Available online www.ijpras.com

International Journal of Pharmaceutical Research & Allied Sciences, 2020, 9(3):1-13

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Macro- and Microelements in the Branches of some Salix Genus Species in the Flora of Ukraine

Natalia Borodina[1], Ain Raal[2]*, Volodimir Kovalyov[1], Oleh Koshovyi[1], Tetiana Ilina[1]

[1] *Pharmacognosy Department, The National University of Pharmacy, Pushkinska st 53, 61002 Kharkiv, Ukraine.*
[2] *Institute of Pharmacy, Faculty of Medicine, University of Tartu, Nooruse 1, 50411 Tartu, Estonia.*

***Email:** ain.raal@ut.ee*

ABSTRACT

Determination of the mineral composition is important for further study and standardization of the dried branches of the species from the Salix (willow) family genus and extracts from the plant material. The study aimed to determine the qualitative and quantitative content of macro- and microelements in some representatives of the Salix spp. growing in Ukraine in comparison with soils and extracts of plant material. The elemental composition of the branches of Salix cinerea L., S. incana Schrank, S. caprea L., S. sachalinensis F. Schmidt, S. acutifolia L., S. fragilis L., S. caspica Pall., S. rosmarinifolia L., and S. myrsinifolia Salisb was determined using atomic emission spectrometry with photographic registration for the first time. The method used allowed us to determine the presence of 5 macro- (K, Na, Ca, P, Mg) and 14 microelements (Fe, Si, Mn, Al, Sr, Zn, Ni, Mo, Cu, Co, Pb, Cd, As, Hg), and the content of 14 elements were quantified in the dried branches of the Salix spp. plants. The ability to accumulate and a relatively high content of micro- and macroelements, as well as low content of toxic microelements (Co, Pb, Cd, As, Hg) in willow branches, allow considering them as a promising but safe source of biologically available microelements. There were typically not correlations between concentrations of micro- and macroelements in plant material and soils. The maximum levels of macroelements and microelements were found in Salix species collected in the Kharkiv region, and in the Zakarpatye region, respectively. The elemental composition in the Salix sachalinensis F. Schmidt was quite different if compared with all others.

Key words: *Salicaceae family, branches, mineral composition, safety, Ukraine.*

INTRODUCTION

Willows have one of the first places in the species diversity in the dendroflora of Ukraine. The genus *Salix* (Willow) family *Salicaceae* (Willow) includes about 350 species, which are united in 29 sections, and sections close in evolutionary-morphological relation - in three subgenuses: *Salix, Vetrix,* and *Chamaetia*. According to the literature, there are naturally about 25 species of willow in Ukraine. The Carpathians are home to the largest number of native willow species. Among the plants of the willow family (Salicaceae) that are artificially grown to obtain wood raw materials, as well as for landscaping, creating reclamation, recreational plantations, willow (genus *Salix* L.) species, and forms are important. Willows are indispensable for the creation of anti-erosion plantations, preliminary soil-improving crops in the reclamation of disturbed lands, as well as for energy plantations designed to produce the biomass suitable for biofuels [1-3].

The leader countries by area of land covered by energy crops in Europe are Italy - 57 thousand hectares (the largest area in Europe), Poland - 13 thousand hectares, Sweden - 12 thousand hectares, Germany - 11 thousand hectares, Denmark - 10 thousand hectares, Finland - 8 thousand hectares. According to the estimates of the Bioenergy Association of Ukraine, the potential bioenergy 21 million tons, incl. about a third - 7 million tons AD - energy crops on 2 million hectares of land. The total area of land involved in cultivation energy crops in Ukraine - about 4 000 hectares for August 2018. Most of them (approx. 3,000 ha) located on the territory of Volyn, Kyiv, and Zhytomyr regions [1, 2].

In the process of care and thinning of these plantations, there are a huge amount of waste remains - young willow branches, which would be advisable to use in medical and pharmaceutical practice to create new supplements and medicines.

They are the promising objects for phytochemical study; they are the sources of highly active natural compounds used in folk medicine for a long time as an anti-inflammatory, diuretic, antipyretic, disinfectant, hemostatic, astringent, sedative, wound healing, choleretic and antirheumatic drug to treat many diseases [4-8]. Some species of the *Salix* genus are officinal in the European countries for medicinal usage. The plant material of various species of the *Salix* genus, such as *S. purpurea* L., *S. fragilis* L., *S. daphnoides* Vill., have been included in the European Pharmacopoeia, British Herbal Pharmacopoeia, French Pharmacopoeia, and *S. acutifolia* Willd. is in the Russian Pharmacopoeia (2008) [1, 9, 10].

The Department of Pharmacognosy of the National University of Pharmacy conducted the study of amino acid composition, phenolic and volatile compounds, and carboxylic acids of branches of different species in the *Salix* genus from the Ukrainian flora [4, 11, 12]. The study of the chemical composition of the plant material of *Salix* spp. branches help to improve methods for quality control of both the raw material and pharmaceutical products from it.

Continuing these studies, it is advisable to study the composition of macro- and micronutrients in raw materials of some willows species from Ukrainian flora and extracts from them, since this is of great importance in their standardization and affects their overall pharmacological effect.

