

RESEARCH ARTICLE

Essential and Toxic Microelements in the Medicinal Remedy *Hyperichi herba* by Different Producers

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ABSTRACT:

The elemental composition of herbal remedy, *Hyperichi herba*, grown in different regions of Ukraine and supplied by three different producers, was studied by flame atomic absorption spectroscopy. The concentrations of essential microelements Fe, Cu, Mn and Zn, as well as toxic metals Pb, Cd, Cr and Co were determined and compared with the available literature data. The significant difference between samples from different producers is observed for Mn, Co, and Cd. The higher the Fe content, the lower Mn and Zn concentrations are detected in individual samples. The concentration of Cu is rather stable. Cadmium is detected in samples of one producer only while in others it is below the detection limit. Relatively small concentrations of Pb are detected in all samples. Chromium is below the detection limit in all studied plants. The elemental composition of plants collected by one producer is characterised by higher Mn and Cd concentrations compared to other samples. The observed Mn enrichment correlates well with an increased content of this element in regional rivers which is virtually independent of an anthropogenic impact on the environment. The principal component analysis was used to minimise the number of variables. Three principal components composed of the concentrations of all essential and some non-essential microelements are sufficient to explain 91% of the total variance.

KEYWORDS: Medicinal plants, atomic absorption spectroscopy, Elemental composition, toxic metal contamination, Principal component analysis.

INTRODUCTION:

Last decades, the use of medicinal plants has been remarkably increasing that is in line with the global trend of people returning to nature-cure^{1,2}. It is often taken as an axiom that herbal medicines are less toxic for the human organism compared to synthetic substances^{3,4}. However, such an opinion oversimplifies the real situation⁵⁻⁷. Conventional medicines are purified compounds, and clinical trials establish their safe doses.

Ingress of any extraneous substances is controlled in all manufacturing stages. On the contrary, medicinal plants may contain a large variable number of chemical compounds and elements. The quality assessment of medicinal plants mainly focuses on the availability of active pharmaceutical ingredients (APIs) while the content of various metals and metalloids may vary in a wide range. There has been an increasing number of research focusing on the elemental compositions of medicinal plants to assess their safety for public consumption⁸⁻¹⁰. Medicinal plants are cultivated with a range of agricultural practices under different ecological conditions. Since the high number of species and different plant organs are used, a high botanical variability in plant compounds is also observed¹¹⁻¹³. All these factors determine diversity in the chemical composition of medicinal plants.

For example, medicinal plants can accumulate toxic metals. The accumulation degree depends on their individual properties and the concentration of heavy metals in soil, air and water¹⁴⁻¹⁶. As heavy metals threaten human and animal health, their content in plants used for consumption or medicinal purposes must be limited. The international organisations recommend checking medicinal plants for the concentrations of heavy toxic metals, and maximum allowed limits are set for Hg, Pb, Cd and As. Nevertheless, there are known many studies documenting high concentrations of certain toxic elements compared to their safe/tolerable exposures¹⁷⁻¹⁹.

Some other microelements have a direct influence on the development of plant organisms. They participate in biochemical processes and are known as essential elements for plants¹⁵. Many elements being essential for the plant development may be simultaneously toxic to human health depending on dose²⁰. In contrast to toxic heavy metals, the content of essential elements is not controlled and restricted in herbal medicines.

In spite of a great variety of the chemical composition, the availability of medicinal plants is almost entirely unrestricted, as no prescription from doctors is required to purchase. Producers of herbal medicines readily describe plants and their parts used for remedy manufacturing, specify moisture and storage conditions. Sometimes they specify a region of herb gathering or assert that plants were grown and collected under ecological conditions. They often declare that a remedy was produced by good agricultural and collection practice (GACP). According to the GACP, seeds or planting stock and cultivating conditions are standardised with compulsory documenting²¹⁻²³. The influence of impurities on the finished medicinal product is believed to be ruled out by controlling the cultivation of plants from the time of seed.

