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Original Research

Effects of Trace Elements on Polyphenolic Compounds in *Millefolii* Herba

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Abstract

Achillea millefolium L. is commonly found as a ruderal plant in Europe and northern parts of Asia and North America, in the temperate climate zone. Numerous phytochemical studies into this species have shown that it is rich in pharmacologically active compounds. The main groups of chemical compounds present in yarrow consist of terpenes, polyacetylenes, flavonoids, coumarins, pyrrolidine alkaloids (such as stachydrine and betonicine), and tannins. This study presents the results of research into the content of select elements and the total of polyphenolic compounds, including the total of phenolic acids. An attempt has been made to determine the correlation between the presence of polyphenolic compounds and the investigated elements. Spearman's rank correlation between the total of phenolic acids and polyphenolic compounds and the selected elements show that the content of phenolic acids was mainly related to barium, boron, titanium, and iron, whereas the content of polyphenolic compounds was connected with aluminium, boron, chromium, molybdenum, and iron. This was proven by the value of the correlation coefficient, which was greater than 0.6000. In order to illustrate "the data structure" a cluster analysis was used. The total of phenolic acids turned out to be mostly related to the total of polyphenolic compounds, which make direct and the least distant connection with barium, and only then with other metals. These interactions do not necessarily have to be reflected by the values of the rank correlation coefficients as they are typical for linear correlation, while the studied effects may be correlations of a higher degree.

Keywords: *Millefolii* herba, trace elements, Spearman's rank correlation, cluster analysis, polyphenolic compounds

Introduction

Common yarrow (*Achillea millefolium* L.) belongs to the genus *Achillea* L. from the family *Asteraceae*, one of the largest groups of vascular plants. Yarrow is also referred to as milfoil, millefolium, and nosebleed; foreign names include Herbe de millefeuille, Herbe de charpentier (French), Schafgarbenkraut, and Achilleskraut (German) [1-2]. The most popular species are *Achillea millefolium* L. (Common yarrow), *Achillea filipendulina* Lam. (Fernleaf yarrow), *Achillea collina* Becker ex Rchb. (Hilloced yarrow), *Achillea pannonica* Scheele, and *Achillea ptarmica* L. (Sneezewort yarrow). The species *Achillea millefolium* L. shows great cytogenetic and morphological polymorphism. Because of polyploidy, the taxonomic classification of some varieties and subspecies may differ in indi-

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vidual monographs [1, 3]. Yarrow polyploidy affect the differences in the occurrence and content of azulene, responsible for the anti-inflammatory effect. Plants of tetraploid karyotype contain proazulenes (precursors of azulenes) while hexa- and octaploid are types without azulene. *A. millefolium* L. *sensu stricto* (*A. millefolium* L. ssp. *Millefolium*) is a hexaploid species, but crossing the taxonomic units results in new varieties that do not usually differ significantly, which makes their classification difficult. Therefore, many new taxons are classified as the species *A. millefolium* L. in pharmacopoeial monographs (the term *sensu stricto* is not used then, sometimes the phrase *sensu lato* appears) [1].

Achillea millefolium is commonly found as a ruderal plant in Europe and northern parts of Asia and North America, in the temperate climate zone. The plant prefers dry and sunlit settings, but does not demonstrate any special soil requirements; it commonly grows on meadows, waysides, fields, and wastelands. The material used in the production of preparations and herbal mixtures is mainly cultivated in southeastern Europe (Poland) and certain regions of Germany (Bavaria, Saxony, Thuringia) [1, 4].

Phytochemical studies into the species *Achillea millefolium* L. have shown that it is rich in pharmacologically active compounds, which suggests its high bioactivity and explains its wide application in phytotherapy. The main groups of chemical compounds consist of terpenes (including sesquiterpene lactones), polyacetylenes, flavonoids, coumarins, pyrrolidine alkaloids (such as stachydrine and betonicine), and tannins.

The healing properties of common yarrow have been used in folk medicine for a long time. It is known that Achilles used this plant to treat his soldiers' battle wounds during the Trojan War, which may suggest its haemostatic activity [5]. A high and diverse content of bioactive compounds in the plant also has guaranteed great popularity and wide application in phytotherapy. The healing use of yarrow has been different depending on geographical location and, most importantly, on the health problems of a given ethnic group.