Formation and accumulation of biologically active substances in medicinal plants are dynamic processes changing in the ontogenesis of plants and depending on numerous environmental factors, including anthropogenic ones [10, 13-15]. This is especially important in the environmentally hostile conditions in Ukraine, especially after the tragedy at the Chernobyl NPP. The specificity of accumulation of heavy metals in a certain species of tree vegetation that is part of urban phytocoenosis has been proven, and it harms the quality of the plant material collected.

On the other hand, the medicinal plants, which accumulate a considerable number of essential microelements, can be used for the prevention and complex treatment of many diseases appearing because of the imbalance of microelements and macroelements in the human body [4, 16]. Also, the trace elements have an important role in the functioning of the human body, they act as cofactors for various enzymatic systems or possess regulatory activity [17].

This study aimed to determine the qualitative composition and the quantitative content of several macroelements and microelements (K, Na, Ca, P, Mg, Si, Fe, Mn, Al, Sr, Zn, Ni, Mo, Cu, Co, Pb, Cd, As, and Hg) in dried branches of 9 species of *Salix* genus naturally growing in Ukraine and their dry extracts, in comparison with soils.

MATERIALS AND METHODS

Sample Description. The branches of the nine *Salix* genus species were collected from 2015 to 2016 in Zakarpatye, Rivne, Kharkiv, and Kyiv regions (Table 1). As these samples of plant material are planned to be studied for introduction into medical and pharmaceutical practice, the raw material was harvested in an appropriate industrial planting and wildlife. Branches were collected from at least ten different trees and formed a combined sample, which was further studied to establish the content of macroelements and microelements and determine the purity of the raw materials. The tops of branches (length 30-40 cm), which included leaves and stems in the block, were cut off from the willow plants and not dried. They collected all at the same level and in the same development stage. The plant samples were dried at room temperature for 10 days, and the dried plant material preserved in well-closed bumper bags at room temperature before analyses. At the same time, average soil samples were taken under the same trees at a depth of 10 to 50 cm. The soil moisture was considered when

conducting the experiments [18, 19].
All *Salix* species studied were identified by the taxonomic guide [20, 21] and using the expert opinion of the botanist Horelov A.M. from the M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv). The voucher specimen (No 98-107) is deposited in the herbarium of the Pharmacognosy Department, The National University of Pharmacy, Kharkiv, Ukraine.

Table 1: Studied branches of different species of *Salix* genus collected in Ukraine

Sample	Species	Origin	Geographical coordinates
1	*Salix cinerea* L.	Near the river Tisa, Steblivka village, Khust district, Zakarpatye region	48° 4' 50.081" N 23° 24' 29.376" E
2	*S. incana* Schrank		
3	*S. caprea* L.		
4	*S. sachalinensis* F. Schmidt	Near the Shubkiv village, Rivne district, Rivne region	50° 41' 21.656" N 26° 29' 48.314" E
5	*S. acutifolia* L.	Near the Shelestove village, Kolomatsk district, Kharkiv region	49° 51' 54.709" N 35° 12' 16.081" E
6	*S. fragilis* L.	Near the river of Klenove village, Bohodukhiv district, Kharkiv region	50° 8' 56.076" N 35° 41' 49.416" E
7	*S. caspica* Pall.	M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv)	50° 24' 56.837" N 30° 33' 46.429" E
8	*S. rosmarinifolia* L.		
9	*S. myrsinifolia* Salisb.		

Preparation of extracts. Based on the preliminary chemical and pharmacological studies, and the prospect of using an industrial scale [4, 5, 11, 12] only samples 1, 4, 5, and 8 were selected for obtaining extracts [22]. 100 g of each sample of the crushed air-dry raw material (0.5-2 mm) were extracted with 1000 ml 70% ethyl alcohol (in the ratio of raw material-extractant 1:10) at 90 °C for 2 hours with occasional stirring. The operation was repeated 2 times. The third extraction was carried out with hot water under the prerequisites. The obtained extracts were combined, evaporated, and dried in a rotary evaporator at the temperature 85-90 °C to obtain dry extracts. The yield of the dry extracts was 18-23 % [23].

Macro-and Microelements Analysis. The elemental composition of the plant material, the soil samples, and the dry extracts were determined using a DFC-8 atomic emission spectrophotometer at the premises of the State Scientific Institution "Institute for Single Crystals" of the NAS of Ukraine. The weighed quantities of the crushed material (particle size passed through a 0.2 mm plastic sieve) were treated with sulfuric acid and carefully coaled in a muffle furnace (temperature was not more than 500 °C) for 1 hour. The samples were evaporated from the craters of graphite electrodes. There were the following conditions of spectra photographing: the amperage of the arc AC – 16 A, the ignition phase – 60°, the frequency of the pulses ignited – 100 discharges per second; the analytical interval – 2 mm; the width of the spectrograph slit – 0.015 mm; exposure – 60 s; as a source of spectra excitation IBC-28 was used. The spectra were recorded on photographic plates using a DFS-8 spectrograph with a three-lens system of the slit illumination and diffraction screen of 600 lines/mm. Measurement of the line intensities in the spectra of the samples analyzed was performed using an MF-4 microphotometer at a wavelength from 240 to 347 nm compared to the standard samples of elements – calibration samples. Photographic plates were developed and dried; then the following lines (nm) in the spectra of the samples and the standard samples of the mixture of mineral elements, as well as the background near them were measured.
According to the results of photometric measurements for each element, the differences of the blackening of the line and the background for spectra of the samples and the standard samples of the mixture of mineral elements were calculated. Then the calibration graph was plotted in the following coordinates: the average value of the difference of blackening of the line and the background – the logarithm of the element content in the standard sample of the mixture of mineral elements. According to this graph, the content of the element in ash (a)

