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Original Research

Bioaccumulation and Translocation of Some Transition Metals in *Mentha spicata* and *Mentha longifolia*

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Abstract

The aim of this paper is to evaluate the potential mechanisms of the absorption and distribution of metals in the aromatic herbs *Mentha spicata* and *Mentha longifolia*, as well as their potential for the bioaccumulation of these elements. The herbs were sampled from the slopes of Mount Bjelasica in Montenegro. After the microwave processing of the herb samples and the associated soil, the content of the investigated metals Cu, Zn, Ni, Fe and Mn were determined using atomic absorption spectrophotometry. Taking average values from various parts of both types of mint, the metals were present in the following descending order: Fe (570 mg/kg) > Mn (54.0) > Zn (30.6) > Cu (27.2) > Ni (2.45). The metals under investigation are most commonly present in the roots of the mint, except in the case of Ni in *M. longifolia*. Manganese is more commonly found in the tissues of *M. longifolia* than in *M. spicata*, while the other investigated metals are found relatively uniformly in both types of mint. The bioaccumulation potential of *M. spicata*, from the soil to the roots, is greatest for Cu and Fe, and generally uniform, with the next highest potential being shown by Zn.

Keywords: medicinal plants, mint, *Mentha spicata*, *Mentha longifolia*, tea

Introduction

Medicinal plants are perhaps most widely used to make tea, which is one of the most extensively consumed drinks on the planet. In addition, medicinal plants form the basis for many herbal preparations, remedies and supplements [1, 2]; these have been used as important medicines for a range of illnesses since ancient times [1, 3]; as well as passive biomonitors [4]; their potential as phytoremediators is increasingly being investigated [5-6]; as flavours, aromas and biopreservatives in various foodstuffs [7]; as dietary supplements, given they contain a range of important nutrients (vitamins, minerals and amino acids) [8]; and as as suitable alternatives to synthetic pesticides [9].

Metal ions in plants affect their medicinal and nutritional properties, and may make them toxic above certain concentrations specific to each element [10].

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An awareness of the concentration of certain metals is important in understanding the pharmacological effects of these medicinal plants and in ascribing the correct dosage [3]. Certain metals play an important role in the biosynthesis of complex bioorganic compounds in plants [3]. Metals engage in a range of chemical reactions in plants, including as cofactors for some enzymes [3, 11] or else independently in redox or catalytic reactions [12].

There are a number of reasons why it is important to determine and monitor the levels of particular metals in medicinal plants. Plants play a major role in the transfer of metals from the earth to humans, mostly through the food chain [3]. About 80% -85% of the world's population uses various medicinal plants, as primary health care and as preparations made from herbs or other herbal medicines [13-14]. In China, 85.20% of the total number of COVID-19 cases were successfully treated with herbal preparations in all stages of infection [15-16]. Moreover, there is a general misconception that natural herbs and preparations are safe to consume in and of themselves. However, there have been numerous reports of incidents caused by toxicity or other side effects associated with the use of medicinal plants, or plant-based remedies, around the world [17-18].

It is also true that the same plant can differ significantly in terms of its microelement content due to being grown in different ecological contexts, while different species of plant will accumulate different quantities of the same microelements from the same biotype or habitat [11].

Despite their significant potential, and the floral richness of our meadows, pastures and forests, medicinal plants and herbs are still not sufficiently widely exploited. The collection of medicinal plants is still in the main an individual activity, which is disorganized and not sufficiently regulated. One of the most famous medicinal plants in our region is mint. Although it grows wild, like a weed, it is a very good means of alleviating various health problems.

There is very little data in the literature on the content of trace metals in the medicinal plants found in Montenegro. As far as we know, no study has been conducted to measure heavy metals in mint from Montenegro.

Samples taken from higher mountain areas can be assumed to be relatively unpolluted and free of anthropogenic influence, and as such we can material from these locations as control or reference samples.

In our paper, we determine the levels of content of various trace metals (Cu, Zn, Ni, Fe and Mn) in the parts of *Mentha spicata* L. and *Mentha longifolia* L., sampled from the slopes of Bjelasica in Montenegro.