In Ukraine, the GACP rules are not compulsory conditions for herbal medicines. By this reason, perhaps, no information on the elemental composition is shown on the boxes with medicinal herbs. Nevertheless, many plants can accumulate a considerable amount of microelements. One can suppose that the lack of regulation on the manufacturing of herbal medicines allows potentially harmful quantities of both toxic and essential trace elements to be in the products under certain conditions. Therefore, the stability of high quality of herbal raw materials is still questionable. Therefore, determining elemental compositions of medicinal plants should be of particular concern. The paper goal is to study elemental composition of samples of the herbal remedy, *Hyperici Herba*, grown and collected in different regions of Ukraine to estimate

possible effect of a location of the herbs picking.

MATERIALS AND METHODS:

Sample Preparation:

The specimens of herbal remedy, *Hyperici Herba*, commonly known as St John's wort (SJW) and supplied by three different producers, were bought at local pharmacies in Kiev, Ukraine. Producers 1, 2 and 3 supply medicinal plants collected in the central (Zhytomyr Region), eastern (Zaporizhzhya Region) and western (Ternopil Region) parts of the country respectively which are separated by a distance of 400-500 km from each other. Before analysis, the samples were ground in a high-speed rotor mill to obtain a homogeneous sample with grain diameters of ≤ 1 mm and stored in polyethylene containers.

All chemicals purchased from commercial sources were of analytical grade. Accurately weighed plant samples (approx. 2 g) were placed into a Teflon reaction crucible and treated with 10 ml of 30% hydrogen peroxide (H_2O_2) / concentrated 65% HNO_3 (1:4, v/v). Decomposition of the samples was carried out in a closed-vessel microwave digestion system. The digestion program consisted of three stages and was as follows: 80% power for 15 min, 100% for 5 min and 80% power for 20 min. After cooling, the clear digested solutions were transferred quantitatively into clean volumetric flasks and made up to 50 ml with twice distilled water. Blank experiments were carried out in the same way. Three independent digestions were performed for each plant specimen. The digests were used to determine concentrations of trace elements by flame atomic absorption spectroscopy (FAAS).

Instrumentation:

The concentrations of Cu, Zn, Fe, Mn, Pb, Cd, Co and Cr were determined using a double-beam Solaar S4 AA Spectrometer (Thermo Electron Co., USA), applying standard conditions in air/acetylene flame and using D_2 correction. The quantitative determinations were carried out by external calibration with the use of certified reference materials for metallic ions produced by Bogatsky Physics and Chemistry Institute (Odessa, Ukraine).

The working range for each element was within a linear range of the method. Calibration intervals were adjusted according to the expected concentrations of elements. The sensitivity of the method with respect to each metal was evaluated using the resulted slope of the calibration curves. Measurement of each sample was repeated three times, and the mean value was calculated. Table 1 illustrates the instrument operating conditions.

Table 1: Operating conditions for the flame AAS method

Element	Analyte wavelength, nm	Working range, µg/l	Slit width, nm	Characteristic concentration, µg/l
Fe	248.3	0.5 ÷ 5.0	0.2	0.061
Mn	279.5	0.5 ÷ 3.0	0.2	0.035
Cu	324.7	0.5 ÷ 4.0	0.5	0.033
Zn	213.9	0.5 ÷ 2.0	0.2	0.030
Pb	283.3	0.5 ÷ 10.0	0.2	0.320
Cd	228.8	0.5 ÷ 2.0	0.5	0.023
Cr	357.9	0.5 ÷ 5.0	0.5	0.100
Co	240.8	0.5 ÷ 4.0	0.2	0.077

Statistical Analysis:

Both own experimental results and available literature data were analysed by statistical methods using IBM SPSS Statistics 20 software. All data were tested for normal distribution with the Shapiro–Wilks model and variance homogeneity with the Levene’s test. The results were expressed either as the means (C_m) with standard errors of the mean (SEM) for data with a normal distribution or medians (C_{med}) and quartiles (Q_{25} , Q_{50} and Q_{75}) for asymmetric distributions.

One-way analysis of variance (ANOVA) was used to analyse possible differences among the mean concentrations. The significance level α was set at or below 5% ($\alpha \leq 0.05$). If the significant differences were found to exist among the means, then post hoc pairwise multiple comparisons were applied to make direct comparisons between two means from two individual groups and determine which means differ. Depending on the results of Levene’s tests, either the least significant difference or Tamhane’s T2 methods were used in post hoc comparisons for equal or unequal variances respectively.

