 1-100 |
| **22** | Sn | 118 | 0.999325 | 1-100 |
| **23** | Ba | 134 | 0.999285 | 1-100 |
| **24** | Ce | 140 | 0.999836 | 1-100 |
| **25** | Au | 197 | 0.998311 | 1-100 |
| **26** | Hg | 202 | 0.999368 | 1-100 |
| **27** | Tl | 205 | 0.998117 | 1-100 |
| **28** | Pb | 208 | 0.999704 | 1-100 |

**Table 5.** Amount of metals in plant samples and %RSD values

| | | Sample-1 | | Sample-2 | | Sample-3 | | Sample-4 | |
|---|---|---|---|---|---|---|---|---|---|
| No | Elements | %RSD[a] | Quantification (mg Analyte/kg Sample) ppm | %RSD[a] | Quantification (mg Analyte/kg Sample) ppm | %RSD[a] | Quantification (mg Analyte/kg Sample) ppm | %RSD[a] | Quantification (mg Analyte/kg Sample) ppm |
| 1 | Li | 1.168 | 6.561 ± 0.077 | 2.071 | 0.561 ± 0.012 | 3.365 | 5.447 ± 0.183 | 0.106 | 2.15 ± 0.002 |
| 2 | Be | 1.815 | 0.005 ± 0 | 5.237 | 0.012 ± 0.001 | 4.005 | 0.01 ± 0 | 5.613 | 0.05 ± 0.003 |
| 3 | B | 2.481 | 349.3 ± 8.668 | 2.131 | 21.66 ± 0.462 | 1.908 | 48.02 ± 0.916 | 0.934 | 17.75 ± 0.166 |
| 4 | Na | 4.667 | 1352 ± 63.11 | 4.319 | 324.9 ± 14.03 | 3.572 | 1085 ± 38.76 | 17.89 | 175.1 ± 31.32 |
| 5 | Mg | 2.762 | 5315 ± 146.8 | 1.046 | 1987 ± 20.79 | 2.19 | 1688 ± 36.98 | 1.627 | 1348 ± 21.93 |
| 6 | Al | 3.079 | 176.6 ± 5.439 | 1.312 | 510.4 ± 6.695 | 1.283 | 472 ±6.054 | 0.543 | 2091 ± 11.36 |
| 7 | K | 1.928 | 7246 ± 139.7 | 1.261 | 3231 ± 40.74 | 2.382 | **13220 ± 3148** | 5.825 | 2105 ± 122.6 |
| 8 | Ca | 3.867 | **11760 ± 454.8** | 0.732 | **46900 ± 343.1** | 1.763 | 5052 ± 89.08 | 1.688 | **10270 ± 173.4** |
| 9 | V | 4.868 | 0.368 ± 0.018 | 1.544 | 1.075 ± 0.017 | 1.177 | 0.897 ± 0.011 | 0.397 | 3.541 ± 0.014 |
| 10 | Cr | 5.93 | 0.318 ± 0.019 | 1.183 | 0.964 ± 0.011 | 1.208 | 0.769 ± 0.009 | 0.304 | 1.869 ± 0.006 |
| 11 | Mn | 3.647 | 58.2 ± 2.123 | 1.237 | 33.43 ± 0.413 | 1.523 | 29.85 ± 0.455 | 0.655 | 32.31 ± 0.212 |
| 12 | Fe | 13.76 | 154.6 ± 21.27 | 4.861 | 626 ± 30.43 | 7.308 | 551.6 ± 40.31 | 5.005 | 900.5 ± 45.07 |
| 13 | Co | 4.411 | 0.32 ± 0.014 | 0.864 | 0.485 ± 0.004 | 2.757 | 0.246 ± 0.007 | 0.533 | 0.697 ± 0.004 |
| 14 | Ni | 3.916 | 6.561 ± 0.193 | 0.289 | 8.899 ± 0.026 | 2.767 | 3.72 ± 0.103 | 0.805 | 7.213 ± 0.058 |
| 15 | Cu | 4.070 | 4.826 ± 0.196 | 0.345 | 3.78 ± 0.013 | 1.458 | 4.383 ± 0.064 | 0.484 | 4.589 ± 0.022 |
| 16 | Zn | 3.647 | 21.34 ± 0.778 | 0.882 | 12.67 ± 0.112 | 1.729 | 13.06 ± 0.226 | 0.959 | 13.14 ± 0.126 |
| 17 | As | 3.332 | 0.188 ± 0.006 | 2.703 | 0.364 ± 0.01 | 3.61 | 0.168 ± 0.006 | 0.225 | 0.497 ± 0.001 |
| 18 | Se | 1.622 | 0.832 ± 0.013 | 5.337 | 0.098 ± 0.005 | 3.268 | 0.301 ± 0.01 | 21.6 | 0.047 ± 0.01 |
| 19 | Sr | 4.620 | 194.9 ± 9.003 | 1.141 | 838.1 ± 9.56 | 1.610 | 95.51 ± 1.537 | 0.437 | 317 ± 1.387 |
| 20 | Ag | 5.109 | 0.013 ± 0.001 | 1.948 | 0.03 ± 0.001 | 1.917 | 0.015 ± 0 | 0.378 | 0.04 ± 0 |
| 21 | Cd | 1.953 | 0.135 ± 0.003 | 3.163 | 0.088 ± 0.003 | 2.804 | 0.181 ± 0.005 | 0.696 | 0.377± 0.003 |
| 22 | Sn | 3.857 | 13.49 ± 0.521 | 2.868 | 12.19 ± 0.35 | 1.495 | 13.02 ± 0.195 | 1.172 | 9.375 ± 0.110 |
| 23 | Ba | 3.939 | 75.91 ± 2.99 | 1.222 | 163.4 ± 1.996 | 1.399 | 8.467 ± 0.119 | 0.640 | 24.75 ± 0.159 |
| 24 | Ce | 2.787 | 0.148 ± 0.004 | 1.379 | 0.401 ± 0.006 | 0.839 | 0.37 ± 0.003 | 1.851 | 1.523 ± 0.028 |
| 25 | Au | - | Nd[b] | - | Nd[b] | - | Nd[b] | - | Nd[b] |
| 26 | Hg | - | Nd[b] | - | Nd[b] | - | Nd[b] | - | Nd[b] |
| 27 | Tl | 0.804 | **0.001 ± 0** | 2.93 | 0.004 ± 0 | 1.764 | **0.002 ± 0** | 2.693 | **0.011 ± 0** |
| 28 | Pb | 3.458 | 0.076 ± 0.003 | 1.432 | **0.003 ± 0** | 0.99 | 0.999 ± 0.01 | 0.414 | 0.496 ± 0.002 |

