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DETERMINATION OF HEAVY METAL CONTENTS IN STINGING NETTLE FROM DIFFERENT LOCALITIES IN SERBIA

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Abstract

The stinging nettle (Urtica dioica L.) belongs to the Urticaceae family and represents a perennial plant. Stinging nettle is abundant species occurring in various types of forest, road verges and grassland sites. In many countries is used as both medicine and food. Stinging nettle is a weed and its seeds, leaves and even roots are used for medicinal purpose. Thanks to its high content of nutrients and bioactive compounds like poly phenols, vitamins and minerals, nettle possesses a great nutritional value and a large number of pharmacological effects, including anti-proliferative, anti-inflammatory, antioxidant, analgesic, immunostimulatory, anti-infectious, hypotensive, antiulcer activities and cardiovascular disease prevention. It is a reservoir of minerals (especially iron), vitamin C and pro-vitamin A.

Last decades the popularity of herbal medicine is rapidly increasing all over the world. However, heavy metal toxicity in plant materials has a great impact and importance on herbal plants and consequently affects the quality of herbal raw materials, herbal extracts, the safety and marketability of drugs. This paper presents determination of content seven heavy metals (Cu, Fe, Mn, Ni, Zn, Cd and Pb) in leaves samples Urtica dioica L. collected from five localities in Republic of Serbia. The sample preparation procedure involved dry digestion in triplicate and dissolution of the ash in 6M HCl and then in 0.1 M HNO_3. All elements were analyzed using an inductively coupled plasma-optical emission spectrometry (ICP-OES). The contents of Ni, Cu, Zn, Mn and Fe in the leaves were found to be in the ranges of 0.54±0.00 to 1.87±0.10 < 2.12±0.69-12.79±0.53 < 18.18±0.46-28.48±1.37 < 30.63±1.45-68.44±5.11, and 109.75±4.01-244.41±13.50 mg/kg dry weight, respectively. Value of toxic heavy metal Pb in two samples is 0.37±0.0 mg/kg dry weight, whiles content Cd has been below the detection limit. The content of heavy metals in samples were used to calculate target hazard quotients values (THQ) and hazard index (HI).

Key words: *Urtica dioica L, trace elements, heavy metals, Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES).*

Introduction

Wild plants are interesting due to their pharmacological potential and deliberated interest in the research due to their chemical composition.[1] Its chemical composition is very complex, consisting of flavonoids, alkaloids, enzymes, minerals and trace elements etc. [2-3] They are very often a source of medicinal raw materials.[4] In recent times, the popularity of herbal medicine is rapidly increasing all over the world. On same tame a rapid development in the diet studies focused on the determination of trace elements, which reflect their role in human health and nutrition. [5-6]. Medicinal plants are either direct or indirect source of minerals in human diet.[7] Metal ions in the chemical components of plants determine the medicinal, nutritious and toxic properties of the plant [8]. In report of World Health Organization, trace elements have been classified into three groups from the point of view of their nutritional importance in humans, in the following way: (1) essential elements; (2) elements which are probably essential; and (3) potentially toxic elements, some of which may nevertheless have some essential functions at low levels [9].

The stinging nettle (*Urtica dioica L.*) belongs to the Urticaceae family and represents a perennial plant. Stinging nettle is abundant species occurring in various types of forest, road verges and grassland sites. In many countries is used as both medicine and food a since stinging nettle are not aggressive and do not have severe side effects. [10-11] Stinging nettle is a weed and its seeds, leaves and even roots are used for medicinal purpose. Thanks to its high content of nutriments and bioactive compounds like poly phenols, vitamins and minerals, nettle possesses a great nutritional value and a large number of pharmacological effects, including anti-proliferative, anti-inflammatory, antioxidant, analgesic, immunostimulatory, anti-infectious, hypotensive, antiulcer activities and cardiovascular disease prevention.[12] It is a reservoir of minerals (especially iron), vitamin C and pro-vitamin A

The same plant species differ in microelement content under different ecological conditions, while diverse species in the same biotope accumulate different amounts of microelements [13-14] The human body needs proper concentration various microelement to maintain normal function and sustain life. [1] There are many metal ions are play very important roles in biological activities in the human body. Metals such as Cu, Fe, Zn, Ni and Mn are effectively used in biochemical processes in the human body.