We used the method of principal component analysis (PCA), the simplest type of factor analysis, with an orthogonal Varimax rotation and Kaiser normalisation. It allows one to simplify structures and illustrate large data sets, by calculating a smaller number of meaningful linear combinations (principal components) from a large number of variables (element concentrations). In essence, this method consists in the selection of a new orthogonal coordinate system in observation space. As the first principal component (PC), a direction, along which an array of observations has the largest variance,

is selected. In other words, the first task of PCA is to select interacting variables, whose cross-correlation determines the largest share of the total variance. These variables constitute the first PC. Then the first PC is excluded from further consideration. Next PCs are also selected to maximise a remaining part of the total variant. Orthogonality between all PCs is an additional condition for PC mapping. Evidently, a part of the total variance linked to a given PC decreases with its number.

RESULTS:

The measured concentrations of microelements in individual samples are shown in Table 2. The data obtained allow one to estimate a general level of concentrations and elemental variability in going from one producer to another. The concentrations of Fe and Mn range roughly from 26 to 55 µg/g and from 55 to 138 µg/g respectively. The Zn and Cu concentrations are typically much lower. They change from 17 to 31 µg/g and from 5 to 12 µg/g respectively. Pb is detected in all studied samples. Cadmium is detected in samples of producer 3 only. Cobalt is observed in samples of producers 1 and 3. The Cr concentrations are below the detection limits in all studied samples. Some of the studied elements, such as Fe, Mn, Cu and Zn, have significant metabolic functions in the human body and plants, thus being essential elements. Chromium has stimulating effects on plant growth. However, it is not considered as an essential microelement for plants¹⁵ because its functions are not completely recognised yet. Cobalt is known to be an essential microelement in plants because it is involved in symbiotic N₂ fixation and valence changes stimulation synthesis of chlorophyll and proteins¹⁵.

Table 2: Element concentrations (µg/g) in individual samples supplied by different producers

Producer	Fe	Cu	Mn	Zn	Cd	Pb	Co	Cr
1	42.53	9.45	105.77	26.39	<0.023	0.413	0.185	<0.376
1	26.74	11.85	103.99	20.89	<0.023	0.328	0.190	<0.376
1	46.17	7.63	97.39	31.42	<0.023	0.325	0.263	<0.376
2	46.06	5.02	64.08	22.41	<0.023	0.385	<0.077	<0.376
2	55.52	6.50	77.01	16.55	<0.023	0.320	<0.077	<0.376
2	49.50	5.52	55.96	19.88	<0.023	0.345	<0.077	<0.376
3	26.11	7.77	137.72	28.80	0.891	0.335	0.085	<0.376
3	35.67	8.04	132.29	28.20	0.834	0.612	0.318	<0.376
3	29.73	7.00	135.00	30.05	0.851	0.539	0.210	<0.376
Average 1-3	39.78 ±3.55	7.64 ±0.69	101.02 ±10.2	24.96 ±1.73	0.859 ±0.02	0.400 ±0.04	0.205 ±0.03	<0.376

Cadmium and lead are among the most toxic elements for both human body and plants. Both Co and Cr, being essential elements for the human body, nevertheless, are rather toxic. Modern pharmacopoeias attribute Cd and Pb to Class 1, Co to Class 2A and Cr to Class 3 and establish permitted daily exposures (PDE) based on their toxicity²⁴. Due to their pronounced toxicity, we combine

Cd, Pb, Cr and Co into a group of toxic metals for convenience. More conclusions can be formulated, if the plants under study are arranged in order of increasing or falling concentration of a particular element. The mean concentrations of essential and toxic microelements in samples of SJW supplied by producers from different regions are shown in Fig. 1.

