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Letter to Editor

Determination of Zinc, Iron, Nitrogen and Phosphorus in Several Botanical Species of Medicinal Plants

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Abstract

The total concentration of zinc, iron, nitrogen and phosphorus, as well as their water and acetic acid extractable forms – nitrate nitrogen, ammonium nitrogen and phosphate phosphorus – were determined in St. John's wort herb (*Hypericum perforatum* L.) yarrow herb (*Achillea millefolium* L.), nettle leaves (*Urtica dioica* L.) and birch leaves (*Betula pendula* Roth.), collected from four natural locations in northern Poland. The content of total Zn and Fe was determined by FAAS, whereas of total N, total P and their extractable forms by UV/Vis spectrophotometry. The obtained results showed that particular plant species differed significantly in the contents of Zn, Fe, P and P-PO_4. The area of harvest didn't have major effects on the concentration of analyzed elements in the studied plant species. Significantly, 13 inter-element correlations were obtained for the studied elements, mainly between iron and nitrogen, iron and phosphorus, phosphorus and nitrogen and among their water-extractable forms, indicating their involvment in metabolism of medicinal plants. Nutritional and toxicological aspects of the analyzed plants used commonly as folk medicines in Poland were evaluated by comparison of the obtained results with the WHO/FAO norms.

Keywords: speciation analysis, essential elements, water extracts of medicinal plants, correlation analysis, WHO/FAO norms

Introduction

It is well known that medicinal plants are rich sources of bioavailable forms of elements for humans. Environmental conditions like type of soil, rainfall, vicinity of industry and extensive agricultural activity, influence the level of bioavailable elements in plants [1-3]. Because of this, the analysis of chemical composition of plants used as medicinal raw materials should take into consideration not only the total concentration of elements, but also their water-extractable, bioavailable chemical forms, which may have strong biological effects [4-7]. Therefore, the nitrate form of nitrogen, known as potentially dangerous for health, was often analyzed in raw medicinal plant materials or natural drugs [8-11]. It was also found that several plant species, like *Achillea millefolium* L. or *Helichrysum arenarium* Moench, contained higher amounts of nitrates than other plants [11]. On the other hand, phosphate phosphorus is known to react with essential metals, like iron, which diminishes bioavailable forms of that indispensable metal to humans [12]. Because of this, content of this inorganic form of phosphorus is often determined in medicinal plants, and the phosphate phosphorus was found in acetic acid extracts in concentrations from 14.5% to 91.4% of total phosphorus [13].

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Taking the above into account, the aim of the studies was to investigate the influence of harvest area of selected medicinal plants collected in Northern Poland, on the level of total zinc, iron, nitrogen and phosphorus, as well as to recognize inter-relations among elements and their extractable forms. The areas of harvest were selected as free from industrial contamination, but they are located in the neighborhood of Gdańsk and Olsztyn, therefore this influence can not be totally excluded. The plants selected for the investigation – St. John's wort (*Hypericum perforatum* L.), yarrow (*Achillea millefolium* L.), nettle (*Urtica dioica* L.) and birch (*Betula pendula* Roth.), are popularly collected and used by people as water extracts, infusions or other forms of natural drugs. Moreover, the goal of the investigation was to relate the results of the determination of bioavailable species of nitrogen and phosphorus to the recommended dietary allowances (RDA) [14] and to the acceptable daily intakes (ADI) [15].

Experimental Procedures

Plant Material

Three areas of harvest were situated in Northern Poland near The Baltic Sea, in the suburbs of Gdańsk (Oliwa and Brzeźno) and in a small village – Piaski on the Vistula Sand Bar, the fourth area was located in the northeastern region of Poland in the suburbs of Olsztyn, as shown in Fig. 1. The medicinal plants are set in Table 1. The whole