expressed in percent was determined. The elemental composition in the plant material was calculated according to the formula:

$$x = \frac{a \bullet m}{M}$$

where m – is the mass of ash (g); M – is the weighed quantity of the dried plant material taken for analysis (g); a – is the element content in ash found by the graph (%). The method used is intended for determining trace impurities. The interval of the content specified (wt % to ash) is: Mn from 2 10^{-4} to 1, Cu - from 1 10^{-4} to 5 10^{-2}, Ni, Ge, Pb, Ga, Ag, Sn from 5 10^{-4} to 1 10^{-2}, Cd - from 5 10^{-3} to 1 10^{-2}, V, Mo, Co, Cr from 2 10^{-4} to 1 10^{-2}, Ni - 5 10^{-4} to 1, Sr - from 1 10^{-2} to 1, Zn - from 1 $^{10-2}$ to 2. Calibration graphs in the range of the concentrations of elements measured were plotted using the standard sample solutions of metal salts (ICOPM-23-27) [24-26].

Statistical Analysis. The mean and standard deviation (SD) of the sample was calculated according to the monograph "Statistical analysis of the results of a chemical experiment" of the State Pharmacopoeia of Ukraine, 2.2. The average sample μ was calculated as the arithmetic mean of all variants (n=5 of combined samples). At the same time, the spread of options around the average is characterized by the magnitude of the standard deviation s. The uncertainty of this estimate is characterized by the value of the confidence interval, in which the true value μ is given with the given two-way probability P_2. Under uncertainty, the confidence interval is understood, usually for the 95% significance level. Limit values of the confidence interval were calculated using Student's criterion. Quantitative data are presented as the mean ± SD. Linear regression between concentrations of micro- and macroelements in branches and soil analysis was used (Pearson correlation coefficients); the level of statistical significance was set at not more than p>0.05.

RESULTS AND DISCUSSION

Elemental composition of the plant material. The elemental analysis of medicinal plants for the content of different elements is required to check them for health and safety [27]. The method used allowed determining the content of 5 macro- (K, Na, Ca, P, Mg) and 14 microelements (Fe, Si, Mn, Al, Sr, Zn, Ni, Mo, Cu, Co, Pb, Cd, As, Hg) in the dried plant material studied, 8 of them also have been quantified in this study. The results presented in Table 2 give a detailed idea of the differences in the elemental composition of the samples studied.

Table 2: Content of macroelements and microelements in the branches of the *Salix* spp. Plants

Sample	The content of the elements. Mean ± SD (n=10)				
	Macroelements (g/kg)				
	P	Mg	Ca	Na	K
1	1.502±0.341	2.603±0.385	6.900±0.585	0.640±0.055	10.750±1.024
2	1.800±0.164	3.600±0.513	7.203±0.650	0.601±0.049	15.002±1.411
3	1.953±0.105	3.352±0.345	8.950±0.789	0.840±0.092	11.204±1.140
4	1.301±0.108	2.800±0.130	5.605±0.343	0.351±0.029	17.501±1.100
5	2.250±0.211	3.653±0.471	13.005±0.996	0.400±0.059	20.250±2.011
6	1.005±0.097	1.900±0.195	6.405±0.480	0.381±0.035	22.404±2.058
7	1.552±0.187	3.300±0.422	6.051±0.657	0.500±0.047	11.002±1.043
8	1.401±0.100	1.803±0.170	6.900±0.730	0.600±0.050	12.002±1.084
9	1.200±0.110	2.951±0.278	5.903±0.488	0.530±0.061	17.703±1.371

Sample	Microelements (mg/kg)								
	Fe	Si	Al	Mn	Ni	Mo	Cu	Zn	Sr
1	430.2± 41.1	3451.0±584.5	431.0±39.2	430.8±41.1	4.3±0.3	<0.3	4.4±0.4	86.2±18.1	86.5±12.5
2	540.4± 46.5	3603.9±768.5	150.8±17.1	150.2±13.7	3.1±0.4	<0.3	6.0±1.1	120.1±13.9	60.2±7.6
3	561.1± 63.1	4502.4±381.2	280.3±18.3	220.6±19.1	2.8±0.6	<0.3	5.6±0.7	112.2±15.0	112.5±14.7

4	102.3± 11.1	420.3±32.9	170.5±14.1	121.4±12.6	8.4±1.1	0.7±0.1	7.7±0.8	70.4±7.0	35.1±11.0
5	650.3± 48.2	3651.5±366.1	650.1±42.4	320.6±44.1	1.3±0.3	<0.3	10.1±1.3	40.3±3.8	20.0±2.4
6	160.0±12.9	1150.8±149.7	140.2±15.2	48.4±2.9	0.5±0.1	0.3±0.1	9.6±1.7	51.1±7.1	76.4±6.3
7	441.0±51.0	2203.3±281.5	165.2±17.2	55.0±2.8	0.6±0.1	<0.3	5.5±1.1	55.4±4.9	110.4±23.0
8	240.5±17.3	1800.1±110.6	150.3±16.8	30.8±4.9	4.2±0.3	<0.3	3.0±0.5	180.2±16.7	60.1±17.1
9	411.3±52.0	2950.5±233.2	147.3±13.9	59.4±13.1	3.1±0.2	<0.3	6.0±0.2	118.0±9.9	88.4±11.3