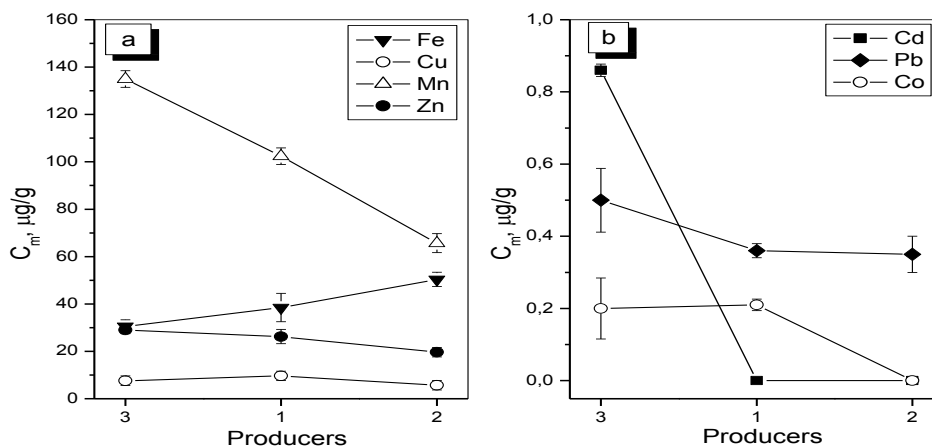


Fig. 1: Mean concentrations C_m, with SEM (µg/g) of essential (a) and toxic (b) microelements in samples of SJW supplied by three different producers (1-3)

The significant differences between samples of the various suppliers for Mn, Co and Cd and no differences for Zn, Cu, Fe and Pb were found by ANOVA tests (Table 3). Samples of producer 1 contain more Mn compared to samples of producer 2, but the highest

concentration of Mn is in samples of producer 3. The significant difference in Mn content can be considered as an evidence of substantial contamination of some samples by Mn. The higher the Mn concentration, the lower the Fe concentration is detected.

Table 3: ANOVA tests for comparison of C_m in samples of different producers

		Sum of squares	df	Mean square	F	α
Fe	Between groups	2781.003	2	1390.502	3.227	0.076
	Within groups	5171.230	12	430.936		
	Total	7952.233	14			
Cu	Between groups	12.708	2	6.354	1.556	0.251
	Within groups	49.009	12	4.084		
	Total	61.717	14			
Mn	Between groups	9241.624	2	4620.812	8.468	0.003 (*)
	Within groups	8184.982	15	545.665		
	Total	17426.606	17			
Zn	Between groups	221.745	2	110.873	1.141	0.352
	Within groups	1165.656	12	97.138		
	Total	1387.401	14			
Cd	Between groups	1.475	2	0.738	2545.080	0.000 (*)
	Within groups	0.002	6	0.000		
	Total	1.477	8			
Pb	Between groups	173.500	2	86.750	0.942	0.441
	Within groups	552.648	6	92.108		
	Total	726.148	8			
Co	Between groups	0.091	2	0.045	6.260	0.034 (*)
	Within groups	0.044	6	0.007		
	Total	0.134	8			

(*) Differences are significant at the 0.05 level (2-tailed)

DISCUSSION:

Volatility of chemical composition of plants:

Figure 1 illustrates the observed differences between samples in the mean concentrations which are

statistically significant for Mn, Cd and Co. The studied samples of producer 3 are apparently more contaminated with Cd and characterised by a higher Mn content than the samples of other producers. The Co concentration is

below the detection limit in the samples of producer 2 that differences them from other plants. The concentration differences between samples for all other elements are within experimental errors. There are known many published works which are devoted to the study of the elemental composition of St John's wort. We analysed the results of 12 publications^{16,25-35}. All these results from now on referred to as the literature data and marked with index *lit* are compared with some experimental data (index *exp*) obtained in the given

research. A large spread in the measured elemental concentrations is typical for the literature data. As follows from the Shapiro–Wilks test, only Co, Cd and Mn distributions obey the normal law while all other elements show asymmetrical distributions with pronounced skewness and kurtosis. Both mean values with SEM and medians with other characteristics of asymmetrical curves were calculated and shown in Table 4 to characterise the literature data.