Iron (Fe) is an important element for oxygen transport and ATP production. As redox-active metal it is involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis, production and scavenging of reactive oxygen species and pathogen defense.

Copper is of utmost importance for life and is an important coenzyme and cofactor in biochemical functions. Copper is essential for photosynthesis and mitochondrial respiration, for carbon and nitrogen metabolism, for oxidative stress protection, and is required for cell wall synthesis. Copper is an important component and catalytic agent of many enzymes and proteins in the body, so it can influence human health through multiple mechanisms.

Zinc is the second most abundant transition metal present in living organisms second only to iron. It is critical for the growth and survival of cells. Zinc is important as a component of enzymes for protein synthesis and energy production and maintains the structural integrity of biomembranes.

Nickel is essential in numerous prokaryotic enzymes like dehydrogenases, hydrogenases, and methyl-reductases.

Manganese plays a crucial role as a co-factor in various enzymes are known as "manganoproteins." These proteins include enzymes, like oxidoreductases, transferases and hydrolases, which are necessary for metabolic functions and antioxidant responses. Manganese plays a significant role in host defense, blood clotting, reproduction, digestion and various other functions in the body.

Pb and Cd are not biometals, but according to their physico-chemical characteristics they can replace biometals in certain biomolecules Pb and Cd show toxic effects on any levels have been known no beneficial properties and can be present in the plants in concentrations that are high enough to be a risk of acute poisoning. The presence of the toxic metals in plants are as a result of the anthropogenic influences. Generally, Pb in medicinal plants and spices, Cd in food are commonly caused by environmental pollution.[13] Some medicinal plants may cause health risks owing to their toxic element contents. It is necessary to determine the heavy metals concentration to know that the use of certain plants in the diet will not cause any harmful effects. Owing to the importance of minerals present in wild crafted plants in this paper presents determination of content seven heavy metals (Cu, Fe, Mn, Ni, Zn, Cd and Pb) in leaves samples Urtica dioica L. collected from five localities in Republic of Serbia. All elements were analyzed using an inductively coupled plasma-optical emission spectrometry (ICP-OES). These methods employ highly sensitive spectroscopic techniques and generally require destruction of the sample matrix to render a solution of the analyte ready for analysis. [14-19].

Materials and Methods

Materials and apparatus

All chemicals were of analytical grade, nitric acid (p.a. 65%, Merck, Germany), hydrochloric acid (p.a. 35%, Lach-Ner, Czech Republic), and deionized water of high purity was used (conductivity 0.05 µScm-1). Standard solutions of Cu, Fe, Ni, Mn, and Zn at a concentration of 1 mg/kg (Accu TraceTM Reference Standard, USA),

Heavy metals (Cu, Fe, Ni, Mn, Zn) concentrations were determined by Thermo Scientific iCAP 7000 Series model- inductively coupled plasma optical emission spectrometry (ICP- OES).

Sampling and Sample Preparation

All samples used in this research represent stinging nettle collected from five localities in Republic of Serbia. No other industrial developments exist within the region. The samples were collected by a standard procedure: at least 300 m from the main road, 100 m from the local road, and 5 m from the forest road at the depth of 0-30 cm. All samples were washed thoroughly and separatelly. Running tap water was employed to remove the dust and adhered particles. The samples were later rinsed thrice with deionized water and later on dried on the filter paper

Analytical Measurements

The sample preparation procedure involved dry digestion in triplicate. The samples of the plant material were burning and dissolving the ash in a suitable acid. In dry digestion, about 3 g of the sample (exact mass measured to 4 decimal places) was calcined gradually by raising the temperature by 50 °C/h, from room to 450 °C, at which the sample was kept for 8 h. The resulting ash was dissolved in 5 mL hydrochloric acid (c = 6 M) and the solution was evaporated in water bath to dryness. The residual precipitate was dissolved in ~10.0 mL nitric acid (c = 0.1 mol/dm^3), filtered, and washed with deionized water. The final volume was 50 mL.[20] The blank sample was prepared in the same way with no stinging nettle. All elements were analyzed using an inductively coupled plasma-optical emission spectrometry (ICP-OES) [21]. We have repeated this analysis three times with each sample collected from each site/region to get much more precision and less errors in our results.