The name of our paper is to analyse the relationship between the content of the metals in the samples of mint, and their distribution throughout the plant tissue, which may reveal uptake pathways, their translocation ability and their potential for bioaccumulation in the investigated plants.

Materials and Methods

Study Area

Mount Bjelasica (Fig. 1) occupies an area in the central-continental part of Montenegro, lying between 42° and 43° north in latitude and 19° and 20° east in longitude, and having circular shape which is 30 km in diameter in total [19].

The massif of Bjelasica is 30 km long and covers a total area of 851 km^2. Mount Bjelasica is among the most important mountain regions in southern Europe in terms of the richness and wealth of its flora and fauna [20].

Bjelasica is particularly famed for the wealth and variety of its flora. Thanks to the very significant concentration of different species found there (numbering between 1200 and 1400 different taxa, including species and subspecies), the area has been

Fig. 1. Locations of the sampling stations around Mount Bjelasica.

identified as one of the so-called biodiversity hotspots for vascular flora in Montenegro. Indeed, among the high mountain flora found on Mount Bjelasica, a great number of species are endemic. Eleven of the habitats listed in Appendix I of the Berne Convention (i.e. habitats covered by the EMERALD and NATURA 2000 projects) are present on Mount Bjelasica. Due to the presence of an extremely large number of endemic plant species and habitats, the area of Biogradska gora (in the wider Bjelasica region) is recognized as an IPA area (Important Plant Area), and is also a protected national park and a UNESCO Biosphere Reserve [20].

Sampling

The samples of *M. spicata* and *M. longifolia* were collected from four locations on the southwest slopes of Mount Bjelasica while the mint was in flower, during the first half of August 2021. The selected locations were all situated within about 500 m of each other, at around 1000 m above sea level. All the sampling sites were at least 100 m from rural roads, settlements and other buildings. 5-7 healthy plants of a similar size were sampled from each location and packed in paper packaging for transport to the laboratory. The identification of the species of mint was performed at the Department of Biology of the Faculty of Natural Sciences and Mathematics of the University of Montenegro.

Soil samples were also taken from the same sites as the mint samples, from a depth profile of 0-25 cm. Larger plant remains and stones were removed on the spot. The sampled soil was then stored in plastic boxes for transportation to the laboratory.

Analysis of the Plant and Soil Samples

The plant samples were dried in air, at room temperature, then separated into root, stem and leaf and ground with a plant material grinder. The soil samples were also air-dried, then ground in a mortar and sieved through a 1.5 mm sieve.

The "pseudo"-total forms of the examined metals in the plant material was determined after the preparation of samples in a closed microwave system, using a Milestone Ethos 1, under high pressure according to the method of US EPA 3051A [21]. A sample of 0.5000 g (±0.0001 g) was then dissolved using 10 mL of concentrated HNO_3 and 2 mL of concentrated H_2O_2 at 180ºC for 15 minutes. After being dissolved, the solutions were diluted with distilled water in normal 50 mL vessels.

The mineralization of sieved sediment samples weighing 1.0000 g (± 0.0001 g) was performed under pressure and at high temperature, using a mixture of 37% (w/w) HCl : 65% (w/w) HNO_3 (3:1 v/v) [21]. After being dissolved, the solutions were diluted with 2 M HNO_3 in normal 100 mL vessels.

Some soil chemical analyses were carried out according to the methods described by [22]. In order to determine the amount of mobile and bioavailable metals, extraction using 0.1 M EDTA was performed. In this way, the real conditions prevailing in the environment were simulated. The soil/0.1 M EDTA mixture (1 g: 50 mL), pH 6, was stirred for 1 hour using a magnetic stirrer and then filtered.

The contents of the tested metals in the samples were determined using an atomic absorption spectromety (PinAAcle 900, Perkin Elmer). The determinations were performed using an air-acetylene flame, with openings 0.7 nm for Cu and Zn and 0.2 nm for Fe, Ni and Mn. The lamp current was 25 mA for Cu, Mn and Zn and 40 mA for Fe and Ni. The wavelengths at which Cu, Zn, Ni, Fe and Mn were determined were (nm): 324.8; 213.9; 232.0; 248.3 and 279.5, respectively.

