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Content of Mobile Elements in the Soil and their Accumulation in the Shoots and Fruits of Highbush Blueberry (*Vaccinium corymbosum* L.) and Bogbillberry (*V. uliginosum*)

Nataliya Vorobets [1], Nataliya Yavorska [1], Roman Fafula [2,*], Tetyana Zazuliak [3,*]

[1] Pharmacognosy and Botany Department, Danylo Halytsky Lviv National Medical University; vorobets_natalia@meduniv.lviv.ua (N.V.);
[2] Department of Biophysics, Danylo Halytsky Lviv National Medical University; roman_fafula@ukr.net (R.F.);
[3] Central Research Laboratory and the Laboratory of Industrial Toxicology of Danylo Halytskyi Lviv National Medical University; CsRL_dept@meduniv.lviv.ua (T.Z.);
* Correspondence: vorobets_natalia@meduniv.lviv.ua (N.V.);

Scopus Author ID 55203239100

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Abstract: *Vaccinium uliginosum* L. is aboriginal plant species in Ukraine, and *Vaccinium corymbosum* L. is from the American continent but is widely cultivated in Europe and Ukraine for fruit production. The vegetative part of these plants is practically not used, and their chemical composition has not been studied in detail, although the native Europeans and Americans used the plants for medicinal purposes. Contents of selected elements were determined in samples of forest *V. uliginosum* L. shoots and fruits harvested in Western Ukraine and cultivated *V. corymbosum* L. (three cultivars) fruits and shoots. The atomic absorption spectrophotometry with sample aspiration in air-acetylene flame was used to analyze the metal concentration. Chemical analyses of shoots showed significantly greater content for essential elements (Cu, Zn, Mn, Co, Cr) than in fruits of both investigated species. In the fruits of *V. corymbosum* is much more Cu, Zn, Ni, Mn (2.13-3.17; 3.58-8.84; 0.74-1.18; 3.36-6.52 mg·kg^{-1} DW) compare to *V. uliginosum* ones. *V. corymbosum* varieties differ significantly in content elements in the shoots and fruits (especially Co, Cr, Cu, Cd). Correlation between the concentrations of elements in shoots and fruits of *V. corymbosum* cultivars as well as in shoots and fruits of *V. uliginosum* and the mobile metal concentrations in soil of their growth were calculated, but direct connections were not found. The element composition of *Vaccinium corymbosum* shoots and fruits has proven to be promising tools.

Keywords: *Vaccinium uliginosum*; *Vaccinium corymbosum*; shoots; fruits; element analysis.

1. Introduction

The content of mineral elements in the composition of plants is important for several reasons: they are a source of replenishment of essential components with food and medicinal plant raw materials; changes in the composition and content of mineral components cause changes in the synthesis and accumulation in plants of many organic compounds that are biologically active; the excessive content of many micro and macronutrients in plant raw materials make it limited or impossible to use. Blueberry species, which are now widely used, have attracted our attention: *Vaccinium uliginosum* L. and *Vaccinium corymbosum* L. (Ericaceae).

Vaccinium uliginosum (bog bilberry) (BB) is a deciduous shrub with up to 0.8 m stems, glabrous and brownish; 6-25x4-20 mm leaves, flowers 4- or 5-merous in racemes; fruit is a berry, bluish-black globose to ellipsoid 5-10 mm, sweet and edible. BB is distributed throughout Europe, including the northern regions of Ukraine – Polissia and all areas of the Carpathians' subalpine and alpine zones on oligotrophic marshes in the forest zone [1], as well as in northern Asia and Northern America**.** BB's fruits have been used for many centuries in cooking and traditional medicine by the population of northern Ukraine - in places of natural growth of this species [2] and now known as antiseptic, astringent, carminative, hypnotic, and hypoglycemic in various countries [3, 4].