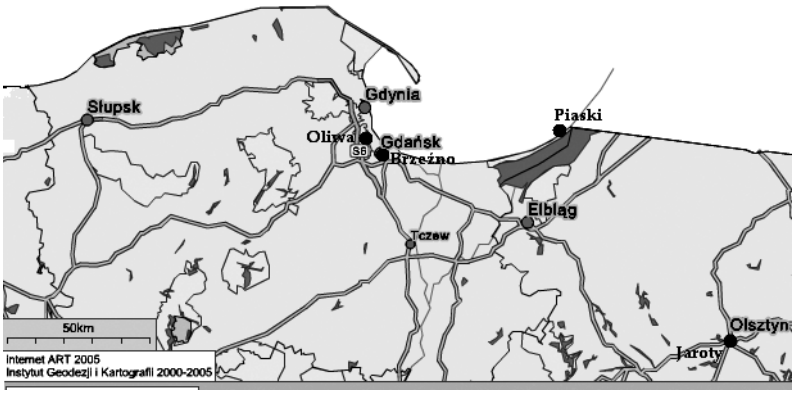

Fig. 1. Harvest areas of the studied medicinal plants.

Table 1. Botanical classification, place of harvest and medical properties of the studied plants.

Sample number	Plant name, Plant species, Botanical family	Collection area	Medical use
1	St. John's wort *Hypericum perforatum* L. Hypericaceae	Gdańsk-Brzeźno	antidepressive, digestive, metabolic
2		Piaski (on Vistula Sand Bar)	
3		Olsztyn-Jaroty	
4	yarrow *Achillea millefolium* L. Compositae (Asteraceae)	Gdańsk-Brzeźno	metabolic, sedative, antihaemorrhoidic, antipyretic
5		Gdańsk-Oliwa	
6		Piaski (on Vistula Sand Bar)	
7		Olsztyn-Jaroty	
8	nettle *Urtica dioica* L. Urticaceae	Gdańsk-Brzeźno	diuretic, antirheumatic, antiphlogistic, antidiabetic, metabolic
9		Gdańsk-Oliwa	
10		Piaski (on Vistula Sand Bar)	
11		Olsztyn-Jaroty	
12	birch *Betula pendula* Roth Betulaceae	Gdańsk-Brzeźno	diuretic, antirheumatic, antipyretic
13		Gdańsk-Oliwa	
14		Piaski (on Vistula Sand Bar)	
15		Olsztyn-Jaroty	

plants – St. John's wort (*Hypericum perforatum* L.) and yarrow (*Achillea millefolium* L.), as well as the leaves of nettle (*Urtica dioica* L.) and birch (*Betula pendula* Roth.) were collected, then dried in shadow at less than 35°C, [16], then ground using the Knifetec (Foss-Tecator, Denmark) sample mill, and kept in polyethylene containers prior to analysis.

Microwave Digestion

For preparation of plant material for the determination of total zinc, iron and phosphorus, microwave digestion was used. An accurately weighed plant sample (0.5 g) was digested with the mixture of 30% H_2O_2 (POChem, Poland) and concentrated 65% HNO_3 (Selectipur, Merck, Germany), (3:5, v/v). Digestion of samples was done in the Uniclever BM-1z (Plazmatronika, Poland) unit using the following temperature program: heating up to 250°C – 7 min. (step 1), cooling down to 100°C – 5 min. (step 2) and waiting until 25°C – 10 min. (step 3). After this process, the samples were transferred to 50 mL volumetric flasks and diluted with the twice distilled water obtained from the quartz-glass system (Heraeus, Switzerland).

Wet Digestion

In order to prepare the plant material for the determination of total nitrogen and to digest the water extract before the determination of the ammonium nitrogen, the wet digestion was performed. Therefore. an accurately weighed plant sample (0.1-0.3 g) or 5 mL of the water extract of a plant, was transferred to Kjeldahl flask. Next 5 mL of 30% H_2O_2 solution and 10 mL of concentrated H_2SO_4 solution (both from POChem, Poland) were added and the sample was digested until the solution became clear. Then it was transferred to the volumetric flask and volume up to 100 mL was made up with the twice-distilled water.

Extraction

The twice distilled water of temperature of 85°C was used for the extraction. To the accurately weighed plant sample (1.0 g) 30 mL of hot water was added, then stirred with electromagnetic stirrer for 30 min. and filtered through a paper filter with medium–sized pores (Filtrak, Germany). The filtrate was collected in the volumetric flask and diluted to 50 mL with the twice-distilled water.