The sesquiterpene lactones present in the essential oil determine the bitter taste of the material. Bitter substances are applied in gastritis usually connected with insufficient secretion of gastric acids, in dyspepsia and lack of hunger (which is used in preparations for children and elderly people with observed underweight or inadequate absorption of nutrients) [6]. The oil contained in yarrow also regulates the liver and gallbladder secretion activities. Its constituents such as camphene, eucalyptol, borneol, or α- and β-pinene have cholagogic and biligenic activity. The material itself may be used as a spasmolytic agent within the digestive system as it relaxes the smooth muscles of the alimentary tract, with an accompanying carminative effect, which is useful in tympanites [2, 6-8]. Yarrow has been used to relieve menstrual pain, too [6]. The flavonoid compounds, i.e. flavone C-glycoside derivatives such as shaftoside and isoorientin, have shown hepatoprotective properties [1]. Such activity probably results from great anti-oxidant activity conditioned by the presence of polyphenols. It is also manifested in vessel protective effects, anti-atherogenic, and antiaggregatory activity [6].

The substances present in the essential oil demonstrate anti-inflammatory and decongestant activity. The former is used externally in order to alleviate skin and mucosa irritation and inflammation as well as to heal wounds [6]. Some of the substances found in common yarrow show antimicrobial and antiviral activity, and may also inhibit cancer development. The compounds that influence the development of cancer cells are flavonoids and sesquiterpenes. Their activity towards cancer differs, though. They may inhibit the growth of cancer cells, lead to apoptosis, or act synergistically with the anti-cancerous medicines already applied [9].

Using common yarrow may restrict frequently occurring hypersensitivity to plants from the family *Asteraceae*. It is connected with the presence of sesquiterpene lactones containing an α-methylene-γ-lactone ring in the plants of this family. Thanks to this grouping, the material displays high biological activity, but also a great allergic potential. Compounds containing this structure (e.g. α-peroxyachifolid) frequently cause contact allergy.

Many researchers examine the influence of heavy metals on plants [10-20].

Experimental Part

Preparations for the Study

The material for the study consists of the herb of *A. millefolium* L. picked during the flowering period of the plant and, next, dried at room temperature, in draught and shadow. The materials were collected from regions under different influence of anthropogenic pressure in 2011 and 2012. Because of the variations in the intensity and quality of the pollution emitted into the atmosphere, the herb samples were collected from potentially ecologically clean areas, such as meadows by the Zielonka landscape park or an apple tree orchard, as well as potentially polluted ones, i.e. a green belt by a busy road where a sewage treatment plant and an electrical power and heating plant were located, and a meadow next to a chemical plant producing fertilizers. When choosing the herb collection area, the presence of industrial plants and car traffic intensity had been taken into account as agents generating pollution and affecting the content of specific elements in the plant. The full characterization of the samples has been presented in Table 1.

Apparatus

Determination of the Content of the Select Elements

The samples of yarrow in which the select trace elements were determined were mineralized in a Mars 5 microwave digestion system (CEM Corporation, USA), and then the content of the selected elements was investi-

Table 1. Characterization of the material to be studied.

Sample number	Date of picking	Air temp., weather conditions	Description of the area where material was picked
MHE1	16.09.2011	20ºC sunny, windless day	Meadow by Puszcza Zielonka, close to Kliny
MHE2	16.09.2011	20ºC sunny, windless day	Meadow by the road Poznań-Wierzonka, right behind Kicin
MHE3	16.09.2011	20ºC sunny, windless day	Green belt by the road Poznań-Czerwonak, vicinity of the sewage treatment plant in Koziegłowy and Thermal Power Station Karolin, heavy traffic
MHE4	02.09.2012	25ºC slightly cloudy, windless day	Green area in the middle of a housing estate, lots of pedestrians, little traffic
MHE5	03.09.2012	20ºC sunny, windless day	Playground next to a nursery school, lots of pedestrians, little traffic
MHE6	11.09.2012	20ºC sunny, windless day	Wood edge, no traffic, about 3 km away from chemical plant
MHE7	11.09.2012	22ºC sunny, windless day	Meadow by chemical plant, heavy traffic, vicinity of the road Luboń-Mosina
MHE8	11.09.2012	28ºC, sunny, windless day	Apple orchard in Szczepankowo, about 100 m from a housing estate road
MHE9	08.09.2012	25ºC, sunny, windless day	Meadow on the periphery of the city, little/no traffic
MHE10	08.09.2012	25ºC, sunny, windless day	Meadow in the vicinity of building plots, away from traffic
MHE11	Commercial preparation of the herb of yarrow Millefolii herba		

Table 2. Mineralization parameters.