Notes: Co and Pb<0.03; Cd, As and Hg<0.01 mg/kg

Sample 1 - *Salix cinerea*, 2 - *S. incana*, 3 - *S. caprea*, 4 - *S. sachalinensis*, 5 - *S. acutifolia*, 6 - *S. fragilis*, 7 - *S. caspica*, 8 - *S. rosmarinifolia*, and 9 - *S. myrsinifolia*

All analyzed microelements were determined quantity, but 5 ones (Co, Pb, Cd, As, Hg) were not found within the sensitivity of the method (was found in very small quantities (Co, Pb, Cd, As, Hg)) [28]. Iron, cobalt, manganese, copper, molybdenum, and zinc are among the most important essential elements. Fortunately, in willow branches such toxic elements as cobalt and lead (both <0.03 mg/kg), cadmium, arsenic, and mercury (all <0.01 mg/kg) were absent or not within the range of determination by the method of emission spectrometry. In branches of the species studied such microelements as potassium (10.750–22.404 g/kg), calcium (5.605–13.005 g/kg), magnesium (1.803–3.653 g/kg), phosphor (1.005-2.250 mg/kg), sodium (0.351-0.840 mg/kg), and microelements as silicon (420-4502 mg/kg), iron (102-650 mg/kg), zinc (51-180 mg/kg), manganese (31–431 mg/kg) were accumulated in the greatest amount. On average, the maximum content of macroelements was noted for species collected in the Kharkiv region (samples 5 and 6), and the maximum content of microelements was observed for the branches collected in the Carpathians (samples 1-3). The content of both macroelements and microelements in sample 4 is much more different if compared with all others. This is probably due to the soil and climatic conditions of the plants *S. caspica, S. rosmarinifolia,* and *S. myrsinifolia*, which branches were collected in M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv), accumulated microelements at the sufficiently high level. The branches of *S. caprea, S. acutifolia,* and *S. fragilis* can be recommended as the richest sources of different macroelements, the most microelements had *S. acutifolia* (Al, Mn, Ni, Mo, Cu), and *S. caprea* (Fe, Si, Zn, Sr).

The concentration of cadmium and lead was low, less than 0.03 mg/kg. According to The Commission of the European Communities [6, 29], the maximum allowed concentrations for cadmium and lead are 0.05 and 0.2 mg/kg/bw for vegetables, berries, and fruits, which are detected much more in *Salix* species by us. Also, the leaching of toxic elements such as cadmium and lead from plant material by water extraction was very little in the study performed by Drozdz et al. [17]. It was found [30] that 21% of the analyzed 54 samples of medicinal plants contain both cadmium and lead above their permissible limits.

As much the level of iron in different willows was between 102 and 650 mg/kg, it can be considered safe as its iron concentration is below the WHO limit 3000 mg/kg [31]. On the other hand, the iron concentration in 25 woody trees and shrubs in the northeast of Mexico was 66-276 mg/kg [32], but in our study more than 300 mg/kg in six samples of nine *Salix* spp. It is interesting to mention that the ferrous type of natural mineral water (Fe > 1 mg/L) is suggested for anemia and iron deficiency [33]. Plants contain less heme iron than nonheme iron which bioavailability is low (2-20%) [34]. Thus, the rather high concentration of iron in the *Salix* genus studied and its real influence on human health stays unclear. On the other hand, there is no correlation between the content of iron in plant material and soils (correlation coefficient was 0.491, p=0.202).

By Meos et al. [35] the lead content in dry pot marigold (*Calendula officinalis* L.) inflorescences was 9.3 mg/kg, in dry leaves 11.6 mg/kg, and in soil 0.65 mg/kg. There was a strong positive correlation between the number of precipitations and lead content of plant leaves indicating the soil as primarily the source of increased lead content. The main indigenous and primary source of elements in medicinal plants associated with soil [36]. As much the willow branches studied did not have direct contact with soil, there is no risk for contamination from the soil but stays possible from wind/dust. Willow tissues can accumulate relatively high cadmium concentration; therefore, it can remove, restore, and stabilize pollutants in the environment [37]. The content on silicon (420-4502 mg/kg) was also quite high (140-10840 mg/kg) in eight medicinal plants growing in Iraq [38]. Several heavy metals with health risks (Pb, Cr, As, Zn, Cd, Cu, Hg, and Ni) can be accumulated in plant tissues from the soil [39, 40]. On the other hand, the anthropogenic contamination of also water and atmosphere results in contamination of the medicinal plants and formulations [27].

Elemental composition of the soils. Analyses of micro and microelements also in different parts of *Salix* spp. growing in Ukraine may be informative in the future. It was shown [38] that the concentrations of copper and zinc varied between *Salix* plant parts and species, for example, copper concentrations decreased in the order twigs > leaves > wood > bark. Higher concentrations of Cd, Pb, and Zn were generally noted for the stems than for the leaves whereas Mn and Cu were consistently present at higher concentrations in the leaves of *Salix subfragilis* [41].

The content of macroelements and microelements in raw materials is greatly influenced by their composition in soils where it grows [41-46]. Thus, a study of the elemental composition of the soils has been also performed (Table 3). The research showed that the soils were rich in macroelements such as P, Mg, Ca and K, and microelements – Fe, Al, Mn, Mo, and Cu.

