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WATER-EXTRACTABLE MAGNESIUM, MANGANESE AND COPPER IN LEAVES AND HERBS OF MEDICINAL PLANTS

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Abstract: Since herbal teas, infusions and decoctions prepared from medicinal plants are popular remedies, it remains a topical question whether these herbal drugs can be treated as sources of essential elements for humans, who often use them in their everyday diet. Therefore, total and water-extractable contents of Mg, Mn and Cu were determined in 41 leaves originating from four botanical species of *Plantago lanceolata, Arctostaphyllos uva-ursi, Rubus fruticosus* and *Betula sp*., as well as in 33 samples of herbs represented by three species of *Urtica dioica, Hypericum perforatum* and *Achillea millefolium*. The highest level was determined in the case of Mg (in a range from 2.0 to 7.0 mg/g of dry mass [d.m.]), followed by Mn (from 50.0 to 1300.0 mg/kg d.m.), and lowest of all, Cu (from 3.5 to 19.5 mg/kg d.m.). Student's *t*-test showed that a statistically significant difference exists between samples originating from different plant species regarding the total content and water-extractable forms of Mg, Mn and Cu. By analysis of the relations between elements, it was observed that total level of Cu correlated with total levels of Mg and Mn, which indicates a synergistic interaction between the essential elements under study. With regard to Dietary Reference Intakes (DRIs), the leaves of *Rubus fruticosus* contained the highest amounts of a water-extractable bioavailable form of Mn, which guarantees from 160 to 200% of the daily requirement of Mn for women and men, respectively. On the other hand, the extract obtained from *Urticae folium* gave water-extractable Mg in the amount of 76 mg/500 mL, which constitutes about 20% of daily requirement. The plant material richest in water-extractable Cu was *Hyperici herba*, containing 154.5 µg/500 mL, or 17% of DRI for both sexes.

Keywords: leaves, herbs, medicinal plants, water-extractable forms of Mg, Mn and Cu, DRIs

Herbal teas, infusions and decoctions prepared from medicinal plants are popular remedies often employed to support the treatment of several chronic diseases, but also to help in the general well-being of the human organism, as well as to assist the correct functioning of various organs and to regulate metabolic processes. They are considered, in comparison with chemical substances, to be less toxic and with negligible or reduced adverse effects for the human organism. Therefore, the question as to whether herbal drugs, through frequent use in everyday diet, can be treated as sources of essential elements for humans is indeed a topical one.

There have been many studies concerning the analysis of toxic and essential elements present in medicinal plants and the herbal drugs obtained from them. In some of these investigations the important issue was the determination of water-extractable chemical forms of elements in relation to their total level, which can be treated as a potentially bioavailable species for the human organism (1–5). In other cases, the relations between the micro- and macro-elements studied in herbal drugs were investigated in order to recognize their effects, be they synergistic or antagonistic, on a living organism (6–11).

The choice of essential elements to study was made on the basis of their indispensable role for life in both plants and human organisms (12). Mg is both a component of many enzymes and of chlorophyll structures, not to mention its catalytic functions as a macro-element. Mn plays a role in the synthesis of nucleic acids, in the photolysis of water during the reaction of photosynthesis, it stabilizes chloroplast structure and also influences the metabolism of the mucopolysaccharides and activates several enzymes, such as superoxide dismutase, arginase and malate. Cu is involved in energy metabolism, N metabolism, oxidizing systems, and also plays catalytic functions in numerous redox reactions.

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Since Mg, Mn and Cu are essential elements for the living organism, and given the fact that medicinal plant raw materials can be treated as sources for the bioavailable forms of these elements, the aim of this study was recognition of the relation between the level of Mg, Mn and Cu in leaves and herbs and in their aqueous extracts (herbal teas). Another goal of the investigations was a comparison of the concentration of water-extractable chemical forms of the elements studied with the norms of Dietary Reference Intakes – DRIs (13) in order to observe whether these forms can be sources of these indispensible elements.

EXPERIMENTAL

Plant material

The medicinal plant raw materials originated from the herbal company of *Kawon* and were purchased in a pharmacy. Their characterization is given in Table 1. Before analysis, the samples were ground using the Knifetec (Foss-Tecator, Denmark) sample mill and stored in polyethylene containers.