Power [W]	Temperature rise time [min]	Pressure [PSI]	Maximum temperature [ºC]	Temperature maintenance time [min]
600	20	195	210	10

gated on a Varian ICP- OES VISTA-MPX emission spectrophotometer and on a Varian ICP-MS (inductively coupled plasma-mass spectrometer).

Determination of the Total of Phenolic Acids and Polyphenols

The total of phenolic acids and that of polyphenols were determined by means of colorimetric methods on the UV VIS Lambda 35 Elmer-Perkin spectrophotometer.

Methods of Determination

Trace Elements

Mineralization

Individual sample weights of the materials were mineralized in 10 ml of concentrated nitric acid Ultranal in the Mars 5 microwave digestion system (CEM Corporation, USA), and then deionized water was poured to bring the volume to 15 ml. The mineralization parameters are presented in Table 2.

Determination of Trace Elements

After having prepared calibration curves for the selected elements, their content in the investigated samples was determined. The study was conducted on a Varian ICP-OES emission spectrometer. The result of the measurement was the average of six determinations. In the cases when the concentration of a given element was below the limit of quantification the analysis was repeated on an ICP-MS spectrometer, which is renowned for greater sensitivity.

The Total of Phenolic Acids

Extract Preparation

About 3.0 g of powdered material was placed in a round-bottom flask and poured over with 50 ml of distilled water. Extraction was performed using an ultrasonic cleaner for 30 minutes at 40ºC. The obtained extract was strained through cotton wool into a conical flask. The extraction was repeated twice in identical conditions, pouring 50 ml of distilled water over the material each time. All 3 obtained extracts were mixed and placed in a round-bottom flask. Then the extract was thickened to about 30 ml, quantitatively moved to a 50 ml volumetric flask, and poured over with distilled water (stock solution B).

Determination of the Content of Phenolic Acids in the Investigated Extract

5 ml of distilled water and 1 ml of the stock solution B were placed in 11 10 ml volumetric flasks. Next, the samples were acidified with 1 ml of 0.1 M hydrochloric acid

solution. After stirring, 1 ml of Arnov's reagent followed by 1 ml of 0.1 M sodium hydroxide solution were added to each flask. Distilled water was poured into the flasks to obtain a volume of 10 ml and the contents were thoroughly mixed. A blank test was performed similarly by replacing 1 ml of the stock solution with 1ml of distilled water.

1 minute after the addition of the sodium hydroxide solution, absorbance of the solutions was measured at wavelength λ=490 nm in relation to the blank test.

The content of phenolic acids in the investigated samples, calculated as caffeic acid, at absorbability $a^{1\%}_{1cm}$= 285, was calculated by means of the following formula:

$$X = (A \times 1.7544)/m$$

...where A – absorbance of solution A, m – sample material weight in grams [Polish Pharmacopoeia VI, 2002]

Determination of the Total of Polyphenols

Calibration Curve for Standard Caffeic Acid

A water solution of the standard caffeic acid was prepared at concentration of 0.2 mg/ml. 6 10 ml volumetric flasks wrapped in aluminium foil were filled with 4 ml of distilled water and 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 ml of the water solution of the standard caffeic acid, respectively. 0.5 ml of Folin-Ciocalteu reagent was added to each flask, and after a minute 2.0 ml of 20% solution of sodium carbonate were put in. Distilled water was poured into the flasks to bring the contents to a volume of 10 ml (the samples contained 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mg of caffeic acid, respectively). Thirty minutes after the addition of the solution of Na_2CO_3, absorbance of the solutions was measured at wavelength λ=760 nm. The blank test was obtained by adding 0.5 ml of Folin-Ciocalteu reagent to 7.5 ml of distilled water and, similarly to the preparation of the samples to be determined, 2.0 ml of 20% sodium carbonate were added after a minute.