Table 3: Content of macroelements and microelements in the soils of the *Salix* spp. plants

Sample	The content of the elements, mg/100 g, Mean ± SD (n=5)				
	Macroelements				
	P	Mg	Ca	Na	K
1	250.0±21.5	1200.0±101.2	1500.0±181.6	3000.0±151.5	1800.0±111.7
2	180.0±11.7	1000.0±81.3	600.0±53.2	1600.0±168.2	1500.0±173.2
3	300.0±31.2	1000.0±94.5	1000.0±108.4	1600.0±201.6	1300.0±110.1
4	90.0±8.1	280.0±31.1	300.0±31.2	500.0±15.8	380.0±31.8
5	280.0±18.8	1100.0±121.5	1300.0±145.8	1600.0±179.3	1300.0±181.9
6	250.0±26.2	1150.0±131.8	1200.0±112.3	1400.0±135.2	1500.0±201.6
7	250.0±19.7	850.0±82.6	2300.0±302.6	1500.0±201.6	2200.0±303.7
8	350.0±36.2	1000.0±81.9	5000.0±381.2	1600.0±151.6	1800.0±210.5
9	300.0±34.6	1000.0±103.4	3500.0±323.5	2000.0±234.2	1600.0±188.4

Sample	Microelements								
	Fe	Si	Al	Mn	Ni	Mo	Cu	Pb	Sr
1	4000.0±386.6	33000.0±561.2	8000.0±89.5	40.0±8.5	7.0±0.8	12.0±3.2	150.0±8.2	10.0±0.5	10.0±1.5
2	3000.0±258.8	33000.0±538.7	7000.0±101.2	45.0±1.7	6.5±1.2	5.0±0.6	80.0±3.5	7.0±0.7	5.0±0.8
3	3000.0±311.7	38000.0±395.5	8000.0±111.7	55.0±3.9	6.0±0.2	20.0±1.2	60.0±2.1	6.0±1.2	12.0±0.6
4	1200.0±81.2	28000.0±681.4	1800.0±65.7	11.0±1.2	7.0±0.9	6.0±0.9	48.0±1.2	2.6±0.2	3.5±0.2
5	2800.0±265.8	32000.0±355.7	5000.0±116.6	50.0±4.9	6.0±1.4	10.0±0.7	130.0±3.7	6.5±0.7	8.0±0.6
6	3000.0±301.6	36000.0±581.7	6000.0±98.8	17.0±2.2	4.5±1.3	12.0±1.1	60.0±4.5	5.0±0.5	9.0±1.1
7	3200.0±334.2	33000.0±486.5	5000.0±123.2	17.0±3.8	5.0±0.5	10.0±1.3	45.0±1.9	6.0±0.6	5.0±1.2
8	2800.0±284.5	34000.0±575.5	6000.0±181.7	25.0±5.2	5.0±0.7	12.0±0.5	60.0±2.2	15.0±0.9	5.0±0.7
9	3500.0±401.8	36000.0±521.8	6500.0±201.5	20.0±1.8	3.5±0.4	18±0.8	22.0±1.5	3.0±0.5	10.0±0.4

Notes: Co <0.03; Cd, As and Hg<0.01 mg/kg

Sample of soils of: 1 - *Salix cinerea*, 2 - *S. incana*, 3 - *S. caprea*, 4 - *S. sachalinensis*, 5 - *S. acutifolia*, 6 - *S. fragilis*, 7 - *S. caspica*, 8 - *S. rosmarinifolia*, and 9 - *S. myrsinifolia*

The environmental situation in Ukraine influences some soils which were contaminated by Pb and Sr. Therefore, it is necessary to control the content of the sum of heavy metals and these elements in particular when harvesting raw materials. A comparative analysis of the soils and the branches of the *Salix* spp. plants showed that the plants studied accumulate P, Mg, Ca, and K from the soil, among the microelements - Fe, Mn, Ni, Zn, and, unfortunately also Sr. The last result must be considered when using this raw material further. On the other hand, Mo and Cu are practically not accumulated by these plants.

There were typically not correlations between concentrations of micro- and macroelements in plant material and soils, just the content of Mn showed a moderate correlation (coefficient 0.663, p=0.043). The concentrations of such elements as P, Ca, K, Mn, Mg, and, unfortunately, Sr, in the raw materials, on average, increases in 7, 7, 13, 5, 3,6, and 10 times accordingly in comparison with the soils. It is interesting to mention that some microbiological indicators, such as the proportion of actinomycetes, bacteria, and fungi in soil layers, may indicate the level of heavy metals in soils [47].

Elemental composition of the dry extracts. Upon manufacturing of galenic and new galenic medicines from raw materials, concentration, or vice versa the removal of some elements from final products can take place. Therefore, the elemental composition of the most promising extracts of *Salix* spp. was also studied (Table 4).
The results of the analysis showed a large amount of K in the extracts, while Na is practically not extracted. The microelements P, Mg, and Ca were extracted but Fe, Al, Mn, Ni, Cu, and Sr are practically not when extracts are obtained.