Vaccinium corymbosum L. (highbush blueberry) (HB) is a crown-forming deciduous shrub with two to five stems arising from a single bole which typically grows from 2 to 3 m in height. Its fruit is a sweet, juicy, blue-black berry about 7 to 10 mm in diameter. It was introduced outside the natural range in North America for commercial berry production and later distributed in other regions, especially after creating high-yielding varieties. It's known that HB grows best on hummocks or raised bogs that provide moist, acidic (pH value between 2.7 and 6.6), well-aerated [5]. Such growth conditions can be easily created in different Ukraine regions, and HB varieties are very popular nowadays. The value of HB fruits is that they accumulate significant amounts of important biologically active compounds that determine their nutritional properties and medicinal herbs [4, 6]. There are only several articles about microelements' content in fruit of *V. uliginosum* and *V. corymbosum* species [7-10], and less about their shoots and leaves [9-11].

Therefore, the present work aimed to investigate the level of several elements (Cu, Zn, Ni, Mn, Co, Cr, Ca, Pb, Cd) in samples of forest-grown bog bilberry (*Vaccinium uliginosum* L.) fruits and shoots harvested in Western Ukraine and three cultivars of highbush blueberry (*Vaccinium corymbosum* L.) fruits and shoots. Besides, our objective was to establish correlative connections between content elements in plants and soils on which they are grown.

2. Materials and Methods

2.1. Plant material.

Shoots and fruits of wild-growing *Vaccinium uliginosum* L. (bog bilberry) were collected in the Volyn region, Shatsky district, village Gaivka, in a pine forest, 171 m above sea level (51°34'0310" N, 23°53'3597" E) and authenticated using keys for botanical determination. The field-grown *Vaccinium corymbosum* cv. Bluejay, Bluecrop, and Elliott were planted at the nurseries LLC Berry Partner in the Lviv region of Ukraine (49°79′28.01″ N, 24°01′00.39″ E). Plant material was harvested in 2018-2020 in the fruiting phase during the ripe fruit stage. In each experiment, the central ten plants from each plot were used for data collection. For experiments, 50-shoot and 50-fruit (berry) samples were taken from each plot.

Berry and shoot materials were collected at each site as a composite sample from locations representative of the planting or woodland area in berry harvest time (July-August for highbush blueberries and August for bog blueberry). All the BB and BH samples were dried in the shade after harvesting, and the dry material was used for mineral detection.

2.2. Soil material.

The soil used in this study was collected from 0-25 cm, and at each site, soil subsamples were combined in a composite sample.

According to the method described by [12], the metal content in the soil was estimated in the air-dried soil samples, which were sieved through a 1 mm sieve. Preparation of soil samples for analysis on the gross content of heavy metals was carried out by sequential processing of pre-calcined soil at 450° C first with HF, then the mixture of HCl and HNO_3, the ratio 3:1. Movable metal forms were determined after extraction in acetate-ammonium buffer (pH 4.8) according to standardized methods (DSTU 4770.1:2007; DSTU 4770.2:2007; DSTU 4770.3:2007; DSTU 4770.5:2007; DSTU 4770.6:2007; DSTU 4770.7:2007; DSTU 4770.8:2007; DSTU 4770.9:2007) (Ukr.). The metal content was expressed in mg · kg^{-1} of dry soil.

2.3. Mineral analysis of plant material.

The samples' mineral composition studies were performed based on the Central Research Laboratory and the Laboratory of Industrial Toxicology of Danylo Halytskyi Lviv National Medical University. The sample for the analysis was prepared following DSTU 7670 (2014) (Ukr.) by the method of dry mineralization, which is based on the complete decomposition of organic matter by burning the sample in an electric furnace based on the Central Research Laboratory and the Laboratory of Industrial Toxicology of Danylo Halytskyi Lviv National Medical University. In this case, pieces of raw material in the crucibles were treated with ethyl alcohol at the rate of 5 cm^3 per 1 g of dry matter, kept for 24 hours, and then placed on an electric stove and carefully conducted charcoal until the release (emission) of smoke. The charred specimens in the crucibles were placed in an electric furnace, and the charcoal was continued at a temperature of (250 ± 25)° C, gradually increasing the temperature to (450 ± 25)° C until gray ash was obtained. The cooled ash was further moistened with a minimum amount of nitric acid and kept in an electric oven at (300 ± 25)° C for 0.5 h. This cycle was repeated several times until a white or slightly colored ash was obtained.