Extract Preparation

About 0.5 g of the powdered material (*Millefolii* herba) was placed in a round-bottom flask and poured over with 100 ml of distilled water. Extraction was performed using an ultrasonic cleaner for 30 minutes at 40ºC. The obtained extract was strained through cotton wool into a conical flask. The extraction was repeated three times in identical conditions, pouring 100 ml of distilled water over the material each time. All 3 obtained extracts were mixed and placed in a round-bottom flask. Then, the extract was thickened to about 50 ml, quantitatively moved to a 100 ml volumetric flask and poured over with distilled water (stock solution A).

Determination of the Total Content of Polyphenols in the Investigated Extract

4 ml of distilled water were placed in 9 × 10 ml volumetric flasks wrapped in aluminium foil. Next, 1 ml of the

Table 3. Results of determination of the elements in the investigated material by the ICP-OES method with standard deviations.

[ppm]	Al		B		Ba		Cr		Cu		Fe		Mn		Ni		Ti		Zn		Ca		Mg	
		SD		SD		SD		SD		SD		SD		SD		SD		SD		SD		SD		SD
MHE1	66.0	2.2	36.3	0.6	18.5	1.1	0.3	0.0	8.0	0.1	92.0	3.0	120.1	3.0	1.3	0.2	0.5	0.1	29.4	1.4	8,955	110	1,599	26
MHE2	53.6	6.6	26.1	0.4	12.7	0.1	0.3	0.0	8.9	0.3	73.5	23.4	36.5	0.5	0.6	0.1	1.1	0.1	18.2	0.1	9,467	216	1,739	27
MHE3	224.8	28.0	30.2	0.3	7.8	0.1	1.1	0.1	17.9	0.5	144.6	23.1	22.0	0.2	1.5	0.1	0.4	0.0	41.6	0.8	9,304	164	2,203	62
MHE4	103.0	15.3	38.0	1.3	7.1	0.1	0.7	0.1	14.3	0.1	133.6	11.1	58.6	1.3	1.2	0.2	2.7	0.7	46.4	0.5	9,740	170	1,929	32
MHE5	86.4	0.9	70.3	1.8	4.2	0.1	0.6	0.3	11.2	0.3	144.9	10.3	72.9	2.8	2.3	0.5	2.3	0.0	29.9	0.5	15,086	525	2,148	67
MHE6	180.2	1.9	32.7	0.7	10.9	0.2	0.8	0.1	13.6	0.5	228.6	16.5	171.2	5.6	2.1	0.2	1.6	0.4	44.4	1.1	14,539	128	4,292	47
MHE7	146.5	17.0	69.3	1.4	7.9	0.2	0.9	0.0	10.2	0.1	256.9	21.8	43.6	0.5	2.1	0.2	4.1	0.7	63.2	0.3	11,393	362	2,618	66
MHE8	109.6	11.5	53.5	1.2	7.5	0.1	0.5	0.0	15.3	0.3	160.3	9.3	49.4	0.7	0.8	0.2	2.4	0.6	25.0	0.4	13,313	326	2,074	26
MHE9	61.3	0.8	29.2	0.5	6.2	0.1	0.4	0.0	13.4	0.3	103.3	0.9	52.4	1.3	1.8	0.1	1.5	0.0	118.7	1.3	11,260	111	2,638	23
MHE10	219.8	26.2	48.7	1.3	7.7	0.4	0.6	0.0	15.2	0.1	288.4	28.2	65.2	2.2	2.5	0.2	4.1	0.8	49.6	0.5	13,699	182	2,277	24
MHE11	54.1	3.9	27.8	0.7	8.2	0.1	0.2	0.0	12.1	0.2	86.7	2.3	111.1	0.7	1.3	0.2	1.3	0.1	26.7	0.4	12,818	122	2,721	28

Table 4. Results of determination of the elements by the ICP-MS method.