[a]Relative standard deviation, [b]Not detected.

Cobalt (Co) participates in enzymatic activities, such as thyroid hormone production, and promotes erythropoiesis by boosting iron absorption (Yamada, 2023). Chromium (Cr) plays a crucial role in the synthesis of carbohydrates and lipids, regulates blood sugar levels by enhancing insulin function, increases cardiac muscle activity, aids in the elimination of toxins, heavy metal salts, and radionuclides from the body, and promotes muscle growth (Anderson, 1997). As and Cd have a therapeutic effect on the body at low doses. They influence oxidation and other metabolic processes in mitochondria and interact with protein and amino acid thiol groups (Nordberg et al., 2007).

## 4. CONCLUSION

The table shows that Ca (11760 ppm in sample 1, 46900 ppm in sample 2, and 10270 ppm in sample 4) and K (13220 ppm in sample 3) were the most abundant detectable elements, while Tl (in samples 1, 3, and 4) and Pb (in sample 2) were the least abundant.

The contents of Ca (10270-46900), Na (175.1-1352), Mg (1348-5315), Fe (154.6-900.5), and Zn (12.67-21.34) found in the samples suggest that they are potential sources for these elements. The amount of Se is low in the samples. Fe concentrations varied from 154.6 to 900.5 mg/kg, with the maximum concentration found in Sample 4. Therefore, O. bobrovi could be a possible natural supplement for this microelement. The concentrations of Zn in this study ranged from 12.67 to 21.34 mg/kg, which is equal to the maximum permissible limit set by the World Health Organization (27 mg/kg) (FAO/WHO. 2001).Table 5 shows the concentrations of various elements, including hazardous trace elements such as As, Co, Cd, Cr, and Pb, which were identified at extremely low levels. (WHO. 1996).

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