The accuracy and precision of the method were evaluated using the certified reference material, in this case NCS ZC73014 Tea leaves (National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA) and the relevant standard for trace elements in the soil (SRM 2709). The reproducibility of the results was between 8.40-10.2 % of the certified values.

Chemical and Reagents

The deionized water (electrical resistivity >10 MΩ cm) was used in all experiments. Nitric acid (Merck), hydrochloric acid (Sigma Aldrich, Steinheim, Germany), and hydrogen peroxide (Merck) with analytical purity were used in the digestion processes. Basic standard solutions of Cu, Zn, Ni, Fe and Mn concentrations of 1000 mg/l were made by Merck. The standard solutions were diluted with deionized water to bring the concentration of the elements into a suitable concentration range.

Statistical Analysis

The Microsoft Excel 2000 package was used to calculate the mean and standard deviations. The significance of the variations in the content of the tested metals between the different organs of *M. spicata* and *M. longifolia* was assessed using one-way analysis of variance (ANOVA I and II). The post-hoc test was applied when the differences were significant (SPSS 2012). Fisher's LSD test of the least significant difference was also used to compare the mean values between the individual metals in both mints.

The bioaccumulation factor, BCF, as an indicator of the plant's ability to absorb metal from the soil, was calculated as follows: $BCF = [Metal]_{part\ of\ plant} / [Metal]_{soil}$ [1]. The translocation factor, TA, as an estimate of metal mobility through plant organs, was calculated as follows : $TA = [Metal]_{above\text{-}ground\ part\ of\ the\ plant}/[Metal]_{root}$ and $TA = [Metal]_{leaf}/[Metal]_{stem}$ [1].

Correlation analysis was used to determine the relationship between the contents of the individual metals (Cu, Zn, Ni, Fe and Mn) in the examined mint

Table 1. Some chemical characteristics of the soil.

	pH	*Exchangeable* pH	Carbonates (%w/w)	Total organic carbon (%w/w)	Organic matter (%w/w)
Min-max	5.92-6.71	5.43-6.41	2.14-5.91	1.92-3.96	3.30-6.84
Average ± SD	6.41±0.39	5.95±0.42	3.32±1.75	3.27±0.93	5.63±1.61

samples. The correlation coefficient (Pearson, r) was calculated using the IBM SPSS Statistic 20 software package.

Results and Discussion

Table 1 gives some chemical characteristics of the soil from which the plant samples were taken.

Table 2 presents the results of the analyses of the content of the tested metals in the ambient soils of the tested mints, as well as estimates of their bioavailability and content by parts of *M. spicata* and *M. longifolia*.

The mean "pseudo"-total content of the tested metals in the soil from which the mint samples were taken decrease in the following order: Fe (2384 mg/kg) > Mn (822 mg/kg) > Zn (176 mg/kg) > Cu (66.4 mg/kg) > Ni (60.1 mg/kg). However, the total metal content in the soil does not mean that all of it is bioavailable to the plants. Plants can absorb only easily soluble, accessible and mobile forms of metal. The descending order of the content of bioavailable forms of metals in the soils from which *M. spicata* was sampled was: Fe (469 mg/kg) > Mn (276 mg/kg) > Cu (24.7 mg/kg) > Zn (12.2 mg/kg)> Ni (6.75 mg/kg). The amount of bioavailable Ni (9.94 mg/kg) was greater in the soil samples from which *M. longifolia* was taken than the quantity of Zn (3.37 mg/kg). The descending order of the share of bioavailable amount of the metals in relation to the "pseudo"-total content, taken together for both sampled soils was: Cu (38.4% wt) > Mn (29.2% wt) > Fe (14.0% wt) ~ Ni (13.8% wt) > Zn (4.46 % wt).

Table 2. Metal concentrations (mg/kg dw) in soil and parts of *Mentha spicata* and *Mentha longifolia*, minimal and maximal concentrations and average concentrations±standard deviation; n = 3.