Table 4: Statistics for the literature data^{16, 25-35}

	Zn	Cd	Co	Pb	Cr	Cu	Fe	Mn
Distribution	Skew	Normal	Normal	Skew	Skew	Skew	Skew	Normal
C_m(lit) of peak 1	39.92	0.06	0.45	2.00	0.98	12.04	148.63	15.5
SEM of peak 1	5.79	0.03	0.03	0.20	0.14	2.05	21.92	2.4
C_m(lit) of peak 2		0.8						124.5
SEM of peak 2		0.3						30
C_{med}(lit)	33.70	0.49	0.44	1.80	0.49	9.50	74.00	21.90
C_{min}(lit)	7.00	0.05	0.10	1.60	0.12	1.60	1.20	2.40
C_{max}(lit)	400.00	1.78	0.85	2.70	5.00	120.00	760.00	261.00
Skewness	7.10	0.89	0.23	1.13	1.79	5.50	2.19	1.26
Kurtosis	54.75	0.44	0.70	0.24	3.99	32.02	4.27	1.01
Q₂₅	24.50	0.07	0.34	1.65	0.27	7.53	49.50	11.36
Q₅₀	33.70	0.49	0.44	1.80	0.49	9.50	74.00	21.90
Q₇₅	42.01	0.80	0.51	2.45	1.60	11.29	171.50	89.00

As seen from Table 4 and Fig. 1, the measured mean concentrations of Zn and Cu are rather close in magnitude to the median values derived from the literature. On the contrary, the C_m(exp) values for Pb, Fe, Co and Cr are much lower than the C_{med}(lit) or C_m(lit), being located within the quartile Q₂₅ in the published datasets. The concentration distributions of the literature data for these elements have positive skewness and are characterised by longer right tails. In other words, very high concentrations of Fe, Pb, Cr or Co were observed by some authors, such as e.g. 1200 µg/g Fe (16 times as many as C_{med}(lit)²⁶), 8 µg/g Pb³⁶, 2.6 µg/g Co³⁷, 3.7 µg/g Cr²⁶, etc. Such abnormally high data shift both the mean and median concentrations to a

higher concentration range compared to the mean values measured in our experiments.

In turn, both Mn and Cd by the literature data demonstrate two Gaussian peaks. For example, the Cd concentration is less than 0.5 µg/g with an average C_m(lit)~0.06 µg/g in 29 of 64 published results (Fig. 2). In turn, the mean Cd concentration approximates to 0.8 µg/g in the rest 35 samples which are more contaminated and form the second Gaussian peak. By analogy with Cd, two peaks close to 65 and 135 µg/g are observed for Mn (Fig. 2). Therefore, two modes of SJW plants with lower and higher concentrations of Mn and Cd are observed in the literature.

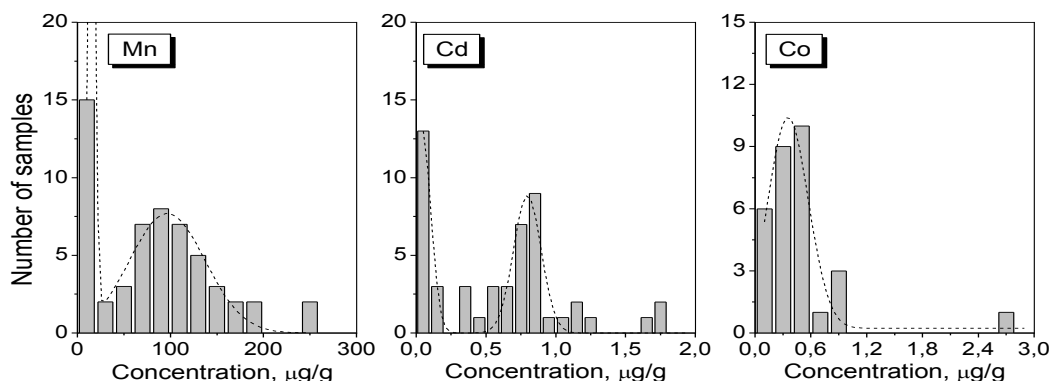


Fig. 2: Histograms illustrate Mn, Cd and Co concentration distributions by the literature data

The compositions of the studied samples qualitatively agree with the literature data. In particular, the samples

of producer 3 show the highest concentrations of Mn (C_m(exp) = 135 µg/g) and Cd (C_m(exp) = 0.86 µg/g)

among all samples. On the contrary, the samples of producer 2 are characterised by the lowest Mn concentration ($C_m(\text{exp}) = 65 \mu\text{g/g}$) and together with samples of producer 1 they have the lowest Cd content (below the detection limit). Therefore, the samples of producers 2 and 3 can be considered as weakly and strongly contaminated plants respectively. The mean experimental concentrations of Mn and Cd of producer 3 samples are very close to the mean values of peaks 2 calculated from the literature data (Fig. 2) while these concentrations are somewhat higher than the $C_m(\text{lit})$ values of peak 1 in samples of producer 2.