Table 4: Content of macroelements and microelements in the extracts of the *Salix* spp. branches

Sample	The content of the elements, mg/100 g, Mean ± SD (n=5)				
	Macroelements				
	P	Mg	Ca	Na	K
1	145.0±11.5	440.0±70.7	715.0±101.1	75.0±6.8	2970.0±110.9
4	86.0±9.6	490.0±62.4	800.0±99.6	61.0±5.5	3400.0±208.8
5	145.0±10.2	340.0±54.2	680.0±72.2	78.0±10.1	2745.0±178.4
8	190.0±18.9	385.0±50.6	645.0±47.3	170.0±18.9	2580.0±187.9

Sample	Microelements							
	Fe	Si	Al	Mn	Ni	Cu	Zn	Sr
1	0.5±0.1	55.0±5.7	0.5±0.1	3.5±0.2	0.5±0.1	0.5±0.1	5.5±0.7	1.3±0.1
4	4.9±0.3	43.0±5.1	0.8±0.2	3.7±0.5	1.8±0.7	1.5±0.3	7.4±0.9	1.8±0.3
5	2.0±0.7	98.0±6.8	1.2±0.4	3.4±0.1	0.3±0.1	0.9±0.2	7.3±1.1	1.5±0.2
8	1.5±0.2	70.0±5.5	0.5±0.1	1.7±0.2	0.4±0.1	1.0±0.3	7.7±1.3	0.9±0.1

Notes: Co, Mo, and Pb<0.03; Cd, As and Hg<0.01 mg/kg
Sample of the extracts: 1 - *Salix cinerea*, 4 - *S. sachalinensis*, 5 - *S. acutifolia* and 8 - *S. rosmarinifolia*

Our studies broaden the information regarding the chemical composition of the dried branches of the *Salix* genus plants and give a theoretical justification of the possibility of the complex use of medicinal plants taking considering the environmental factors. In sum, the results of our study also give guidance to the pharmaceutical manufacture of galenic and traditional remedies.

CONCLUSIONS

For the first time the elemental composition of branches of nine species of willow (*Salix cinerea*, *S. incana*, *S. caprea*, *S. sachalinensis*, *S. acutifolia*, *S. fragilis*, *S. caspica*, *S. rosmarinifolia*, and *S. myrsinifolia*) grown in Ukraine in comparison with soils and their extracts, has been determined. The ability to accumulate and a relatively high content of micro- and macroelements, as well as low content of toxic microelements (Co, Pb, Cd, As, Hg) in willow branches, allow considering them as a promising but safe source of biologically available microelements and substances. The maximum levels of macroelements and microelements were found in *Salix* species collected in the Kharkiv region, and in the Zakarpatye region, respectively. The elemental composition in the *Salix sachalinensis* F. Schmidt was quite different if compared with all others. The willow species studied accumulate P, Mg, Ca, and K from the soil, among the microelements - Fe, Mn, Ni, Zn, and, unfortunately also Sr, only the concentrations of Mn showed a moderate correlation in branches and soils (0.663, p=0.043).
The concentrations of P, Ca, K, Mn, Mg, and Sr, in the raw materials increases in 7, 7, 13, 5, 3,6, and 10 times accordingly in comparison with the soils.
The extracts from these raw materials contain a large amount of K, while Na is practically not extracted. Also, in the proposed technology among the microelements, P, Mg, and Ca are extracted, unlike Fe, Al, Mn, Ni, Cu, and Sr.
It is noteworthy that the extracts contain a high content of Si, which in the extract can be found only in the form of silicon-organic compounds, which indicate the prospect of studying these substances as litholytic agents. Mn

and Zn, which are important microelements and cofactors of some enzymes, also go into the extracts, which will undoubtedly affect the overall pharmacological effect of these products.

Funding. The research was funded by the Ministry of Health Care of Ukraine at the expense of the State Budget.

Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

1. Afonin A.A. Methodological principles for the creation of sustainable highly productive willow stands (for example, autochthonous species Salix of the Bryansk forest). RIO of Bryansk State University; Bryansk; 2005 (in Russian).
2. Fuchilo, Y.D., Sbitna, M.V., Fuchilo, O.Y. Indigenous willow of Ukraine, Science Newsletter NLTU of Ukraine, 2013, 23: 68-72 (in Ukrainian).
3. Kuzovkina, Y.A., Schulthess, C.P., Zheng, D. Influence of soil chemical and physical characteristics on willow yield in Connecticut. Biomass and Bioenergy, 2018; 108: 297-306.
4. Borodina, N.V. The study of volatile components Salix caprea L., Proc. 4th European Conference on Biology and Medical Sciences, 2015: 209-213.
5. Borodina, N.V., Kovalyov, V.V., Koshovyi, O.M. Comparative analysis of the amino acid composition of shoots Salix purpurea L., Salix viminalis L., Salix fragilis L., Bulletin of the South Kazakhstan State Pharmaceutical Academy, 2014, 68: 53-55 (in Russian).
6. Breslavskyi, V.B., Kurin, V.A. Plants of Salicaceae family and propolis are promising sources of phytomedicines, Traditional Medicine, 2011, 5: 159-163 (in Russian).
7. Furchilo, Y.D., Sbitna, M.V. Salix of Ukraine (biology, ecology, exploitation). Logos; Kyiv; 2009 (in Ukrainian).
8. Shinkovenko, I.L., Kashpur, N.V., Ilyina, T.V., Kovalyova, A.M., Goryacha, O.V., Koshovyi, O.M., Toryanyk, E.L., Kryvoruchko, O.V. The immunomodulatory activity of the extracts and complexes of biologically active compounds of Galium verum L. herb, Ceska a Slovenska Farmacie, 2018; 67: 25-29.
9. Oketch-Rabah, H.A., Marles, R.J., Jordan, S.A., Low Dog, T. United States Pharmacopeia Safety Review of Willow Bark (Review), Planta Medica, 2019, 85(16): 1192-1202.
10. Salam, M.M.A., Mohsin, M., Kaipiainen, E., Villa, A., Kuittinen, S., Pulkkinen, P., Pelkonen, P., Pappinen, A. Biomass growth variation and phytoextraction potential of four Salix varieties grown in contaminated soil amended with lime and wood ash, International Journal of Phytoremediation, 2019, 21: 1329-1340.
11. Borodina, N.V., Kovalyov, V.V. Comparative analysis of phenolic compounds in shoots of Salix caprea L., Salix purpurea L., Salix viminalis L. in the flora of Ukraine, Paper presented at the IX International Symposium: Phenolic compounds: fundamental and applied aspects, Moscow, 2015. (in Russian).
12. Borodina, N.V., Kovalyov, V.V., Koshovyi, O.M., Akhmedov, E.Y. The chromatography-mass spectrometry study of Salix rosmarinifolia L., Azerbaijan Pharmaceutical and Pharmacotherapy Journal, 2016, 16: 15-20.
13. Gontova, T., Ilyinska, N., Golembiovska, O. A study of the component composition of phenolic compounds obtained from Dahlia varieties Ken’s Flame herb, Der Pharma Chemica, 2016; 8(18): 455-459.
14. Hrishko, V.M., Danilchuk, O.V. The accumulation of some heavy metals in poplars and the peculiarities of the migration of elements in the soil-plant system, Plant Introduction, 2007, 3: 84–91 (in Ukrainian).
15. Kokhno, M.A., Hordienko, V.I., Zakharchenko, H.S. Dendroflora of Ukraine. Wildlife and cultivated plants and shrubs. Higher school; Kyiv; 2001 (in Ukrainian).
16. Gonzalez Rodriguez, H., Maiti, R., Duenas Tijerina, H.A., Kumari, A., Sarkar, N.C. Macro and micronutrient contents of 25 woody trees and shrubs in northeast of Mexico, International Journal of Bio-Resource & Stress Management, 2015, 6: 478-483.
17. Drozdz, P., Seziene, V., Pyrzynska, K. Mineral composition of wild and cultivated blueberries. Biol Trace Elem Res, 2018; 181: 173-177.

18. Zabolotnyi, O.V., Zabolotnyi, V.A., Koshevoi, M.D. Conditionality examination of the new testing algorithms for coal-water slurries moisture measurement, Naukovyi Visnyk Natsionalnoho Hirnychoho Universytetu, 2018, 1: 51-59.
19. Zdoryk, O.A., Khokhlova, K.O., Georgiyants, V.A., Vyshnevska, L.I. Investigation of physical and chemical stability of ointment with herbals, International Journal of Pharmaceutical Compounding, 2014, 18(3): 248-252.
20. Kochno, M.A., Parkhomenko, L.I., Zarubenko, A.V. Dendroflora of Ukraine. Wild and cultivated trees and bushes. Angiosperms. Phytocenter; Kyiv; 2002.
21. Skvortsov, A.K. Willows of Russia and Adjacent Countries. Taxonomical and Geographical Revision. University of Joensuu; Joensuu; 1999.
22. Ilina, T., Kashpur, N., Granica, S., Bazylko, A., Shinkovenko, I., Kovalyova, A., Goryacha, O., Koshovyi, O. Phytochemical profiles and in vitro immunomodulatory activity of ethanolic extracts from Galium aparine L., Plants, 2019, 8: 541.
23. Borodina, N.V., Kovalev, V.N., Stremoukhov, O.O., A method of obtaining a complex dry extract from vegetable raw materials. Ukraine patent No 98912, 9, 2015 (in Ukrainian).
24. Maliuvanchuk, S.V., Stasiv, T.H., Hrytsyk, A.R., Struk, O.A. The study of macro- and micro-elemental composition of plant raw materials of Aiugareptans L. and Filipendulahexapetala Gilib., Pharmacia, 2018, 65(4): 38-45.
25. Mykhailenko, O., Gudžinskas, Z., Kovalyov, V., Desenko, V., Ivanauskas, L., Bezruk, I., Georgiyants, V. Effect of ecological factors on the accumulation of phenolic compounds in Iris species from Latvia, Lithuania, and Ukraine, Phytochemical Analysis, 2020, 31(1): 1–19.
26. Osmachko, A.P., Kovaleva, A.M., Ili'ina, T.V., Koshovyi, O.N., Komisarenko, A.M., Akhmedov, E.Yu. Study of macro- and microelements composition of Veronica longifolia L. herb and Veronica teucrium L. herb and rhizomes, and extracts obtained from these species, Azerbaijan Pharmaceutical and Pharmacotherapy Journal, 2017, 17: 24-28.
27. Pohl, P., Dzimitrowicz, A., Jedryczko, D., Szymczycha-Madeja, A., Welna, M., Jamroz, P. The determination of elements in herbal teas and medicinal plant formulations and their tisanes, J Pharm Biomed Anal, 2016, 130: 326-335.
28. Ud Din Ahmad, F., Ahmad, N., Masood, K.R., Hussain, M., Malik, M.F., Qayyum, A. Phytoremediation of arsenic-contaminated soils by Eucalyptus camaldulensis, Terminalia arjuna, and Salix tetrasperma, Journal of Applied Botany and Food Quality, 2018, 91: 8-13.
29. Commission of the European Communities Commission Regulation (EC) No 1881/2006 setting maximum levels of certain contaminants in foodstuffs. Off J Eur Union L364, 2006: 5-24.
30. Ababneh, F.A. The hazard content of cadmium, lead, and other trace elements in some medicinal herbs and their water infusions, Int J Anal Chem., 2017, doi.org/10.1155/2017/6971916.
31. Biel, W., Jaroszewska, A. The nutritional value of leaves of selected berry species, Scientia Agricola, 2017, 74: 405-410.
32. Courchesne, F., Turmel, M.C., Cloutier-Hurteau, B., Constantineau, S., Munro, L., Labrecque, M. Phytoextraction of soil trace elements by willow during a phytoremediation trial in Southern Québec, Canada, International Journal of Phytoremediation, 2017, 19(6): 545-554.
33. Quattrini, S., Pampaloni, B., Brandi, L.L. Natural mineral waters: chemical characteristics and health effects, Clin Cases Miner Bond Metabol, 2016, 13: 173-180.
34. Brian J. Alloway. Heavy Metals in Soils. Trace Metals and Metalloids in Soils and their Bioavailability. Third Edition. Springer Dordrecht Heidelberg; New York, London; 2013. DOI 10.1007/978-94-007-4470-7.
35. Meos, A., Jüriado, T., Matto, V., Raal, A. Lead content in pot marigold (Calendula officinalis L.) inflorescences, and leaves: impact of precipitations and vicinity of motorway, Biol Trace Elem Res, 2011, 149: 244-251.
36. Pohl, P., Bielawska-Pohl, A., Dzimitrowicz, A., Greda, K., Jamroz, P., Lesniewicz, A., Szymczycha-Madeja, A., Welna, M. Understanding element composition of medicinal plants used in herbalism – A case study by analytical atomic spectrometry, J Pharm Biomed Anal, 2018, 159: 262-271.
37. Yang, W., Wu, F., Ding, Z., Zhang, X., Zhao, F., Wang, Y., Yang, X. Cadmium accumulation and tolerance in seven ornamental willow genotypes, Bull Environment Contam Toxicol, 2018, 101: 644-650.

