INTRODUCTION

Since ancient times, wild plants have been used in traditional medicine as teas and tinctures, because they contain many compounds which may contribute to preservation of good health, because of their potential antioxidant, antimicrobial, hepatoprotective, antitumor, and anti-inflammatory activities [1]. Plant species *Seseli pallasii*, have been recognized and described as one possessing healing properties [2].

Plants generally have the ability to absorb non-metals (e.g., N, P, S) and metals (e.g., K, Ca, Mg, Cu, Zn, Mn, Ni, Cr, and Mo) which are necessary for their functioning and in the same time can accumulate other potentially harmful metals (e.g., Al, Cd, and Pb) from the environment (soil, water, and air). However, even the essential metals (e.g., Cu, Mn, Ni, Cr) can be toxic for the plant when present in higher concentrations [3]. If the plants are applied for medicinal and veterinary purposes, and/or in diet of people and animals, they represent a source of heavy metals intake in the organism, and in that way pose a risk to their health, because the high content of heavy metals can cause morphological cell abnormalities and mutagen influence in humans [4].

Although the presence and variety of essential elements in many plant species play an important role in the battle against a variety of diseases, heightened content of individual heavy metals in plants can lower their therapeutic activity, or even can be toxic to human health due to their consuming, and therefore their appliance is limited. In accordance to that, concentration of heavy metals in herbs is strictly limited and defined by international standards (i.e. WHO, 1998) [5].

According to established standards as macro-elements can be classified elements which are ingested in amounts greater than 100 mg per day, and represent 1% or less of body mass. This group includes calcium, phosphorus, magnesium sulphur, potassium, chloride and sodium. Macro-elements are structural components of tissues; they have certain functions in the cells and basal metabolism, as well in water balance and acidic-alkaline balance [1].

Micro-elements are necessary in a much smaller quantities, less than 100 mg per day, and they make up less than 0.01% of body mass. Micro-elements are Zn, Fe, Si, Mn, Cu, Cr, fluorides and iodides. Elements which cause adverse effects are categorised as toxic (Pb, As, Cd,...); even though they are mostly present in traces, they pose a significant threat to human health, and therefore, determination of their content and mechanism of action have become an area of special interest and a priority in numerous research.

Bagel & Erdemoglu have given values for daily intake of minerals by consuming herbal teas for persons with the weight of 70 kg: 500 mg Ca, 300 mg Mg, 15 mg Fe,
5 mg Al, 2.8 mg Mn, 15 mg Zn, 2.5 mg Cu, 1.6 mg Sr, 1.1 mg Ba, 0.025 mg Ni, 0.05-0.2 mg Cr, 0.04 mg Co, 0.415 mg Pb and 0.057 mg Cd [6].

Herbs accumulate toxic and heavy metals in different vegetative parts from air, water, and soil and can be used for bio-monitoring or as accumulators of pollutants for the purpose of bioremediation of the environment.

Plants adopt heavy metals from soils through roots, but under certain conditions, they intake them over above-ground parts. Intake of metals usually depends on soil’s properties, the characteristics of the plant species themselves, as well as their vegetative stage. Mobility and availability of heavy metals from soils is generally low, especially under normal pH values (pH=6.8-7.2) of soil, and high concentration of organic matter and clay [7]. Heavy metals are generally poorly mobile in plants, and their content in root is therefore greater than in above-ground parts of plant if the intake is happening dominantly through the root. However, some heavy metals (Cu, Mn, Zn, Mo and Cd) show fair mobility in plants, which has resulted in their transport to leaves [8]. The plant can adopt Pb over its root, but a negligible amount could be transported to the above-ground organs of the plant. In above-ground parts, plant adopts foliarly 90 to 99% of Pb [9].

Plant species from the family Apiaceae possess healing properties and have been used in traditional medicine from olden times. Only after the discovery of new modern methods for analysis and isolation of biological active components from plant material, their composition and function have been studied more in detail, and therefore some plant species have found application in pharmaceutical industry. However, heavy metal content is one of the limiting factors for the application, because the plants are able to accumulate significant amounts of heavy metals, showing no visible signs of damage [9]. Genus Seseli in Serbia is represented by a dozen species, some of which are those that grow in similar ecological conditions in the same territories and can hardly differs one from the other by the laymen, who usually collect and consume these plants. Very little-researched plant species S. pallasii, in that sense, in fact has been applied widely in traditional medicine and as a spice in human nutrition because of insufficient knowledge and confusion of the distinction from eg. Seseli rigidum or Levisticum officinale. This was the main reason to conduct present investigation-determination of concentrations of toxic metals (Cd, Pb, Cr and Ni) in S. pallasii, in order to estimate its safety for medicine and nutrition purposes. Regardless the fact that area from which plants were harvested is considered as unpolluted by human factor, it may ignore the possibility of natural presence of toxic elements in the soil (and thus in water), so it is essential to determine whether the plant is safe for human consumption.

**EXPERIMENTAL**

**Plant material**

Root and aereal parts of plant species *S. pallasii* were collected in flowering and fruting vegetative stage in the summer of 2013. from the Kravlje village area, Serbia. Voucher specimen was deposited in Herbarium of Department of Biology and Ecology, Faculty of Science and Mathematics (HMN), University of Nis, under the register number 7211.