[ppm]	V	Cr	Mo	Pb	Cd
MHE1	0.33	0.68	0.68	0.30	0.15
MHE2	0.25	0.41	0.73	0.43	0.04
MHE3	1.42	2.17	1.91	1.95	0.51
MHE4	0.33	0.91	1.42	0.81	0.15
MHE5	0.32	1.13	1.63	0.65	0.06
MHE6	0.51	1.02	2.19	0.88	0.23
MHE7	0.77	1.22	1.09	1.54	0.19
MHE8	0.46	0.69	1.10	3.54	0.10
MHE9	0.33	0.63	1.14	0.60	0.24
MHE10	0.77	0.84	1.45	2.61	0.17
MHE11	0.29	0.31	0.68	0.75	0.27

Table 5. Results of determination of the total of polyphenols, including phenolic acids, with standard deviations.

	Total of phenolic acids ±SD*	Total of polyphenols ±SD**
MHE1	0.40±0.02	1.15±0.02
MHE2	0.42±0.02	1.27±0.02
MHE3	0.21±0.01	0.81±0.01
MHE4	0.18±0.01	0.73±0.01
MHE5	0.13±0.01	0.65±0.01
MHE6	0.22±0.01	0.58±0.01
MHE7	0.16±0.01	0.88±0.02
MHE8	0.11±0.00	0.47±0.01
MHE9	0.19±0.01	0.94±0.02
MHE10	0.18±0.01	0.82±0.02
MHE11	0.42±0.02	1.41±0.03

*percentage content of phenolic acids calculated as caffeic acid ± standard deviation

**percentage content of polyphenols calculated as caffeic acid ± standard deviation

stock solution A was added. After stirring, 0.5 ml of Folin-Ciocalteu reagent was added to each flask, and after 1 minute 2 ml of 20% solution of sodium carbonate were put in each flask. Distilled water was poured into the flasks to obtain a volume of 10 ml and the contents were thoroughly mixed. The blank test was performed similarly by replacing 1 ml of the stock solution A with 1 ml of distilled water. Thirty minutes after the addition of sodium carbonate, absorbance of the solutions was measured at wavelength λ=760 nm in relation to the blank test.

The total content of polyphenols in the investigated samples, calculated as caffeic acid, was evaluated by means of the linear equation $y = ax + b$.

Results and Discussion

The studies resulted in obtaining information about the contents of copper, chromium, barium, boron, aluminium, iron, manganese, nickel, lead, zinc, calcium, magnesium, cadmium, molybdenum, and vanadium, as well as the total of polyphenols, including the total of phenolic acids, in the material. The concentrations of cobalt, selenium, arsenic, beryllium, and thallium was below the limit of quantification. Table 3 presents the results of the determination of the select elements by the ICP-OES method with standard deviation, Table 4 shows the results of the determination of the elements by the ICP-MS method, and Table 5 contains the results of the determination of the total of polyphenols, including phenolic acids, with standard deviation.

The lowest content of aluminium, iron, copper, and molybdenum were characteristic of the samples with the greatest content of the total of polyphenols (including phenolic acids).

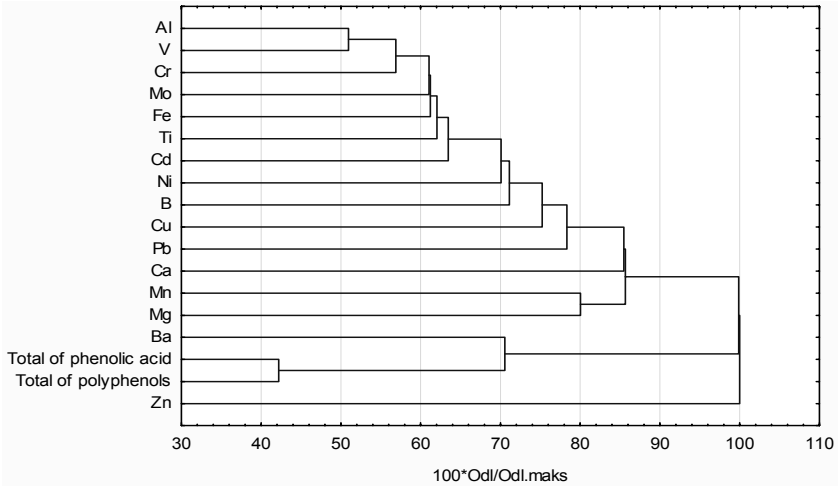

Fig. 1. Cluster analysis.