The analysis method of plant material and soil for the elements' content is the atomic absorption spectrophotometry with sample (aspiration) in air-acetylene flame on the instrument, the C-115 M1 atomic absorption spectrophotometer (Calibration Certificate No. UA/37/261118/001543 dated 22/11/2018). The amount of light adsorption with the wavelength corresponding to the resonance line is proportional to the value of the metal concentration in the analyzed sample.

The analysis solution was prepared by dissolving the ash in a nitric acid solution (1: 1 by volume) at the rate of 1-5 cm^3 of the solution, evaporated to moist salts. The precipitate was dissolved in a 15-20 cm^3 nitric acid solution with a mass fraction of 1% and quantitatively transferred into a volumetric flask. The control solutions were prepared in parallel by the reagents' mineralization, in the same amounts added to the sample, and carried out through all stages of sample preparation for analysis.

The results were processed by absolute calibration using a computer program. This method's measurements' relative total standard uncertainty (us, %) does not exceed 18%. The results were expressed in mg·kg^{-1} DW.

The calibration dependence, the correctness index, and the calibration characteristic's stability were determined using standard samples of the composition of ions solutions of the corresponding metals, which have the proven competence in the established order.

2.4. Statistical analysis.

All the laboratory experiments were carried out in triplicates, and the data reported are mean ± standard deviation (SD). Calculation of correlation coefficient and correlation analysis were carried out using Microsoft Office Excel (2007). The metal content in the studied plants' organs and the soil and biochemical parameters were analyzed, checked for normality and equality of variance. Pearson's correlation coefficient was calculated for assessing the relationship between estimated metal concentrations in the soil and their parameters in the bog bilberry and highbush blueberry shoots and fruits.

3. Results and Discussion

Because many external factors, such as growth environment (soil, geographical conditions), cultivation and fertilization practices are widely diverse in different regions of *V. uliginosum* and *V. corymbosum* growth and production, and they could contribute to the mineral composition of fruits and shoots, it is important to investigate them. Bluecrop, Bluejay, and Elliott are recommended for Ukrainian production regions based on a sufficient horticultural management requirement, and now they are standard cultivars. As a part of the current interest in the properties of plant material of BB and HB, it was carried out to compare the contents of seven biologically essential elements (Ca, Mn, Zn, Cu, Ni, Co, Cr) and two nonessential (Cd and Pb) [13] in berries and shoots of two *Vaccinium* species: cultivated HB (three cultivars) and wild BB.

The obtained results show that the fruits and shoots of the studied *V. uliginosum* and *V. corymbosum* differ significantly in the contents of essential micronutrients. HB fruits were characterized by various contents of essential elements depending on the cultivar (Table 1). Concerning the cultivar, the major Cu and Mn were in Bluejay fruits and least in Elliott, and at the same time, the Cu content in BB was 2.5-3.6 times lower. Zn was prevalent in Bluecrop fruits. Ni's concentration in the fruits of all studied varieties of HB was found to be 2-11 times lower than the concentration of Cu, Zn, and Mn. Co and Cr were found in fruits in trace amounts.

Table 1. Elements content in fruits, mg·kg^{-1} DW (mean ± SD).

Sample	Cu	Zn	Ni	Mn	Co	Cr
V. corymbosum cv. Bluecrop	2.13±0.058***	8.84±0.119***	1.12±0.148$^{\#}$	4.46±0.031**	< 0.01	< 0.01
V. corymbosum cv. Bluejay	3.17±0.058	4.35±0.482	1.18±0.099	6.52±0.344	< 0.01	< 0.01
V. corymbosum cv. Elliott	2.73±0.154**	3.58±0.205*	0.74±0.038*	3.36±0.154***	< 0.01	< 0.01
V. uliginosum	0.87±0.042***	3.07±0.032***	<0.05	<0.05	<0.05	n.d.