The extraction with 2% (v/v) solution of acetic acid was done using the same way, as described above, but in room temperature, without heating of the solution.

Determinations

The concentration of total zinc and iron was determined by flame atomic absorption spectrometry (FAAS), whereas the level of non-metals and their forms in aqueous and acidic extracts by UV/Vis spectrometric methods. In order to check the recovery and accuracy of the applied analytical procedures, total concentration of zinc, iron, nitrogen and phosphorus was determined in the certified reference materials, but of ammonium nitrogen, nitrate

Table 2. Validation of the analytical methods used in the determination of elements in the studied plants.

Element	Spectroscopic method based on	Analytical wavelength [nm]	Certified reference material	Declared concentration [mg/g d. wt.]	Determined concentration [mg/g d. wt.]	Recovery [%] (n=6)	Relative error [%] (n=6)
Total zinc	FAAS, bc	213.9	Mixed Polish Herbs (INCT-MPH-2)	33.5* (certified value)	32.0*	95.5	1.5
Total iron	FAAS, bc	248.3	Virginia Tobacco Leaves 2 (CTA-VTL-2)	1.083 (certified value)	0.997	92.1	5.0
Total nitrogen	Nessler reagent	420	Tomato Leaves (NIST-1573a)	29.20 (information value)	27.79	95.2	9.4
Ammonium nitrogen	Nessler reagent	420	-	2.23	2.09	93.8**	9.8**
Nitrate nitrogen	Nitration	410	-	200*	172.9*	86.5**	9.5**
Total phosphorus	Molybdenum blue	650	Oriental Tobacco Leaves 1 (CTA-OTL-1)	2.89 (recommended value)	2.81	97.2	2.0
Phosphate phosphorus	Molybdenum blue	650	-	2.72	2.49	91.5**	2.9**

* value is in mg/kg; ** determination was done with the standard addition method; bc – background correction was used

Table 3. The results of determination of elements and their water-soluble forms given as the mean ± standard deviation (n=6).

Sample number	Total zinc [mg/kg d.wt.]	Total iron [mg/kg d.wt.]	Total nitrogen [mg/g d.wt.]	Nitrate nitrogen [mg/kg d.wt.]	Ammonium nitrogen [mg/g d.wt.]	Total phosphorus [mg/g d.wt.]	Phosphate phosphorus (aqueous extract) [mg/g d.wt.]	Phosphate phosphorus (acetic acid extract) [mg/g d.wt.]
1	47.02 ± 4.70	7.56 ± 1.16	5.90 ± 0.26	nd	nd	1.73 ± 0.01	1.46 ± 0.02	nd
2	69.69 ± 3.70	26.06 ± 1.50	8.43 ± 0.74	544.48 ± 3.92	0.64 ± 0.05	3.87 ± 0.02	2.19 ± 0.06	1.81 ± 0.03
3	34.91 ± 0.97	32.91 ± 1.54	6.24 ± 0.28	1070.19 ± 16.54	1.09 ± 0.11	3.86 ± 0.01	2.02 ± 0.03	1.84 ± 0.04
4	42.74 ± 2.67	20.79 ± 0.81	7.96 ± 0.66	134.24 ± 6.83	1.23 ± 0.12	3.23 ± 0.08	1.70 ± 0.02	1.55 ± 0.02
5	66.11 ± 0.25	26.54 ± 1.92	9.00 ± 0.30	688.26 ± 11.38	2.99 ± 0.12	4.67 ± 0.07	2.99 ± 0.05	2.80 ± 0.02
6	44.23 ± 0.57	23.26 ± 2.24	5.87 ± 0.28	112.46 ± 11.10	1.07 ± 0.10	3.99 ± 0.05	2.32 ± 0.07	2.11 ± 0.02
7	26.59 ± 3.19	16.36 ± 0.21	9.54 ± 0.54	36.54 ± 3.84	1.97 ± 0.10	4.30 ± 0.01	2.60 ± 0.03	2.24 ± 0.02
8	51.23 ± 1.56	67.01 ± 0.95	10.05 ± 0.42	355.87 ± 7.43	0.31 ± 0.03	4.43 ± 0.03	1.18 ± 0.07	1.63 ± 0.11
9	21.64 ± 0.80	40.17 ± 2.12	6.79 ± 0.20	262.79 ± 19.70	1.08 ± 0.05	5.46 ± 0.09	2.05 ± 0.12	2.31 ± 0.03
10	39.03 ± 1.64	229.48 ± 5.78	24.21 ± 0.46	4699.39 ± 14.93	1.53 ± 0.11	7.37 ± 0.05	1.28 ± 0.06	3.40 ± 0.03
11	20.16 ± 0.80	30.76 ± 2.81	13.38 ± 0.92	151.93 ± 11.50	1.56 ± 0.10	6.03 ± 0.26	1.62 ± 0.06	2.55 ± 0.02
12	103.36 ± 2.60	18.41 ± 0.56	13.23 ± 1.20	230.91 ± 10.71	0.67 ± 0.03	2.46 ± 0.06	1.07 ± 0.04	0.70 ± 0.02
13	147.60 ± 2.47	27.01 ± 0.12	9.84 ± 0.46	177.20 ± 6.18	0.83 ± 0.01	2.36 ± 0.02	0.61 ± 0.05	0.62 ± 0.04
14	170.86 ± 3.20	26.48 ± 0.78	12.02 ± 0.73	nd	nd	3.85 ± 0.05	1.94 ± 0.08	1.38 ± 0.07
15	184.16 ± 2.36	18.02 ± 0.45	15.90 ± 1.54	113.20 ± 11.49	0.22 ± 0.02	3.44 ± 0.03	1.13 ± 0.07	0.86 ± 0.02