Metal		min.-max. Average±S.D.				
		Content in soil		Content in plant		
		„Pseudo"-total	Bioavailable	Root	Stem	Leaf
Cu	*M.s.*	60.7–78.1 68.6±14.3	22.1-23.9 24.7±2.13	32.3-35.2 33.4±1.27 **a(b)**	29.5-36.7 30.8±3.19 **a(b)**	15.2-19.7 17.3±2.05 **a(b)**
	M.l.	58.3-72,6 64.1±12.6	25.1-29.8 26.2±2.51	29.3-34.4 32.8±2.61 **a(b)**	26.1-31-6 28.2±2.33 **a(b)**	17.9-22.8 20.7±2.39 **a(b)**
Zn	*M.s.*	166-201 181±14	8.93-14.8 12.2±2.81	41.3-47.6 44.3±1.16 **ab(b)**	19.4-22.1 25.1±2.03 **bc(b)**	18.2-22.1 20.1±1.89 **bc(b)**
	M.l.	154-195 171±16	2.82-4.19 3.73±0.62	55.1-64.5 58.2±4.47 **a(b)**	8.13-12.9 10.6±1.32 **bc(b)**	21.2-30.7 25.5±4.12 **bc(b)**
Ni	*M.s.*	44.3-80.3 57.4±12.1	3.44-9.32 6.75±1.31	3.72-4.19 3.85±0.18 **a(c)**	0.79-1.21 0.97±0.21 **a(c)**	3.26-3.68 3.52±0.16 **a(c)**
	M.l.	51.4-79.5 62.8±10.3	7.73-12.4 9.94±2.16	2.58-3.42 2.91±0.35 **a(c)**	0.34-0.41 0.37±0.03 **a(c)**	2.85-3.54 3.06±0.32 **a(c)**
Fe	*M.s.*	2 360-2 478 2 423±48	383-512 469±25	991-1 273 1 136±83	282-349 336±31	206-232 214±15
	M.l.	2 344-2 536 2 478±53	208-231 214±16	1 345-1 472 1 457±92	87.3-102 94.1±12	161-205 183±13
Mn	*M.s.*	721-934 826±73	223-376 276±43	65.1-74.2 69.5±3.83 **ab(a)**	32.9-40.2 36.8±3.13 **b(a)**	34.1-38.7 37.6±2.03 **b(a)**
	M.l.	707-919 817±87	171-292 204±32	74.6-83.8 78.8±4.21 **a(a)**	46.0-51.2 49.1±2.21 **b(a)**	43.2-61.3 52.3±4.67 **b(a)**

*The values of individual metals with the same first letter(s) are not significantly different at p = 0.05 between different part of the plants

**The values in individual metals with the same letter(s) in parentheses are not significantly different at p = 0.05 between plants different metals

The application of ANOVA variance analysis to the results of individual metal content shows statistically significantly ($p = 0.05$) greater Fe results compared to the other metals. In this case, there is no significant difference between Cu, Zn, Ni and Mn. Furthermore, there is no significant difference in the metal content between individual parts of mint other than in the case of Fe in the root. Therefore, we excluded Fe in the analysis of variance (Table 2). $F = 16.716$ was calculated for Cu, Zn, Ni and Mn in two mints (6 results). The tabular value of F for the significance level of 0.05 and the number of degrees of freedom $\varphi_1 = 3$ and $\varphi_2 = 20$ is 3.098. To determine which mean values differed significantly from each other, we applied the Tukey-Snedecor test. The tabular value for Q (4.20) = 3.96, so the maximum difference between the mean values was D = 20.440. There was no statistically significant difference between the Cu and Zn content in some parts of the mint. The contents of Mn are statistically significantly greater and Ni are statistically significantly lower. The Cu and Ni content do not differ statistically by individual parts of the mint. The content of Zn and Mn (in the root) differ statistically significantly by individual parts of mint.

The smallest significant difference for $p = 0.05$, based on Fisher's test between the mean values of Cu, Zn, Ni and Mn in the two mints when compared together is LSD = 19.34.

Table 3 presents the bioaccumulation abilities of *M. spicata* and *M. longifolia*, given as the bioconcentration factor, BCF, for the tested metals and their translocation abilities, TA, through the plant organs.