Table 6. Values of Spearman's rank correlation coefficients.

	Al	B	Ba	Cr	Cu	Fe	Mn	Ni	Ti	Zn	Ca	Mg	V	Mo	Pb	Cd	Total of phenolic acid	Total of polyphenols
Al	1.000	0.464	-0.145	**0.849**	**0.673**	**0.827**	-0.118	0.507	0.337	0.418	0.227	0.245	**0.918**	**0.691**	**0.800**	0.345	-0.443	**-0.627**
B	0.464	1.000	-0.464	0.447	0.100	**0.727**	0.091	0.461	**0.702**	0.255	0.482	-0.127	0.345	0.245	0.427	-0.345	**-0.877**	**-0.618**
Ba	-0.145	-0.464	1.000	-0.251	-0.455	-0.300	0.173	-0.297	-0.456	-0.409	-0.409	-0.100	0.000	-0.455	-0.273	0.018	**0.740**	0.482
Cr	**0.849**	0.447	-0.251	1.000	0.484	**0.694**	-0.288	0.443	0.332	0.525	0.137	0.224	**0.712**	**0.735**	0.589	0.297	-0.463	**-0.612**
Cu	**0.673**	0.100	-0.455	0.484	1.000	0.445	-0.245	0.082	0.191	0.191	0.209	0.245	0.545	**0.636**	**0.800**	0.400	-0.374	-0.600
Fe	**0.827**	**0.727**	-0.300	**0.694**	0.445	1.000	0.036	**0.685**	**0.724**	0.509	0.600	0.355	**0.745**	0.573	**0.764**	0.091	**-0.690**	**-0.655**
Mn	-0.118	0.091	0.173	-0.288	-0.245	0.036	1.000	0.315	0.046	0.000	0.409	0.245	-0.173	0.009	-0.273	0.009	0.201	-0.045
Ni	0.507	0.461	-0.297	0.443	0.082	**0.685**	0.315	1.000	0.400	**0.648**	0.575	0.548	0.479	0.539	0.233	0.288	-0.323	-0.215
Ti	0.337	**0.702**	-0.456	0.332	0.191	**0.724**	0.046	0.400	1.000	0.478	0.588	0.187	0.214	0.191	0.519	-0.196	**-0.716**	-0.415
Zn	0.418	0.255	-0.409	0.525	0.191	0.509	0.000	**0.648**	0.478	1.000	0.118	0.473	0.482	0.391	0.191	0.473	-0.324	-0.109
Ca	0.227	0.482	-0.409	0.137	0.209	0.600	0.409	0.575	0.588	0.118	1.000	0.527	0.027	0.464	0.391	-0.091	-0.470	-0.518
Mg	0.245	-0.127	-0.100	0.224	0.245	0.355	0.245	0.548	0.187	0.473	0.527	1.000	0.255	0.364	0.300	**0.727**	0.055	-0.036
V	**0.918**	0.345	0.000	**0.712**	0.545	**0.745**	-0.173	0.479	0.214	0.482	0.027	0.255	1.000	0.464	**0.700**	0.445	-0.333	-0.400
Mo	**0.691**	0.245	-0.455	**0.735**	**0.636**	0.573	0.009	0.539	0.191	0.391	0.464	0.364	0.464	1.000	0.464	0.245	-0.347	**-0.718**
Pb	**0.800**	0.427	-0.273	0.589	**0.800**	**0.764**	-0.273	0.233	0.519	0.191	0.391	0.300	**0.700**	0.464	1.000	0.264	-0.562	-0.600
Cd	0.345	-0.345	0.018	0.297	0.400	0.091	0.009	0.288	-0.196	0.473	-0.091	**0.727**	0.445	0.245	0.264	1.000	0.256	0.155
Total of phenolic acid	-0.443	**-0.877**	**0.740**	-0.463	-0.374	**-0.690**	0.201	-0.323	**-0.716**	-0.324	-0.470	0.055	-0.333	-0.347	-0.562	0.256	1.000	**0.708**
Total of polyphenols	**-0.627**	**-0.618**	0.482	**-0.612**	-0.600	**-0.655**	-0.045	-0.215	-0.415	-0.109	-0.518	-0.036	-0.400	**-0.718**	-0.600	0.155	**0.708**	1.000