Microwave digestion

Microwave digestion of accurately weighed plant samples (approx. 1.0 g) was performed by the use of the mixture: 30% H_2O_2 (POChem, Poland)/concentrated 65% HNO_3 (Selectipur, Merck, Germany) (3:5, v/v) in the Uniclever BM-1z (Plazmatronika, Poland) unit applying temperature from 250 to 350°C and pressure from 31 to 45 atm. After this process, the samples were transferred to 50 mL volumetric flasks and diluted with twice distilled water obtained from the quarz-glass system (Heraeus, Switzerland). Three independent digestions were carried out for each sample.

Extraction

Twice distilled boiling water was used for the extraction. To the accurately weighed plant samples (approx. 2.0 g) 50 mL of boiling water was added, then stirred with an electromagnetic stirrer for 30 min without temperature control and filtered through a paper filter with medium-sized pores (Filtrak, Germany). The filtrates were collected in volumetric flasks and diluted to 50 mL with the twice distilled water. Three independent extractions were obtained for each sample.

Determinations

The concentrations of total and water-extractable Mg, Mn and Cu were determined using the 250 Plus Atomic Absorption Spectrometer (Varian, Australia), applying standard conditions in air/acetylene flame at 285.2 nm (Mg), 279.5 nm (Mn) and 324.5 nm (Cu) and an external calibration method. In the determination procedure of Mg, 1% solution of La(III) was added to each sample before determination. Measurement for each sample was repeated three times and the average value calculated.

Table 1. Characterization of the analyzed medicinal plant raw materials.

Number of samples	Plant material, plant species, botanical family	Medical use
	Leaves	
10	*Plantaginis lanceolatae folium*, *Plantago lanceolata* L., *Plantaginaceae*	*expectorans, bacteriostaticum*
10	*Uvae ursi folium*, *Arctostaphylos uva ursi* L., *Ericaceae*	*bacteriostaticum*
10	*Rubi fruticosi folium*, *Rubus fruticosus* L., *Roseceae*	*adstringentium*
11	*Betulae folium, Betula verrucosa* Ehrh., *Betula pubescens* Ehrh., *Betulaceae*	*diureticum, adstringentium*
	Herbs	
11	*Urticae herba*, *Urtica dioica* L., *Urticaceae*	*diureticum, haemostaticum, vitaminosum*
11	*Hyperici herba*, *Hypericum perforatum* L., *Hypericaceae*	*antidepressivum, adstringentium, spasmolyticum*
11	*Millefolli herba*, *Achillea millefolium* L., *Compositae*	*aromaticum amarum, cholagogum, carminativum*

Table 2. Validation of analytical procedures applied for determination of Mg, Mn and Cu (n = 6).

Validation parameters	Mg	Mn	Cu
Range (µg/mL)	0.04 – 1.20	0.05 – 5.00	0.44 – 5.00
LOD (µg/mL)	0.01	0.02	0.13
LOQ [µg/mL)	0.04	0.05	0.44
Mixed Polish Herb MPH-2 (mg/kg)	2920 ± 180	191 ± 12	7.77 ± 0.53
± SD (mg/kg)	2950 ± 360	170 ± 11	9.22 ± 0,50
RSD (%)	12.2	7.0	5.4
Recovery (%)	101.0	89.0	118.7

Validation of the analytical procedures was achieved with the use of CRM *Mixed Polish Herb MPH-2*. The results are presented in Table 2.

RESULTS AND DISCUSSION

Total content and water-extractable Mg, Mn and Cu

The arithmetical means of total concentration and the water-extractable forms of the elements studied are shown in Figures 1-3. Analysis of the data indicates that total Mg was found in the herb samples in a large range from 2.3 mg/g of dry mass (d.m.) in *Millefolli herba* to 7.2 mg/g d.m. in *Urticae herba*, which differs considerably in comparison with the value obtained from leaves, i.e., 2.0 mg/g d.m. in *Uvae ursi folium* to 4.0 mg/g d.m. in *Betulae folium*. Total Mg is on a comparable level with that reported in literature, e.g., in plants used medicinally in Algeria this element was determined from about 0.3 to more than 7.5 mg/g d.m. (6), or in Indian medicinal herbs from 0.83 to 2.63 mg/g d.m. (8). The water-extractable form of Mg was found in leaves from 0.47 mg/g d.m. for *Uvae ursi folium* to 1.5 mg/g d.m. for *Plantaginis lanceolatae folium*, whereas in the herbs from about 0.8 mg/g d.m. for the samples of *Hyperici herba* and *Millefolli herba* to 1.9 mg/g d.m. for *Urticae herba*, as is also shown in Figure 1. The highest extraction rate, about 43% of total Mg, was noticed in the case of *Plantaginis lanceolatae folium*, while in the other plant samples it varied from 27 to 40% of total level. These values are similar to those obtained for tea samples, which were on average 38% of total Mg (2).