Results and Discussion

Content of Heavy Metals

In this work, the content of essential (Cu, Fe, Mn, and Zn) and probably essential in trace (Ni) was determined using an inductively coupled plasma-optical emission spectrometry (ICP-OES).
All samples contain significant concentrations of essential elements Fe, Cu, Mn, Zn and Ni.

Among the investigated metals Fe was the highest *109.75±4.01-244.41±13.50 mg/kg dry weight.* Manganese was the second highest element found in all tea samples *30.63±1.45-68.44±5.11* mg/kg. The concentrations of Ni, Cu and Zn in the analyzed samples were in the range of *of 0.54±0.00 to 1.87±0.10 < 2.12±0.69-12.79±0.53 < 18.18±0.46-28.48±1.37* mg/kg respectively.

Table 1. Total contents of heavy metals (mg/kg dry weight) in samples of stinging nettle

Sample	Content of heavy metals (mg/kg)				
	Cu	Fe	Ni	Mn	Zn
1	4.89±0.31	109.75±4.01	0.54±0.00	30.63±1.45	18.18±0.46

2	**12.79±0.53**	**181.28±25.12**	**1.87±0.10**	**53.90±7.45**	**23.07±2.95**
3	**8.30±0.73**	**166.96±12.63**	**1.27±0.00**	**32.16±2.89**	**28.48±1.37**
4	**3.97±0.28**	**244.41±13.50**	**0.92±0.00**	**66.66±2.21**	**25.66±0.91**
5	**2.12±0.69**	**120.36±3.70**	**0.97±0.10**	**68.44±5.11**	**20.51±0.48**

Value of toxic heavy metal Pb in two samples is 0.37±0.0 mg/kg dry weight, whiles content Cd in all samples has been below the detection limit. As the results obtained in earlier research [13] showed that nettles have a to accumulate Pb which may be used in phytoremediation of polluted soil. Also, the content of heavy metals should be controlled, a special attention should be paid when someone individually collects the plant material.[22-23]

Calculation of Health Risk Assessment

The content of heavy metals in stinging nettle were used to evaluate the estimated daily intake (*EDI*) of heavy metals, target hazard quotients (*THQ*), and hazard index (*HI*) for adults.

Estimated daily intake (*EDI*) of heavy metals. The *EDI* value (mg/kg body weight/day) of heavy metals is a fundamental parameter for metal transfer from plant to human. It can be calculated using the following equation [24]:

$$EDI = \frac{C \cdot F_{IR} \cdot TR}{W_{AB} \cdot 1000} \quad (1)$$

where *C* is the heavy metal content (mg/kg) in stinging nettle, F_{IR} is the in stinging nettle ingestion rate (g/person/day), *TR* is the transference rate of the heavy metal from in stinging nettle, to infusion and W_{AB} is the average body weight for an adult, which is 70 kg.

Table 2. Estimated daily intake (EDI) (mg/kg bw/day),

No	EDI (mg/kg body weight/day) for adults				
	Cu	**Fe**	**Mn**	**Ni**	**Zn**
1.	$2{,}66\cdot10^{-4}$	$1{,}91\cdot10^{-3}$	$1{,}64\cdot10^{-3}$	$3{,}73\cdot10^{-5}$	$6{,}51\cdot10^{-4}$
2.	$6{,}96\cdot10^{-4}$	$3{,}16\cdot10^{-3}$	$2{,}89\cdot10^{-3}$	$1{,}29\cdot10^{-5}$	$8{,}27\cdot10^{-4}$
3.	$4{,}56\cdot10^{-4}$	$2{,}91\cdot10^{-3}$	$1{,}72\cdot10^{-3}$	$8{,}78\cdot10^{-5}$	$1{,}02\cdot10^{-4}$
4.	$2{,}16\cdot10^{-4}$	$4{,}26\cdot10^{-3}$	$3{,}58\cdot10^{-3}$	$6{,}36\cdot10^{-5}$	$9{,}19\cdot10^{-4}$
5.	$1{,}15\cdot10^{-4}$	$2{,}10\cdot10^{-3}$	$3{,}67\cdot10^{-3}$	$6{,}71\cdot10^{-5}$	$7{,}35\cdot10^{-4}$