Bold - R>0.6000

The total of polyphenols was greatest in samples MHE1, MHE2, and MHE11, and amounted to 1.15%, 1.27%, and 1.41%, respectively. MHE1 and MHE2 came from a meadow by Puszcza Zielonka and one by the road Poznan-Wierzonka, right behind Kicin, respectively, whereas MHE11 was a commercial sample. The content of the total of phenolic acids was also highest in these samples (MHE – 0.40%; MHE2 – 0.42 %, MHE11 – 0.42%). In the other ones, the total of polyphenols ranged from 0.94 to 0.47%, while the content of phenolic acids varied from 0.22 to 0.11%.

An attempt at Spearman's rank correlation between the total of polyphenolic compounds, including phenolic acids, and the select elements showed that the content of phenolic acids was mainly connected with boron, barium, iron, and titanium, whereas the content of polyphenolic compounds was connected with aluminium, boron, chromium, molybdenum, and iron. This was proven by the value of the correlation coefficient which exceeded 0.6000. Spearman's rank correlation test was performed with the programme STATISTICA 10. The results have been given in Table 6. The determined correlation coefficients were significant at $p < 0.05$.

In order to illustrate the "data structure" of the content of the select elements and the total of polyphenols, including phenolic acids, all results of the determinations were converted into standard values, according to the following equation:

$$(x_i - x_{śr})/\mathrm{SD}$$

...where x_i – value of an individual result, $x_{śr}$ – mean value for the investigated parameter, SD – standard deviation of the investigated parameter.

Such a transformation allows us to obtain results in a non-dimensional form and, next, to conduct cluster analysis (of concentrations and connections).

This analysis can be achieved by a few different classification algorithms and it may be used to detect data structures, without interpreting or explaining the correlations. Cluster analysis is not a statistical test, but a set of various algorithms which "group objects into concentrations." Grouping is used in many different research areas. A clear "structure" of data means that there are concentrations of objects similar to each other and in such a case the structure will often be reflected on a hierarchical tree in the form of separate branches. The method of grouping enables to detect concentrations (branches) and their interpretations. Measurements of discrepancy or distances between the objects are used during cluster formation. The most frequently chosen one is Euclidean space, i.e. geometric distance in multidimensional space. It is calculated according to the following formula:

$$\mathrm{Distance}(x,y) = \{\sum \mathrm{i}\ (x_i - y_i)^2\}^{½}$$

The results of the analysis have been presented in Fig. 1. The calculations were performed with the program STATISTICA v. 10.

In accordance with predictions, the total of phenolic acids is mostly connected with the total of polyphenolic compounds. The total of polyphenols, including phenolic acids, is directly and least distantly connected with barium. The concentration of the total polyphenols, including the total of phenolic acids with barium, is directly related to zinc, and then to manganese, magnesium, and other metals. The concentration least connected with the total of polyphenols, including the total of phenolic acids, is the one of aluminium and vanadium as well as the content of chromium.

These relationships do not necessarily have to be reflected in the values of the rank correlation coefficients since the rank coefficient R only characterizes linear connections, while the investigated effects may be of a higher degree type (STATISTICA, v. 10.0).

Conclusions

As a result of our studies, it has been shown that polyphenolic compounds are strongly linearly correlated with aluminium, boron, chromium, iron, and molybdenum, whereas the content of phenolic acids seems to be correlated with boron, barium, iron, and titanium. Cluster analysis allowed us to illustrate the existing "data structures." In accordance with predictions, the total of phenolic acids turned out to be mostly connected with the total of polyphenolic compounds. The total of polyphenols, including phenolic acids, is directly and least distantly connected with barium, and only then with other elements. These relationships do not necessarily have to be reflected in the values of the rank correlation coefficients since the rank coefficient R only characterizes linear connections, while the investigated effects may be of a higher degree type.

Conflict of Interest

The authors declare that they have no conflict of interest.

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