**Preparation of plant material for analysis of heavy metals**

Samples of plants are thoroughly washed to remove any soil particles are present, then air-dried to constant weight, all parts of the plant, root, leaf, flowers and fruit are finely chopped, and finally passed through a 1 mm sieve. All samples were stored at -18°C. The metal content in the plant material was determined after the acidic treatment. First was added concentrated HNO₃, heated up in open glass to a small volume (until red vapors originating from NO₂ are removed); then digestion was continued with 70% HClO₄ and again evaporated to a low volume. Finally, the solutions were transferred to normal vessels, and diluted to a volume of 25 ml [10,11].

**Instrumentation and chemicals**

Analyses were carried out on a iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, UK) with an Echelle optical design and a charge injection device (CID) solid-state detector.

**Table 1. Operating conditions for iCAP 6000 ICP-OES**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flush pump rate</td>
<td>0.783 x g</td>
</tr>
<tr>
<td>Analysis pump rate</td>
<td>0.196 x g</td>
</tr>
<tr>
<td>Nebulizer gas</td>
<td>0.7 L/min</td>
</tr>
<tr>
<td>Coolant gas flow</td>
<td>12 L/min</td>
</tr>
<tr>
<td>Auxiliary gas flow</td>
<td>0.5 L/min</td>
</tr>
<tr>
<td>Plasma view</td>
<td>Axial/radial mode</td>
</tr>
<tr>
<td>Flush time</td>
<td>30s</td>
</tr>
</tbody>
</table>

**ICP-OES validation**

In order to check for matrix effects on the sensitivity and selectivity of detections, calibration lines established from multi-elemental standards were compared with standard addition curves obtained from nut samples spiked with the standard solutions. The best analytical lines were selected by the slope of both kinds of calibration line criteria. The chosen wave lengths, with statistically comparable slopes of calibration lines, limit of detection (LOD), limit of quantification (LOQ), correlation coefficient, intercept, slope, average RSD for repeatability of calibration solutions measurements and m_cal/m_sample ratio, are presented in Table 2.
Cd and Pb are toxic to humans even at low doses. These metals were also detected in plants as a result of increased industrialization and polluted biosphere. Therefore it is necessary to monitor their, even very low concentrations in potential sources, and therefore in the medicinal herbs.

By comparing the results obtained for the content of Cd and Pb with values defined by WHO [12], it can be concluded that the plant is grown in an unpolluted environment, and that there is no increased content of these heavy metals.

A certain amount of Cd and Pb in S. pallasii is comparable with their contents of the medicinal plants from the territory of Serbia [13]. Trace amounts of nickel may be useful for human organism, in particular for the enzyme activation, but at higher concentrations, can be toxic. Also, exposure to higher concentrations of Ni induces oxidative stress. According to Kabat-Pendias and Pendias, toxic concentration of Ni varies between plant species and varieties, ranging from 40 to 246 ppm [14]. Environmental Protection Agency (EPA) recommends a daily intake of Ni up to 1 mg for humans, while the increased concentration is toxic [15].

The concentrations of Ni in comparison to the other metals were relatively low and ranged from 0.62 ppm to 2.22 ppm in the leaves and in the fruits. The results have shown that the content of nickel is in normal concentration range [16], and comparable with the results of herbal teas (Matricaria chamomilla L., Melissa officinalis L., Mentha piperita L. and Foeniculum vulgare Mill.) in Serbia (0.738-6.034 ppm) [17]. Chromium is an essential trace metal that is required for normal metabolism and its deficiency can cause various disorders for both plant and for the consumers. It is known that chromium enhances insulin activity. The highest concentration of Cr was determined in the flowers 0.82 ppm, while in other plant’s parts were registered very similar amounts, 0.22 ppm in the leaves to 0.29 ppm in the roots. Concentration of Cr in all parts of the plant S. pallasii is within the limits of normal levels although it is higher in comparison with the content of Cr in medicinal plants Matricaria chamomilla L., Melissa officinalis L., Mentha piperita L. and Foeniculum vulgare Mill., which are widely used in traditional Serbian medicine. Some metals are mobile, and hence plant them adopt by the root from the soil. However, heavy metals can be adopted by plant through air pollution, but also during pollination (likely transfer via pollen). Based on these findings, the largest amount of all studied metals was found in the flower, except Ni, which may be due to the role of the flower in a vegetative period of the plant and its morphology.