Health Risk Assessment of Heavy Metals in the stinging nettle

This was computed based on the average contents of carcinogenic and noncarcinogenic metals determined in the stinging nettle samples using the probabilistic risk assessment model of the United States Environmental Protection Agency [25-26].

Target hazard quotient (THQ)

The targeted hazard quotient (THQ) was used to estimate the potential noncarcinogenic effects of individual heavy metals present in the stinging nettle. The THQ value was calculated by the following formula:

$$THQ = \frac{EDI}{RfD} \quad (2)$$

where the *THQ* is the dimensionless target hazard quotient, *EDI* is the estimated daily intake of heavy metals (mg/kg body weight/day), and *RfD* is the oral reference dose for the heavy metals (mg/kg body weight/day) [26-27] which does not produce a lifetime harmful effect.

Table 3. Target hazard quotient (THQ) and hazard index (HI) values of heavy metals for adults due to consumption of analyzed of stinging nettle.

No	THQ for adults					HI for adults
	Cu	Fe	Mn	Ni	Zn	
1.	$6{,}66\cdot10^{-3}$	$2{,}73\cdot10^{-3}$	$1{,}17\cdot10^{-1}$	$1{,}87\cdot10^{-3}$	$2{,}17\cdot10^{-3}$	0,1308
2.	$1{,}74\cdot10^{-2}$	$4{,}52\cdot10^{-3}$	$2{,}07\cdot10^{-1}$	$6{,}46\cdot10^{-3}$	$2{,}76\cdot10^{-3}$	0,2377
3.	$1{,}14\cdot10^{-2}$	$4{,}16\cdot10^{-3}$	$1{,}23\cdot10^{-1}$	$4{,}39\cdot10^{-3}$	$3{,}40\cdot10^{-3}$	0,1466
4.	$5{,}41\cdot10^{-3}$	$6{,}09\cdot10^{-3}$	$2{,}55\cdot10^{-1}$	$3{,}18\cdot10^{-3}$	$3{,}06\cdot10^{-3}$	0,2732
5.	$2{,}88\cdot10^{-3}$	$3{,}00\cdot10^{-3}$	$2{,}62\cdot10^{-1}$	$3{,}35\cdot10^{-3}$	$2{,}45\cdot10^{-3}$	0,2739

Hazard index (HI).

The overall potential health risks may product from the influence of more than one metal. The cumulative potential noncarcinogenic effects of multiple heavy metals present in the stinging nettle can be quantitatively evaluated by the hazard index (HI) [26-27]. Different contaminants there are variety potential non-carcinogenic effects. Consequently, exposure to two or more heavy metals may result in additive and/or interactive effects [27-28]. If it is assumed that the toxic effects of heavy metals in herbal tea are additive rather than synergistic or antagonistic to human health, HI can be estimated by the sum of the target hazard quotients of all studied pollutants and can be calculated by the following equation [27]:

$$HI = THQ_1 + THQ_2 + THQ_3 + \cdots + THQ_n \quad (3)$$

where THQn is the THQ value of an individual metal and HI is the hazard index of multiple metals. All determinations were carried out in triplicate. Experimental data was subjected to ANOVA test and statistical significance was obtained at $p < 0.05$. Finally, the data was expressed as mean standard deviation ($\pm$ SD) (mg kg^{-1}, dry weight) .