The highest mean concentration of total Mn, about 1.3 mg/g d.m. was detected in the sample of *Rubi fruticosi folium*, a value more than 20 times higher than that in the other leaves. Such a concentration may even be considered toxic for humans (15), however it must be stressed that this is the total level of the metal and not the amount which passed into the aqueous extract. A quite high Mn level, 370 mg/kg d.m. in *Betulae folium*, was also detected. In the other two samples of leaves, total concentration of Mn was about 50 – 60 mg/kg d.m. An analysis of the level of Mn in the herbs gave similar results, from 380 mg/kg d.m. in *Millefolli herba* to 440 mg/kg d.m. in *Hyperici herba*. Disregarding the extremely high Mn level in *Rubi fruticosi folium*, the range of total Mn is similar to that given in the literature (1, 2, 8, 9). Extraction yields were low, in the range of a single figure percentage of total Mn. The highest extraction rate was obtained for *Plantaginis lanceolatae folium* (approx. 10% of total Mn), followed by *Rubi fruticosi folium* (approx. 8.6% of total level). When comparing the results of extraction yield obtained in this study with those given in the literature, they are lower, at about 20 – 50% of total Mn for the selected medicinal plants (14) and 24% of total Mn for commercially available tea samples (2).

In the case of Cu, the highest total level was found in leaves of *Plantaginis lanceolatae folium (*19.5 mg/kg d.m.), then *Rubi fruticosi folium* (8.3 mg/kg d.m.). In the other two leaves this microelement was determined at 5.6 mg/kg d.m. in *Betulae folium* and 3.5 mg/kg d.m. in *Uvae ursi folium*. In the herbs analyzed, the total concentration of Cu was less differentiated than in the leaves, varying from 7.5 mg/kg d.m. in *Millefolli herba* to 12.4 mg/kg d.m. in *Hyperici herba*. The range of total Cu was similar to other studies (1, 8, 9). Water-extractable Cu was determined from 0.09 mg/kg d.m. in *Uvae ursi folium* to 2.1 mg/kg d.m. in *Plantaginis lanceolatae folium* in the leaf samples analyzed and from 1.1 mg/kg d.m. in *Urticae herba* to 3.6 mg/kg d.m. in *Hyperici herba* in the group of herbs. The extraction yield was from 1.9% of total Cu in the case of

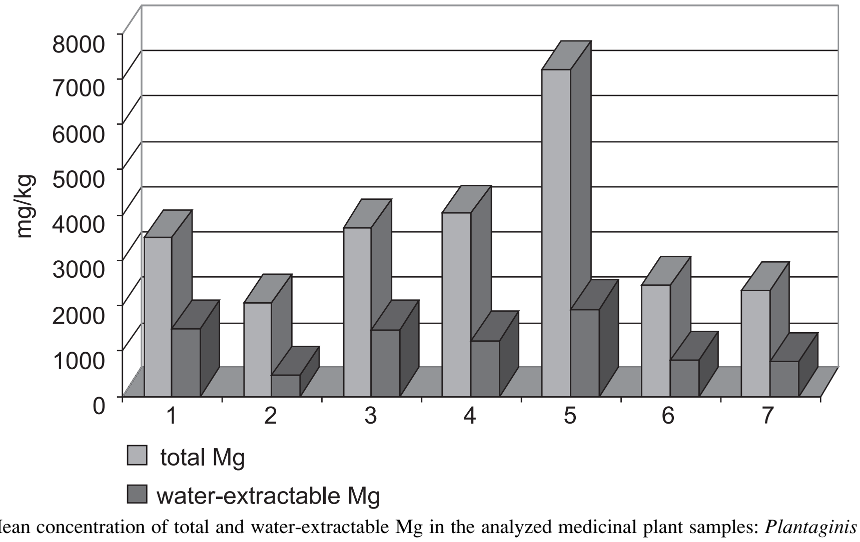

Figure 1. Mean concentration of total and water-extractable Mg in the analyzed medicinal plant samples: *Plantaginis lanceolatae folium* (1), *Uvae ursi folium* (2), *Rubi fruticosi folium* (3), *Betulae folium* (4), *Urticae herba* (5), *Hyperici herba* (6), *Millefolli herba* (7)