M. spicata and *M. longifolia* showed a similar trend of bioaccumulation. The difference is that *M. longifolia* showed a slightly higher bioaccumulation ability for Fe compared to Cu. Manganese and Ni also showed weak bioaccumulation but the rate was slightly higher for Mn compared to Ni. Different BCF values for the tested metals can be found in the literature [1, 5, 23-24]. Obviously, the amount of metal absorbed from the soil does not depend only on the bioaccumulation capacity of the plant for certain metals. It is probable that the accumulation of individual metals also depends on the quantity present and the species in the soil, as well as the climate, the age of the plant, the synergism and antagonism of the binding metals in the plant and so on. The results of this and other works indicate that there is a complex mechanism that determines the intake of metals by plants.

The translocation of the studied metals through the organs of *M. spicata* is greatest for Ni and lowest for Fe. In the paper Patel et al. [25], Ni also shows high translocation through the organs of *M. spicata*. The descending order of translocation of the tested metals through the organs of *M. longifolia* was: Ni > Zn > Fe > Mn > Cu. In Jena et al. [1], the mean values of Cu and Zn translocation through *M. longifolia* tissues are identical. The manganese and Fe translocations are similar and larger than those for Cu and Zn. In the work of Dina et al. [5] data showed inefficient metal translocation between *M. piperita* organs (TA <1).

Table 4 shows the comparative values of the metal content in mint in our work with the results recorded from other regions.

Table 3. Mean values of the bioaccumulation factor, BCF, for the tested metals in the mint parts and mean values of the translocation capacity, TA.

Bioconcentration factor, BCF						
	Metal→	Cu	Zn	Ni	Fe	Mn
Mentha spicata	root/soil	0.49	0.24	0.07	0.47	0.08
	stem/soil	0.45	0.14	0.02	0.14	0.04
	leaf/soil	0.25	0.11	0.06	0.09	0.05
Mentha longifolia	root/soil	0.51	0.34	0.05	0.59	0.10
	stem/soil	0.44	0.06	0.01	0.04	0.06
	leaf/soil	0.32	0.14	0.05	0.07	0.06
Translocation ability, TA						
Mentha spicata	stem/root	0.92	0.57	0.25	0.30	0.53
	leaf/stem	0.56	0.80	3.63	0.64	1.02
	leaf/root	0.52	0.45	0.91	0.19	0.54
Mentha longifolia	stem/root	0.86	0.18	0.13	0.06	0.62
	leaf/stem	0.73	2.40	9.73	1.94	1.06
	leaf/root	0.63	0.44	1.05	0.12	0.66

Table 4. Comparative presentation of metal content in *M. spicata* and *M. longifolia* with results from other regions.

M. spicata					Region	Reference
Cu	Zn	Ni	Fe	Mn		
		/			Eastern Anatolia, Turkey	[7]
					East Serbia	[11]
		/		/	The local markets of Dubai	[17]
					Damour urban area-Lebanon	[26]
/	/		/	/	Sewage Treatment Plants, India	[27]
		/			Cundinamarca (Colombia)	[28]
					North-West parts of India	[29]
M. longifolia						
		/			Chhattisgarh, Central India	[1]
	/				Southeast of Turkey	[3]
		/			Eastern Anatolia, Turkey	[7]
					Greater Cairo, Egypt	[23]
				/	Kurdistan Region - Iraq	[30]
					Bosnia and Herzegovina	[31]
		/			Khushab District, Pakistan	[32]

Higher results in our research

Similar results as in our paper

Lower results of our research

The level of Cu content is generally greater in our study than in other regions, while the levels of Zn and Fe are generally lower. The reason may be that Cu in our soil samples is largely in a variable, easily accessible fraction (39.3% wt). Zinc is the most immobilized metal in the soil samples and only 5.65% wt of its total content is bioavailable to plants. The available Mn results are generally either higher or lower than the results given by other authors. Nickel content almost uniform in scale, either slightly higher or lower than results from other regions.