nd – not detected

Table 4. The correlation coefficients between the elements in medicinal plants; statistically significant values are in bold type ($\alpha < 0.05$).

	Total zinc	Total iron	Total nitrogen	Nitrate nitrogen	Ammonium nitrogen	Total phosphorus	Phosphate phosphorus (aqueous extract)	Phosphate phosphorus (acetic acid extract)
Total zinc	1							
Total iron	-0.21	1						
Total nitrogen	0.25	**0.76**	1					
Nitrate nitrogen	-0.22	**0.96**	**0.71**	1				
Ammonium nitrogen	-0.47	0.16	0.05	0.26	1			
Total phosphorus	-0.44	**0.71**	**0.53**	**0.65**	0.48	1		
Phosphate phosphorus (aqueous extract)	-0.41	-0.22	-0.41	-0.12	0.60	0.25	1	
Phosphate phosphorus (acetic acid extract)	**-0.53**	**0.57**	0.32	**0.57**	**0.72**	**0.92**	**0.53**	1

nitrogen and phosphate phosphorus in spiked samples, as shown in Table 2.

Calculations

All statistical calculations were done using Statistica version 6 software (Statsoft, Poland) .

Results and Discussion

The results presented in Table 3 illustrate the differentiation of the total concentration of zinc, iron, nitrogen and phosphorus in medicinal plants. It could be emphasized that levels of zinc were determined as the highest in all samples of birch leaves, not depending on the area of plant growth. This result confirms the literature data, that birch is known as a zinc accumulator [3]. The same notice can be made concerning total nitrogen in this plant species. In the case of total phosphorus, its highest level was found in the nettle samples, then in the yarrow samples. The particular attention to the sample of nettle (10), harvested from the location in Vistula Sand Bar, should be paid. In this plant the exceptionally high amount of nitrogen and phosphorus was found, and particularly high iron concentrations – one raw of magnitude higher in comparison with the other samples. The reason for this is not known, probably the high concentration of the studied elements in the sample of nettle could be explained by the influence of agricultural wastes (fertilizers) on the level of N, P and Fe in a soil, on which the plant was growing.