* P ≤ 0.05; ** P ≤ 0.01, *** P ≤ 0.001, $^{\#}$ P > 0.5 compared to values in Bluejay c.v., n.d. - not determined.

Chemical nutrient analysis of shoots showed significantly greater content for essential elements than in fruits (Table 2). Only nickel accumulates in shoots and fruits in approximately the same concentration. Among the trace elements studied, in shoots of HB, the highest concentration of Mn, especially in Elliott; the concentration of Zn, Cu, and especially Ni was much lower. However, surprisingly similar Cu and Zn concentrations were found in shoots samples of two cv. of HB and BB. There was a significant effect of cultivar on shoot Co and Cr concentration. Low concentrations of cobalt and chromium (0.05-0.17 mg•kg^{-1} DW) were found in the shoots of Bluecrop, Bluejay, and BB, and only in Elliott, their concentration is 0.93 and 0.88 mg•kg^{-1} DW, respectively.

Table 2. Elements content in shoots, mg·kg^{-1} DW (mean ± SD).

Sample	Cu	Zn	Ni	Mn	Co	Cr
V. corymbosum cv. Bluecrop	5.40±0.259#	5.54±0.055#	1.45±0.042#	20.10±2.336#	0.19±0.032*	< 0.05
V. corymbosum cv. Bluejay	5.70±0.215	5.49±0.070	1.75±0.413	25.96±3.609	0.05±0.045	< 0.05
V. corymbosum cv. Elliott	1.21±0.025***	6.79±0.036***	1.15±0.006#	34.33±0.121#	0.92±0.051***	0.90±0,029
V. uliginosum	5.18±0.032***	5.76±0.038**	1.76±0.093*	2.14±0.644***	< 0.01	< 0.05

* P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; □P > 0.5 compared to values in Bluejay c.v.

The analyzed calcium in BB and HB samples showed that Bluecrop and Bluejay's shoots stored higher Ca levels than fruits: in 3.7 times and 1.2 times, respectively (Table 3). BB's shoots had a lower concentration of Ca than cultivated HBs except for Elliott, which was 2.35-2.86 times lower concentration than other cultivars and 1.89 times lower than BB. Commonly, investigated HB varieties contained similar levels of Ca at shoots as BB.

Table 3. Calcium content of fruits and shoots, mg•kg^{-1} DW (mean ± SD).

Sample							
Fruit				Shoot			
V. corymbosum cv. Blue crop	*V. corymbosum* cv. Blue jay	*V. corymbosum* cv. Elliott	*V. uliginosum*	*V. corymbosum* cv. Blue crop	*V. corymbosum* cv. Blue jay	*V. corymbosum* cv. Elliott	*V. uliginosum*
22.35±0.301 #	55.80±0.529	63,00±0,05*	n.d.	82.90±2.008**	68.10±4.694	29.30±0,424***	54.81±1.04*

P ≤ 0.05; ** P ≤ 0.01, *** P ≤ 0.001, # P > 0.5 compared to values in Bluejay c.v., n.d. - not determined.

The contents of Cd and Pb in the most samples analyzed (shoots and fruits) stored very low concentrations of Cd – below the limit detection (less than 0.01 mg·kg^{-1} DW) (Table 4). However, much more cadmium was concentrated in Bluejay and bog bilberry shoots: 0.51±0.015 and 0.87±0.021 mg·kg^{-1} DW, respectively.

Table 4. Lead and cadmium content in fruits and shoots, mg·kg^{-1} DW (mean ± SD).