Evidently, the volatility of the chemical composition of same plants depends on cultivation conditions, further processing and storage. The primary sources of trace elements are plants' growth surroundings, including soil, water, atmospheric depositions, and nutrient solutions. The uptake of trace elements by plants is affected, in addition to plant-specific ability, by soils factors, of which the most significant are pH, water regime, clay content, nutrient balance, concentrations of other elements, climatic conditions, etc.¹⁵. Plants readily absorb the species of elements that are dissolved in the ground solutions in either ionic or chelated and complexed form. On the contrary, low solubility typically reduces the availability of elements in plants.

As a result, some elements are more susceptible to phytoavailability than others. For example, Ag, Cr, Sn, Ti, and Y are very slightly soluble in soil solution¹⁵. The low solubility complicates their penetration through soil layers into plant tissues, and they are not easily taken up by plants. Other elements, such as As, Hg, Pb and F, are not readily transported to above-ground parts of plants because they are relatively strongly bound with soil components. Many other elements, including Mn, Cd, Zn, B, Co, Cu, Mo, and Ni, are mobile in soil and readily taken up by plants. In SJW, such soluble elements are detected in a majority of research^{16,25-35} in contrast to insolubles.

As follows from Table 2, the medicinal plants of producer 3 collected in Ternopil region are characterised by significantly higher Mn and Cd contents compared to plants collected in other areas. The content and dynamics of seasonal migration of heavy metals, including Mn, Fe, Co, Pb, and Zn, have been recently analysed in small rivers of Ternopil region³⁸. The territory of Ternopil region was divided into 4 parts, namely agricultural (AL), urbanised (UL), recreation lands (RL), and lands transformed by a high anthropogenic impact (TL). All measured concentrations were determined relative to maximum permissible concentrations (MPCs) in the form of pollution coefficients. The MPCs for river water are set down by

government authorities of Ukraine. Only Co concentrations among chemical elements under discussion were below the MPC in all studied lands and seasons. In most cases, the Pb concentrations were below the MPC except for some samples from TL where the Pb pollution coefficient increased to 1.2. A moderate Zn pollution is observed in all lands, and its level varies between 1.3 and 2.7 depending on land types and seasons. In contrast to the elements mentioned above, the river water is heavily polluted with Mn and Fe in all lands. The Mn pollution coefficients are strong functions of seasons and vary between 3.3 and 14 for RL, <1 and 6.1 for UL, 2.5 and 4.2 for AL, and 4.3 and 7.5 for TL. For Fe, the maximal seasonal values the pollution coefficients are 8.8, 3.7, 5.6 and 9.2 for RL, UL, AL and TL respectively.

In fact, no correlation between the detected Mn and Fe contamination of water bodies and the environmental load was observed in³⁸ because both recreation lands and lands transformed by a high anthropogenic impact demonstrate similar levels of the pollution coefficients. In addition to anthropogenic factors, the excess in Mn and Fe concentration in river water is caused by the occurrence of these elements in abiotic components of river valleys, particularly in areas with iron and manganese, alluvial deposits, clay soils with ferrous metal compounds and leaching of elements from rock, soil and forest litter³⁸.

The results obtained attract attention to the problem of variability of the elemental composition of medicinal plants. On the one hand, such volatility enhances risks of bioaccumulation of toxic elements in plants grown even under conditions of recreation lands. On the other hand, it opens new aspects of nutrition of plants by necessary elements to adjust their intake to the food chain.