### Table 2. Parameters of analytical calibration curves

<table>
<thead>
<tr>
<th>Element</th>
<th>Cd</th>
<th>Cr</th>
<th>Ni</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ (nm)</td>
<td>228.802</td>
<td>267.716</td>
<td>341.476</td>
<td>220.353</td>
</tr>
<tr>
<td>LOD</td>
<td>0.0828</td>
<td>0.0588</td>
<td>0.1807</td>
<td>0.2191</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.2759</td>
<td>0.1960</td>
<td>0.3024</td>
<td>0.7304</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.999946</td>
<td>0.999973</td>
<td>0.999741</td>
<td>0.999619</td>
</tr>
<tr>
<td>$b$</td>
<td>0.76</td>
<td>1.96</td>
<td>-2.27</td>
<td>0.09</td>
</tr>
<tr>
<td>$m$</td>
<td>1906</td>
<td>2282</td>
<td>2096</td>
<td>153</td>
</tr>
<tr>
<td>RSD %</td>
<td>4.07</td>
<td>3.46</td>
<td>3.03</td>
<td>0.87</td>
</tr>
<tr>
<td>ratio</td>
<td>1.18</td>
<td>0.94</td>
<td>1.22</td>
<td>0.90</td>
</tr>
</tbody>
</table>

### Quality control

Three measurements were performed for each sample as part of a quality control procedure. In order to annul the effects caused by the reagents, a blank was recorded, which was prepared in the same manner as the samples, only with the reagents used.

### RESULTS AND DISCUSSION

Heavy metals (Cd, Pb, Cr and Ni) were determined in vegetative parts of S. pallasii, root, leaf, flower and fruit. In the Figure 1. content of heavy metals (Cd, Pb, Cr and Ni) in the root, leaf, flower and fruit of plant species S. pallasii was shown.

![Figure 1. Content of Cd, Pb, Cr and Ni in S. pallasii (ppm - mass of the metal per mass of dry plant material basis)](image)

Cd concentrations are in the range of 0.11 to 0.25 ppm. The amount of toxic metal cadmium in the flower and the fruit is approximately the same and is 0.25 and 0.23 ppm, respectively, while the lowest amount of this metal was registered in the root, 0.11 ppm.

Concentration of Pb ranged from 1.42 to 3.14 ppm. The highest concentration of Pb was determined in the flowers- 3.14 ppm, while the lowest was in the leaves-1.42 ppm. In the root and in the fruit were registered approximately the same concentrations of Pb- 2.15 and 2.37 ppm, respectively.

Pb and Cd are trace metals which are not essential for either humans or plants, but can accumulate in biological systems and thus exert their toxic properties. Cd and Pb are toxic to humans even at low doses.
CONCLUSION

Studied toxic metals - Pb, Cd, Cr and Ni in the vegetative parts of the plant *S. palasii* were present in the amount that is acceptable for plant material aimed for use as raw material in pharmaceutical industry and for human nutrition. Their content is far below the prescribed daily intake limits, suggesting that the habitat of the plants is still unpolluted by the toxic metals.

REFERENCES


ACKNOWLEDGEMENTS

This paper is part of the Project by Ministry of Education, Scienceand Technological Development of Republic of Serbia, through grants No. 172047 and No. 172051

BIOGRAPHY

Marija Ilić was born in Aleksinac, Serbia, in 1983. She is PhD student at Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš. Her main areas of research include analytical chemistry, heavy metals in soil and plant, antioxidant activity of essential oils and plant extracts, and LC/IT-MS methods for POPs determination.
SADRŽAJ TOKSIČNIH METALA I
BEZBEDNA UPOTREBA Seseli pallasii
Marija Ilić, Vesna Stankov Jovanović, Violeta Mitić, Marija Dimitrijević,
Jelena Cvetković, Snežana Tošić

Apstrakt: Teški metali, bilo da potiču iz prirodnih ili antropogenih izvora imaju sposobnost biaokumulacije i biotransfera. Mogućnost kontaminacije biljaka teškim metalima zahteva veliku pažnju kada se primenjuju u farmaceutske i prehrambene svrhe jer neki metali u povećanoj koncentraciji mogu imati štetan uticaj na zdravlje ljudi i životinja. Samonikla lekovita biljna vrsta Seseli pallasii primenjuje se u narodnoj medicini kao začin u ishrani, te je utvrđivanje prisustva toksičnih metala u njoj od suštinskog značaja kako bi se se osigurala njena primena neškodljiva za zdravlje ljudi. Vegetativni delovi biljke S. pallasii (koren, list, cvet i plod) sa prostora jugoistočne Srbije analizirani su u pogledu sadržaja toksičnih metala (Cd, Cr, Ni i Pb) primenom indukovano spregnute plazme sa optičkom emisionom detekcijom (ICP-OES).

Rezultati pokazuju da je prosečna koncentracija Cd u nadzemnom delu gotovo duplo viša (0,21 ppm) nego u koren (0,11 ppm), Cr unadzemnom delu je 0,43 ppm, a u koren 0,29 ppm, Ni u nadzemnom delu 0,98 ppm, dok je u koren 0,68 ppm, dok je prosečna koncentracija Pb u nadzemnom delu u koren približno ista, (2,31 i 2,15 ppm, respektivno). Dobijeni rezultati pokazuju da je sadržaj toksičnih elemenata u ispitivanoj lekovitoj biljnoj vrsti ispod preporučenih granica i da se S. pallasii sa ispitivanog područja može bezbedno koristiti kako u tradicionalnoj medicini tako i u ishrani.

Ključne reči: Seseli pallasii, toksični metali, ICP, zaštita.