In our country, there is no regulation as to the maximum permissible concentrations of transition metals in food (including herbal teas). We wanted to evaluate the possibility of using the tested types of mint for medical purposes or as nutritional supplements. Therefore, we compared our results with the Land Register of Concentrations of Elements in Ecosystems [33] and with the normal ranges of Cu, Zn, Ni, Fe and Mn in plants [34-35]. This data is listed in Table 5.

The copper content in the root and the stem of the examined mints are slightly greater than the usual Cu values in plants. Iron content in both the root and the stem of *M. spicata* are greater than the normal values in plants. The zinc content in the stem of *M. longifolia* is slightly lower than the normal plant content. The nickel and Mn contents in the parts of the tested mints are in the range of values that are common to plants.

Sometimes it is not possible to compare the results of the metal content in the same plant species from

Table 5. Normal content of several transition metals in plants (mg/kg).

mg/kg →	Cu	Zn	Ni	Fe	Mn
Average ECCE*	2-20	15-150	00.4-4	5-200	1-700
Normal range in plants**	1.5-20	20-100	0.02-50	30-300	15-150

*Element Concentration Cadastres in Ecosystems (ECCE): (1994): Progress Report. Presented at the 25th General Assembly of International Union of Biological Sciences (IUBS), Paris, France

**Chaney 1989; Kastori et al. 1997

Table 6. Pearson regression coefficient, r, between the content of tested metals in *M. spicata* and *M. longifolia*.

	Cu	Zn	Ni	Fe	Mn
Cu	1	0.58	-0.22	0.68	0.60
Zn		1	0.54	**0.97**	**0.86**
Ni			1	0.46	0.41
Fe				1	**0.89**
Mn					1

different habitats. The metal content in the plant depends on numerous factors: soil characteristics, climate, anthropogenic factors, time and manner of sampling, part of the sampled plant and various others.

Table 6 presents the correlation of the content of the selected metals in two different mint samples.

The connection between the concentrations of the tested metals in both plants is noticeable. The contents of Zn and Fe, Zn and Mn and Fe and Mn in *M. spicata* and *M. longifolia* are significantly positively correlated. Copper is in a medium-high positive correlation with Zn, Fe and Mn, while it is negatively correlated with Ni. Nickel is moderately highly positively correlated with Zn, while it is relatively weakly correlated with Fe and Mn. In the results provided by Mandal [31], in the tissues of *M. longifolia* there is a strong correlation between the following metal pairs: Zn-Cu, Zn-Ni, and Cu-Ni.

Conclusion

Iron is the most common metal in the examined plants, with the mean value of the content of individual parts of mint being 570 mg/kg. This is followed by Mn (54.0 mg/kg), Zn (30.6 mg/kg) and Cu (27.2 mg/kg). Nickel (2.45 mg/kg) is the least common of the tested metal in the tissues of the examined mint.

Manganese is more commonly present in *M. longifolia* tissues than in *M. spicata*. Zinc is slightly more common in *M. longifolia* tissues, while Ni is more present in *M. spicata* tissues. The copper and Fe contents were uniform in both tested species.

The tested metals are most present in the mint root, except for Ni in *M. longifolia*. The order of metal content in the aboveground parts depends on the type of mint and the metal in question. The decreasing content of Cu and Fe by parts of the examined mint are: root > stem > leaf. Zinc and Mn decrease in the order: root > leaf > stem.

The bioaccumulation capacity of the tested mints, from soil to root, is greatest for Cu and Fe, followed by Zn, while the lowest and weakest bioaccumulation is shown by Mn and Ni.

The translocation of the studied metals through the organs of *M. spicata* is greatest for Ni and lowest for Fe. Nickel also showed the highest level of motility through the tissues of *M. longifolia*. However, the least translocation from root to leaf, in contrast to the results for *M. spicata*, was shown by Cu.

The contents of these investigated metals are, with a few exceptions, in the range of values that are normal for plants. Given this, we believe that both *M. spicata* and *M. longifolia* from Bjelasica can be used to make teas and medicinal preparations, at least in terms of the content of the examined metals.

Conflict of Interest

The authors declare no conflict of interest.

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