Safe daily intakes:

In addition to essential elements, plants reveal various tendencies in the uptake of other trace elements. There are known many research works in the literature, reporting heavy contamination of plants by many toxic elements (Hg, Pb, As, etc.). For example, St John's wort was observed to accumulate Cd^{34,35}. Cadmium is detected at a level of 0.8 $\mu\text{g/g}$ in plants of producer 3 but is absent in other studied samples. As seen from Fig. 2, the Cd histogram can be approximated by a two-peak Gaussian. One of these peaks describes Cd-free samples while the mean concentration $C_m(\text{lit})$ of another peak is similar to $C_m(\text{exp})$. Lead is observed in samples of all producers with no significant difference between them. The measured $C_m(\text{exp})$ concentrations of Pb are on average lower than the $C_{\text{med}}(\text{lit})$ value derived from the literature. Many elements, being essential for plant growth, can, however, have toxic effects on cells at

higher concentrations. Hypothetical reactions of plants to increasing concentrations of the essential and non-essential elements are schematically shown in Fig. 3. Essential elements are characterised by an optimal concentration range which provides the best conditions for organism growth while non-essential or toxic elements can impede plant growth from deficient concentrations. Both deficit and excess of essential elements can disorder metabolic cycles and provoke abnormal or stunted growth of the plant.

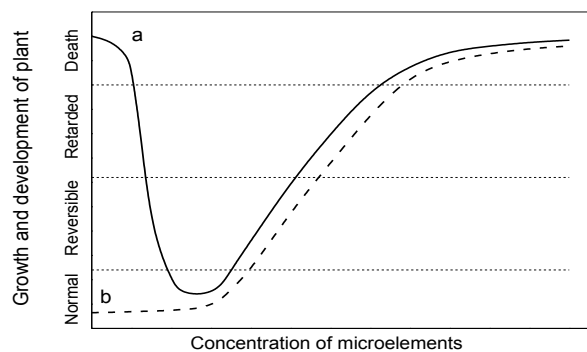


Fig. 3 Schematic diagram²⁷ of plant response to stress from deficiency and toxicity of microelements: a - essential microelements; b - non-essential (toxic) microelements

Many organisations in the world study the influence of chemical elements on human health and develop recommendations and restrictions on their daily consumption. Some recommendations determine optimal daily intakes of elements essential for the human organism and they are referred to as Levels of Optimal Consumption (LOC). Other recommendations focus on the restriction of daily consumption of both essential and non-essential toxic elements. Such recommendations represent Upper Limits of Safe Consumption (ULSC).

The LOC and ULSC ranges by the different authorities^{24,39-44} together with concentration ranges detected in our experiments are shown for in Table 5. The ratios of ULSC to the maximal concentrations observed in the experiment estimate safe limits of daily intakes of the most contaminated herbs from the viewpoint of possible side effect of the microelements, as is illustrated in Table 5. Though all studied samples show microelement contents within the acceptable limits, one-time ingestion of more than 22-80 g of the most contaminated herbs may result to overriding of the ULSC parameter in Cd and Mn respectively.

Table 5: LOC and ULSC^{24,39-44} and experimental concentration ranges

	LOC (min – max), mg ^a	ULSC (min – max), mg ^a	Range of experimental observations, , mg/kg	Safe intake, g/day	Producer
Cu	0,6-10	10-12	5-12	1000	1
Fe	8-60	45	26-56	800	2
Mn	0,3-8	11	56-138	80	3
Zn	8-18	40-50	16-31	1600	1
Co	0.02-0.04	0.6	0.04-0.34	2300	1
Cd		0.02	0.8-0.9	22	3
Pb		0.1	0.3-0.6	160	3

^a Exposures, reported in mg/kg of human body weight, were recalculated into mg assuming an arbitrary adult human body mass of 60 kg for either sex.

Principal Component Analysis:

Evidently, the chemical composition and properties of plants are determined by an ensemble of available chemical elements and compounds. Therefore, a transition from a large number of variables to a limited number of new variables that would adequately describe the collective effect of all presented chemical elements simplifies a multi-component system. We used PCA to describe variability among observed, correlated variables in terms of a potentially lower number of unobserved variables called principal components.

The PCA of St John’s wort samples was performed with the use of the literature data. The composition of 71 samples of SJW was described in^{16,25-35} and 12 elements, namely Al, B, Ba, Ca, Cu, Fe, Mg, Mn, Ni, Pb, Sr and

Zn, were recorded in most of these papers. The task of PCA was to analyse the behaviour of 12 chemical elements in the studied plant with the use of a smaller number of new variables. A data matrix composed of 12 variables and 71 cases was formed. As follows from Table 6, the chemical composition of the studied plant can be described by introducing only three new components. The first PC represents only 38.88% of the total variance, while all three PCs cover 91.4%. Each new component is a linear combination of the concentrations of chemical elements of which it is composed.