38. Hasan, N.M., Jaber, J.K., Fayyadh, I.K., Dawood, S.S. Major and Trace Elements in Some of Iraqi Medicinal Herbs via X-ray Fluorescence Technique, J College Educ, 2016, 3: 55-66.
39. Aricia Evlard, Kjell Sergeant, Bruno Printz, Cédric Guignard, Jenny Renaut, Bruno Campanella, Roger Paul, Jean-Francois Hausman, A multiple-level study of metal tolerance in Salix fragilis and Salix aurita clones. Journal of Proteomics, 2014, 101: 113-129.
40. Wuana, R.A., Okieimen, F.E. Heavy metals in contaminated soils: A review of sources, chemistry, risks, and best available strategies for remediation, ISRM Ecol ID 402647, 2011: 1-20.
41. Kim, H.T., Kim, J.G. Seasonal variations of metal (Cd, Pb, Mn, Cu, Zn) accumulation in a voluntary species, Salix subfragilis, in unpolluted wetlands, Sci Total Environm., 2018, 610-611: 1210-1221.
42. Břendová, K., Kubátová, P., Száková, J., Tlustoš, P. Trace element leaching from contaminated willow and poplar biomass – A laboratory study of potential risks, Biomass and Bioenergy, 2018, 112: 11-18.
43. Gąsecka, M., Mleczek, M., Jutrzenka, A., Goliński, P., Stuper-Szablewska, K. Phenolic compounds in leaves of Salix species and hybrids growing under different soil conditions, Chemistry and Ecology, 2017, 33 (3): 196-212.
44. Krivoruchko, E., Markin, A., Samoilova, V.A., Ilina, T., Koshovyi, O. Research in the chemical composition of the bark of Sorbus aucuparia, Ceska a Slovenska Farmacie, 2018, 67: 113-115.
45. Quiñones Martorello, A.S., Gyenge, J.E., Fernández, M.E. Morpho-physiological response to vertically heterogeneous soil salinity of two glycophyte woody taxa, Salix matsudana x S. alba and Eucalyptus camaldulensis, Plant and Soil, 2017, 416: 343-360.
46. Stolarski, M.J., Szczukowski, S., Tworkowski, J., Krzyżaniak, M., Załuski, D. Willow production during 12 consecutive years. The effects of harvest rotation, planting density, and cultivar on biomass yield, GCB Bioenergy, 2019, 11: 635-656.
47. Hamitowa, S.M., Glinushkin, A.P., Avdeev, Y.M., Nalyuhin, A.N., Beliy, A.V., Zavarin, D.A., Snetilova, V.S., Lebedeva, M.A., Danilova, E.D., Semykin, V.A., Pigorev, I.Y., Lichukov, S.D. Assessment of microorganisms and heavy metals' content in the soils of arboretum named after Nikolai Klyuev. International Journal of Pharmaceutical Research & Allied Sciences, 2017, 6: 47-55.