When the calculated HI value is less than one, no significant risk of non-carcinogenic consequences is expected, but if it is higher, there is a possibility that non-carcinogenic risk effects may occur. Heavy metals have a negative impact on human health, which increases with increasing HI values [30]. If the HI value is greater than 10.0, there is a chronic toxic effect on human health [32-33].

The HI values of all investigated heavy metals via the consumption of stinging nettle infusions are given in Table 3. It was discovered that HI values for all the tested heavy metals, in the five samples were below 1. This enabled us to conclude that there is actually no carcinogenic risk from the drinking of infusions of these stinging nettle, in amounts and with the frequency assumed in this paper.

Conclusion

Five samples of stinging nettle widely consumed among Serbian population were analyzed for concentration of microelements, with an aim of establishing the mineral status and hence the health safety of medicinal plants.

The results of determination of the bioelements (Cu, Fe, Mn, Zn and Ni) content in nettle (Urtica dioica L.) have shown that this plant, contains significant amounts of all examined metals. The highest contents of essential heavy metals were established for Fe and for this reason, these plants are beneficial Fe source for humans. Also, the health risk assessment showed that nettle does not represent a health risk for consumers. The results have shown that this plant, contains amounts of all determination metal which satisfies a certain percentage of the daily requirement for other examined biometals. Considering that it is possible to consuming plants as a supplement and it can be concluded that nettle is an excellent nutrient for fulfilling a high percentage of the recommended daily allowance for adults for examined biometals.

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REFERENCES

C. R. Gupta, (2016). *Nutraceuticals: efficacy, safety and toxicity*, Amsterdam, Elsevier Inc., Pravilnik o kvalitetu čaja, biljnog čaja i njihovih proizvoda. Republic of Serbia (2012).

World Health Organization. WHO, (2002). Traditional medicine strategy 2002–2005. Official Gazette of RS, 4/2012 Geneva;.

A. Mehra, C.L. Baker, (2007)“Leaching and Bioavailability of Aluminium, Copper and Manganese from Tea (Camellia sinensis),” Food Chem., 100(4), 1456-1463.

S. Ražić, V. Kuntić, (2013). Diverse elements in herbal tea products consumed in Serbia using inductively coupled plasma mass spectrometr. Int. J. Food Prop., 16 (1), 1-8.

M. Xie, A. von Bohlen, R. Klockenkämper, X. Jian and K. Günther, Z Lebensm (1998). Multielement analysis of Chinese tea (Camellia sinensis) by total-reflection X-ray. Unters Forsch 207, 31–38. M. J. Gibney, H. V. Vorster, F. J. Kok, (2009).Introduction to human nutrition, Wiley-Blackwell Oxford, United Kingdom.

R. B. Mack, (1995). Lead in history, Clinical Toxicology Bulletin, 3; 7-44.

World Health Organization. WHO, (1996). Trace Elements in Human Nutrition and Health Geneva.

M. L. Leporatti, S. Ivancheva,(2003). Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy, Journal of Ethnopharmacology, 87(2–3) 123–142.

S. Jarić, Z. Popović, M. Mačukanović-Jocić, L. Djurdjević, M. Mijatović, B. Karadžić, M. Mitrović, P. Pavlović,(2007). Anethnobotanical study on the usage of wild medicinal herbs from Kopaonik Mountain (Central Serbia), Journal of Ethnopharmacology, 111(1) 160–175.

I. Arsić, S. Đorđević, D. Runjaić-Antić, M. Ristić (2004). Funkcionalnom hranom protiv tegoba anemije, Zbornik radova 10. Naučno-stručnog skupa Ishrana i zdravlje: Minerali, vitamini, drugi dodaci hrani i njihov zdravstveni značaj, Naučnoistraživački centar Užice, 3D Grafika,, p. 56-60.

Vladimir D. Dimitrijević, Nenad S. Krstić, Maja N. Stanković, Ivana Arsić, Ružica S. Nikolić.(2016). Biometal and heavy metal content in the soil –nettle (*Urtica dioica* L.) system from different localities in Serbia, Advanced technologies 5(1) 17-22.