The PC graph in reciprocal space is shown in Fig. 4. The first component is composed of essential Zn and Fe in combination with impurity metals Al and Ba. Three of four elements show a strong positive correlation with this component, and the fourth element (Ba) has a strong negative correlation.

Table 6: Total Variance Explained

PC	Initial eigenvalues			Rotation sums of squared loading		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.67	38.88	38.88	4.30	35.83	35.83
2	3.58	29.82	68.70	3.87	32.26	68.08
3	2.72	22.70	91.40	2.80	23.32	91.40
4	1.03	8.60	100.00			
5	9.8×10 ⁻¹⁶	8.2×10 ⁻¹⁵	100.00			
6	6.1×10 ⁻¹⁶	5.1×10 ⁻¹⁵	100.00			

The share of PC 2 is almost 30% of the total variance. It is based on essential macronutrient Ca, which together with non-essential Sr and Ni forms a positive correlation with the component 2. Toxic Pb impurity has a strong negative correlation with the component 2. The third PC includes essential macroelement Mg and trace elements Mn and Cu. It explains almost 23% of the total variance. The correlations are positive for Mg and Mn and negative for Cu because component 3 increases with decreasing Cu content. The fact that the studied essential elements belong to different principal components illustrates the relative independence of changes in concentrations of these elements in various samples.

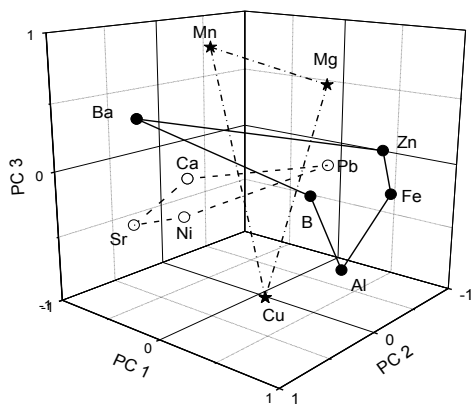


Fig. 4: Principal components plot in rotated space for St John's wort

CONCLUSIONS:

The concentrations of essential microelements Fe, Cu, Mn and Zn and toxic metals Pb, Cd, Co and Cr were studied by FAAS in samples of herbal medicine St John's wort which were grown in Ukraine and supplied by three different producers of medicinal herbs 400-500 km distant from one another. The experimental data obtained were compared with the results of 12 research works which studied 71 samples of St John's wort. The Cu content fluctuates between 5 and 12 µg/g in the experimental samples and is weakly dependent on the producer name. No statistically significant difference in Fe, Zn and Pb concentrations exists between samples of different producers. The Cr concentration is below the detection limits in all studied specimens. In contrast, the concentrations of Mn, Cd and Co in samples of different producers differ from each other. Samples of producer 3

demonstrate the highest Mn and Zn contents and simultaneously the lowest Fe concentration. Samples of producer 3 show measurable Cd concentrations and samples of producers 1 and 3 reveal Co. In other samples, both these elements are below the detection limits. The measured C_m(exp) values are in the vicinity of 0.85 µg/g for Cd and 0.2 µg/g for Co that is very close to the average figures derived from the literature data. The toxic metal, Pb, is detected in all plants, fluctuating between 0.32 and 0.61 µg/g. The measured concentrations of both Pb and Cd still secure against overuse of toxic metals with herbal remedies. However, the limits of tolerable daily intakes are not so far from the doses which can be accumulated in the course of very intensive consumption of at least the most contaminated herbs. The measured average concentrations of Cu, Co, Mn and Cd are comparable in magnitude with the mean concentrations derived from the literature data. The measured C_m(exp) values for Zn, Fe, Pb and Cr are in average lower than C_{med}(lit) reported in the literature. The application of PCA to an array of the published data allows one to explain the total variance with a lower number of variables. In particular, the chemical composition of St John's wort samples is described by introducing only three new principal components which represent 91% of the total variance. Primary essential elements are uniformly distributed among all three principal components. Each component explains 39% to 23% of the total variance.

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