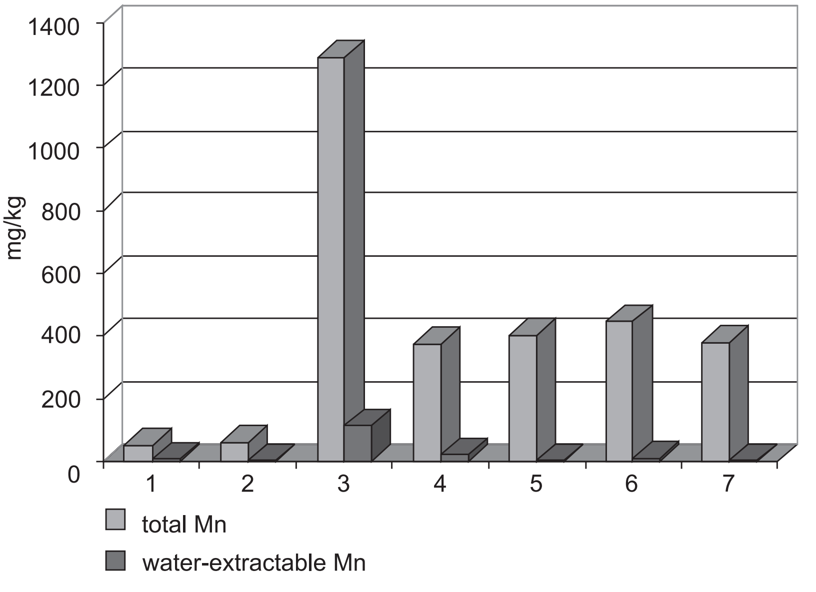

Figure 2. Mean concentration of total and water-extractable Mn in the analyzed medicinal plant samples: *Plantaginis lanceolatae folium* (1), *Uvae ursi folium* (2), *Rubi fruticosi folium* (3), *Betulae folium* (4), *Urticae herba* (5), *Hyperici herba* (6), *Millefolli herba* (7)

Betulae folium to 35% of total Cu in *Millefolli herba*. In comparison with the literature data (2), where the extraction yield for Cu was on average equal to 33% of total Cu in tea samples, our results are lower with the exception given above for the sample of *Millefolli herba*.

Statistical evaluation of the data

It can generally be said that the plant species studied, in the majority of cases, showed a significant statistical difference ($\alpha < 0.05$), both taking into consideration total contents of Mg, Mn and Cu and their water-extractable forms. The exceptions to this general rule are the leaves of *Plantago lanceolata*, *Betula sp.* and *Rubus fruticosus*, which contain similar amounts of Mg, as well as the leaves of *Arctostaphylos uva ursi* and *Plantago lanceolata*, which are not statistically different in their Mn level. In the case of herbs, the samples for analysis originated from only three plant species. The difference