Analyzing the results of nitrate nitrogen concentration in all samples, its range of concentration vary from several mg/kg as determined in a yarrow sample (7) to almost 4.7 mg/g in a sample of nettle (10). The reason for this can be genetic (variety, species of a plant), or high levels of nitrates in soil. Such a great diversification in nitrate nitrogen concentration in plant samples (vegetables), was reported before [17]. Ammonium nitrogen was found in a range of concentration from 0.22 mg/g in a birch sample (15) to almost 3.0 mg/g in one of yarrow plants (sample 5). Since ammonium ion is taken up by roots and produced in several biochemical processes, including nitrate, its level is also much differed in particular plant species [18].

As for the determined phosphate phosphorus, applying water and the solution of acetic acid for extraction, it is possible to notice that it remains on the level of several mg/g of the sample dry mass, indicating, therefore, that a high percentage of total phosphorus can be soluble and treated as bioavailable.

The results of correlation analysis illustrated in Table 4 revealed that a statistically significant relation between iron and phosphorus, also between iron and phosphate phosphorus, exists. This can be important from the point of bioavailability of that essential metal, because the correlation may suggest that a part of iron is bound to phosphates, diminishing by this way its bioavailable pool. The positive correlation of nitrate nitrogen to the total amount of that element is quite justified, the same as the relation between total phosphorus and its phosphate forms. But it is not easy to explain why negative correlation between the total level of zinc and phosphate phosphorus, and between zinc and iron, was detected, because other reports indicate positive correlation between these metals [19, 20].

The level of bioavailable forms of non-metals was compared with the RDI and ADI, and is presented in Table 5. The recommended dietary allowance was established by the US National Academy of Sciences to provide the actual information of required intakes of vitamins and elements for individuals. Acceptable daily intakes, according to WHO, inform on the maximum tolerable doses of chemical compounds like nitrates or other potentially hazardous substances for humans. In order to relate the results of nitrate nitrogen and phosphate phosphorus determination in the studied plant material to the RDI and ADI, the assumption was made that an average adult person of 75 kg body weight prepares 4 infusions of herb in one day using 2.5 g of dry plants. Also, the determined amount of nitrate nitrogen in all plant samples

Table 5. Comparison of the results of nitrate nitrogen and phosphate phosphorus determination with the recommended dietary allowances (RDA) and acceptable daily intakes (ADI).

Sample number	Nitrate nitrogen recalculated into nitrate ion as percentage of ADI	Phosphate phosphorus as percentage of RDA
1	-	2.09
2	8.69	3.13
3	17.08	2.89
4	2.14	2.43
5	1.10	4.27
6	1.79	3.31
7	0.58	3.71
8	5.68	1.69
9	4.20	2.93
10	75.02	1.83
11	2.43	2.31
12	3.70	1.53
13	2.83	0.87
14	-	2.77
15	1.80	1.61

1) Acceptable daily intake for nitrates as nitrate ion – 3.7 mg/kg of body weight

2) Recommended dietary allowance for phosphorus for adult person – 700 mg/day

presented in Table 3 was recalculated into nitrate ion by multiplication by a factor of 4.43, which was obtained by division of the molecular masses of NO_3 by the mass of N. As can be noticed, the maximum value of nitrate ion reaches 75% of ADI for only one herb – the sample of nettle number 10. The other plant samples don't contain nitrates in amounts which could be potentially dangerous for human health. Nevertheless, the level of nitrates in medicinal plants should be monitored to avoid the risk of their contamination with that potentially toxic form of nitrogen. In the case of phosphate phosphorus its level reaches only several percent, from less than 1 to more than 4% of the Recommended Dietary Allowance. Of course the herbal drugs such as teas or infusions are not the only sources of phosphorus available for humans, therefore the total potentially bioavailable amount of this element should comprise all food and beverages taken in one day.

Conclusions

The conclusion can be drawn that the concentration of zinc, iron, nitrogen and phosphorus varied greatly, depending more on genetic factors than on environmental conditions in which the studied plants had grown. However, the exception from the studied plant material can be one of the analyzed samples of nettle, collected in Vistula Sand Bar, in which significantly higher amounts of the elements were determined. Extractable forms of nitrogen – nitrate and ammonium nitrogen were found in a level not exceeding the ADI in relation to nitrate level, but the bioavailable form of phosphorus can only deliver a few percent of RDA.

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