S. M. Petrović, S. R. Savić, M. Lj. Dimitrijević, Ž. B. Petronijević, (2015). The determination of micro and macroelements in chamomile teas (Matricaria chammomilia L.), Advanced technologies, 4(2) 37-42.

S. Razić, A. Onjia, S. Dogo, L. Slavković, A. Popović, (2005). Determination of metal content in some herbal drugs-Empirical and chemometric approach, Elsevier, Talanta, 67 (1) 233–239.

J. Malik, J. Szakova, O. Drabek, et al.,(2008). Determination of certain micro and macroelements in plant stimulants and their infusions. *Food Chem*., 111, 520 – 525.

N. Blagojević, B. Damjanović-Vratnica, V. Vukašinović-Pešić,et al.(2009). Heavy Metals Content in Leaves and Extracts of Wild-Growing Salvia Officinalis from Montenegro, *Pol. J. Environ. Stud*., 18(2), 167 – 173.

N. S. Mokgalaka, R. I. McCrindle and B. M. Botha, (2004). Multielement analysis of tea leaves by inductively coupled plasma optical emission spectrometry using slurry nebulisation*J. Anal. At.Spectrom*, 19, 1375 – 1378.

S. Ražić, A. Onjia and B. Potkonjak (2003). Trace elements analysis of Echinacea purpurea, *J. Pharm. Biomed. Anal.*,33, 845 – 850 (2003).

Official Methods of Analysis of AOAC International, 17th Ed. Official Method 999.11. Gaithersburg, MD, USA: AOAC International (2000).

B. Gouthami and R. R. Nageswara,(2015). "An overview of applications of atomic absorption spectroscopy in the determination of inorganic impurities in drugs an plants and its extracts," *International Journal of Basic and Applied Sciences*, vol. 1, no. 2, pp. 37–45.

G. Brhane and T. Shiferaw,(2014). "Assessment of levels of lead, cadmium, copper and zinc contamination in selected edible vegetables from Bahir dar market, garden of Bahir dar town and Adet agricultural research center in Ethiopia," *International Journal of Innovation and Applied Studies*, vol. 7, no. 1, pp. 78–86.

B. Girmaye, (2014)."Assessment of heavy metals in vegetables irrigated with Awash River in selected farms around Adama town, Ethiopia," *African Journal of Environmental Science and Technology*, vol. 8, no. 7, pp. 428–434.

J. Zhang, R. Yang, R. Chen, et al. (2018). Accumulation of Heavy Metals in Tea Leaves and Potential Health Risk Assessment: A Case Study from Puan County, Guizhou Province, China, Int J Environ Res Public Health.,15(1), 133-155.

Fao/Who,(2012). *Food Additives and Contaminants, Joint Codex Alimentarius Commission*, FAO/WHO Food standards Programme, Rome, Italy.

U.S. Epa, (2015). "Supplementary guidance for conducting health risk assessment of chemical mixtures," *Risk Assessment Forum Technical Panel*, US.EPA, Washington, DC, USA.

Unite States Environmental Protection Agency, Concepts, Methods and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures and Effects: A Resource Document;(2007). EPA/600/R-06/013F, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH, USA.

F. Adusei-Mensah, D. K. Essumang, R. O. Agjei, et al., (2019). Heavy metal content and health risk assessment of commonly patronized herbal medicinal preparations from the Kumasi metropoliJ Environ Health Sci Eng. 17(2), 609-618.

P.F.S. Tschinkel, E. S. P. Melo, H. S. Pereira, et al. (2020). The Hazardous Level of Heavy Metals in Different Medicinal Plants and Their Decoctions in Water: A Public Health Problem in Brazil Biomed Res Int., 1465051.

US Environmental Protection Agency, (2002). Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites, OSWER 9355. Office of Emergency and Remedial Response, Washington.

C. Kamunda, M. Mathuthu, and M. Madhuku,(2016). "Health risk assessment of heavy metals in soils from witwaters-rand gold mining basin, South Africa," *International Journal of Environmental Research and Public Health*, vol. 13, no. 7, p. 663.