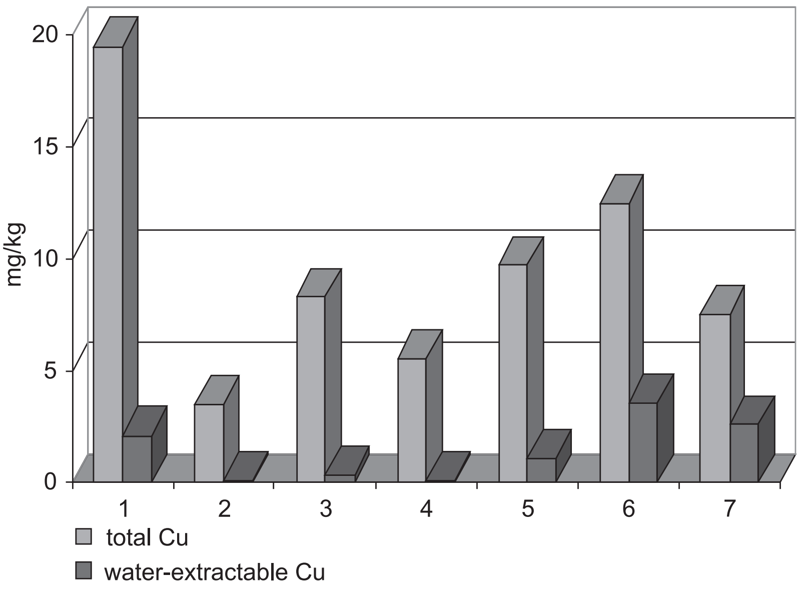

Figure 3. Mean concentration of total and water-extractable Cu in the analyzed medicinal plant samples: *Plantaginis lanceolatae folium* (1), *Uvae ursi folium* (2), *Rubi fruticosi folium* (3), *Betulae folium* (4), *Urticae herba* (5), *Hyperici herba* (6), *Millefolli herba* (7)

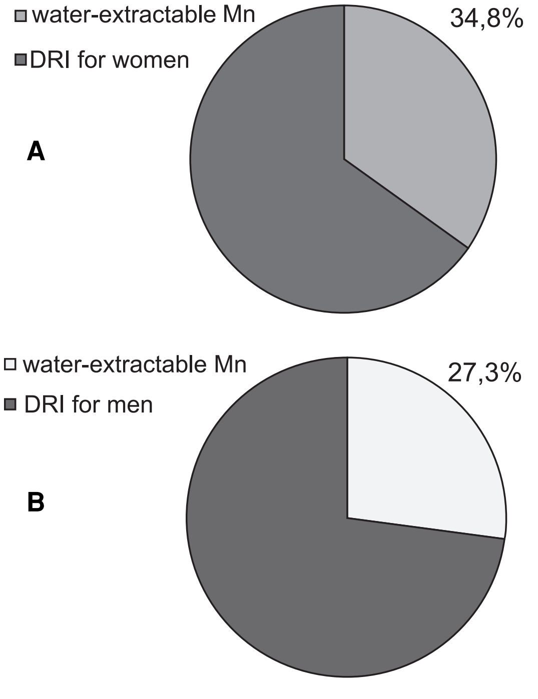

Figure 4.
A. Comparison of water-extractable Mn contents in two glasses of an aqueous extract obtained from *Betulae folium* with the DRI norm for women (19–30 years).
B. Comparison of water-extractable Mn contents in two glasses of an aqueous extract obtained from *Betulae folium* with the DRI norm for men (19–30 years)

in interpretation of the results obtained from herbs and leaves was that only the samples originating from *Urtica dioica* were significantly different statistically from the others in their total and water-extractable Mg content. In the cases of Mn and Cu, the differences between the herbs analyzed were not statistically significant.

Based on the results of correlation analysis, it can be stated that in the leaves of *Plantago lanceolata*, no statistically significant relation was found for any pair of elements. The same was noticed for *Achillea millefolium*, with the only exception being for the relation between Mg extractable and Mn total (r = 0.77). In general, no statistically significant correlation between the total and water-extractable form of the metals was found, with one exception – the relation (r = 0.47; p = 0.009) between Mg total and Mg extractable obtained only in one plant material, *Uvae ursi folium*. Data compiled in Table 3 would also seem to lend support for the statement that total levels of Mg, Mn and Cu are correlated. The correlation between the pairs: Mg total – Cu total and Mn total – Cu total was found in four plant species, whereas between the pairs: Mg total – Mn total in two plant species, which indicates a synergistic interaction between these essential elements. A statistically significant relation between the water-extractable forms of these elements was also

Table 3. Results of correlation analysis. Correlation coefficients statistically significant are in bold font (á < 0,05).

Pair of elements	*Arctostaphylos uva ursi* L. n = 30	*Rubus fruticosus* L. n = 30	*Betula verrucosa* Ehrh., *Betula pubescens* Ehrh., n = 33	*Urtica dioica* L. n = 33	*Hypericum perforatum* L. n = 33
Mg total – Mn total	-0.09 p = 0.015	**0.44**	0.23	-0.09	**0.70** p = 0.000
Mg total – Cu total	-0.07	**0.39** p = 0.034	**0.60** p = 0.000	**0.44** p = 0.011	**0.75** p = 0.000
Mn total – Cu total	**0.38** p = 0.039	**0.82** p = 0.000	**0.38** p = 0.031	-0.31	**0.74** p = 0.000
Mg extr. – Mn extr.	0.18	**0.58** p = 0.001	-0.02	0.33	**0.47** p = 0.006
Mg extr. – Cu extr.	**-0.44** p = 0.014	-0.17	0.25	-0.19	0.11
Mn extr. – Cu extr.	-0.18	0.26	**0.40** p = 0.022	-0.21	-0.27
Mg total – Mn extr.	0.22	-0.04	**-0.45** p = 0.008	0.20	0.27
Mg total – Cu extr.	-0.17	-0.01	0.01	**-0.47** p = 0.005	-0.21
Mg extr. – Mn total	0.16	0.26 p = 0.000	**-0.77**	0.25	-0.30
Mg extr. – Cu total	0.16	**0.36** p = 0.049	0.10	-0.08	0.06
Mn extr. – Cu total	-0.25	-0.01	**-0.83** p = 0.000	**0.51** p = 0.003	0.14

extr. – water extractable species; n – number of measurements; p – significance level

Table 4. The norms of Dietary Reference Intakes – DRIs (13).

Women (19-30 years)	Men (19-30 years)
Mg [mg/day]	
310	400
Mn [mg/day]	
1.8	2.3
Cu [μg/day]	
900	900

observed, as well as between the total and water-extractable forms, but only in several pairs. The most frequently statistically significant correlations between the metals studied occurred in the leaves of *Betula sp.* and *Rubus fruticosus*.

Daily intake of Mg, Mn and Cu

The objective of the investigation was to establish whether water-extractable species of Mg, Mn and Cu could provide a source of these essential elements for humans. Therefore, the concentrations of these forms were compared with the norms of DRIs for women and men at the age interval from 19 to 30 years, according to the Institute of Medicine of the National Academies (13), shown in Table 4. It was assumed that a patient drinks two glasses (500 mL of infusion (or aqueous extract) daily.

In the case of water-extractable Mg, the highest amount was found in the extract obtained from *Urticae folium* at 76 mg/500 mL. This represents 24% of daily Mg requirement for women and 19% for men. In the aqueous extracts prepared from the other plant materials, the contents of this form of Mg was about 40 mg/500 mL in the samples obtained from *Betulae folium, Plantaginis folium* and *Rubi fruticosi folium*. In the case of samples from *Uvae ursi folium*, *Hyperici herba* and *Millefolli herba* the water-extractable Mg form was in the range of several mg/500 mL of extract.

When considering the water-extractable form of Mn, the leaves of *Rubus fruticosus* appeared to be the richest plant material, containing as it did 3.7 mg/500 mL of aqueous extract, or 200% of DRI norm for women and 160% for men. The aqueous extract obtained from the sample of *Betulae folium* is also rich in this form of Mn, having 1.24 mg in two glasses of infusion, as shown as an example in Figure 4. The other herbal extracts can deliver from 5.2% (*Urticae herba*) to 20.4% (*Hyperici herba*) of DRI for women and from 4.0 to 16.0% of DRI for men.

The water-extractable form of Cu present in the aqueous extracts obtained from the medicinal plant samples is rather low in comparison with the DRIs norms. The richest plant material was the sample of *Hyperici herba*, which yielded 154.5 µg/500 mL, or 17% of DRI for both women and men. The aqueous extracts obtained from the samples of *Plantaginis folium, Millefolli herba* and *Urticae herba* would also provide a low percentage of the DRI . The other remaining plant samples contain water-extractable Cu in amounts which total less than 1% of DRI for women and men.

CONCLUSIONS

Based on the investigation performed, it was found that statistically significant differences existed for the total contents and water-extractable forms of Mg, Mn and Cu between the samples originating from different plant species. It was also revealed that total levels of Mg, Mn and Cu correlated, which indicates synergistic interaction between them.

It was also shown that leaves and herbs of the medicinal plants are rather poor sources of the water-extractable forms of Mg, Mn and Cu, potentially bioavailable for human consumption. However, it must be stressed that only in the case of several plant materials, e.g. the leaves of *Rubus fruticosus* and *Betula sp,* and the herb of *Urtica dioica,* did they contain a water-extractable amount of the metallic elements analyzed, especially Mn and Mg, significant enough to be considered valuable for the human diet.

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