Element	Fruit				Shoot			
	V. corymbosum cv. Blue crop	*V. corymbosum* cv. Blue jay	*V. corymbosum* cv. Elliott	*V. uliginosum*	*V. corymbosum* cv. Blue crop	*V. corymbosum* cv. Blue jay	*V. corymbosum* cv. Elliott	*V. uliginosum*
Cd	< 0.01	< 0.01	0.05	0.010±0.002	< 0.01	0.51±0.015	< 0.01	0.87±0.021
Pb	0.06	0.03	0.10±0.006	0.025±0.025	< 0.01	0.02	0.10±0.006	< 0.01

* P ≤ 0.05; ** P ≤ 0.01, *** P ≤ 0.001, # P > 0.5 compared to values in Bluejay c.v., n.d. - not determined.

In the investigated soils, the content of mobile elements, especially Cu, Zn, Ni, Mn, Pb, differs significantly from their total content (Table 5); the changes of Co, Cr and Cd are not significant.

Table 5. Element content in soil of growth sites of investigated species, mg·kg^{-1} DW (mean ± SD).

Locality of growth, Species	Cu	Zn	Ni	Mn	Co	Cr	Pb	Cd
	Total content							
V. corymbosum cv. Bluecrop, Bluejay, Elliott	2.33±0.058	14.30±1.539	10.87±0.65	56.00±4.00	1.97±0.40	78,4±5.76	9.2±0.52	0.20±0.100
V. uliginosum	5.63±0.231	46.2±3	7.87±0.289	102.5±3.57	3.6±0.5	94.43±7.57	43.03±1.84	0.53±0.058
	Content of mobile forms							
V. corymbosum cv. Bluecrop, Bluejay, Elliott, p1	0.2±0 ***	3.667±0.289 **	2.3±0 ***	22.70±0.75 ***	1.1±0. #	57.5±9.01 #	2,33±0.88 **	0.05±0.0 #
V. uliginosum, p2	0.5±0 ***	25.43±4.661 * or **	4.53±1.150 *	21.77±1.10 ***	1.90±0.69 #	12.5±0 ***	12.5±1.40 ***	0.267±0.058 **

p1 - the content of the mobile forms of the elements in the soil of the areas of cultivation of highbush blueberries in comparison with their total content;
p2 - the content of the moving forms of the elements in the soil of the areas of growth of bog bilberry in comparison with their gross content.

A direct connection between the quantities of trace elements mobile forms and their accumulation in highbush blueberry and bog bilberry was not found (Table 6). The results demonstrated some relations between the content of the mobile form of elements in soil and plant samples (Table 6).

Table 6. Correlation coefficient (r) between the content of the mobile form of elements in soil and in plant samples.

Sample	Microelement, Coefficient					
	Cu	Zn	Ni	Mn	Co	Cr
V. corymbosum cv. Bluejay shoots	-1	0.82199	-	-0.9899	-	
V. corymbosum cv. Bluecrop shoots	-0.9177	0.9958	-	0.8093	-	-
V. corymbosum cv. Elliott shoots	-0.7559	0.240192	-	0.670208	-	-
V. corymbosum cv. Bluejay fruits	-0.866	0.987829	-	-0.12915	-	-
V. corymbosum cv. Bluecrop fruits	-0.945	0.536107	-	0.953821	-	-
V. corymbosum cv. Elliott fruits	-0.5	0.87298	-	0.180679	-	-
V. uliginosum shoots	0.5	1	0.132539	-0.12754	-	0.64046

A significant negative correlation was observed between the mobile form of the copper content in soil and plant samples for all varieties of *V. corymbosum* (except *V. corymbosum cv. Elliott fruits*). A significant positive correlation was observed between the content of the mobile form of zinc in soil and in plant samples for all varieties of *V. corymbosum* (except *V. corymbosum cv. Elliott shoots*) and *V. uliginosum* shoots. A significant positive correlation was observed between the mobile form of manganese content in soil and in plant samples of *V. corymbosum* cv. Bluecrop shoots, *V. corymbosum* cv. Bluecrop fruits and *V. corymbosum* cv.

Elliott shoots. However, the content of the mobile form of manganese in soil exhibited a strong negative correlation in plant samples of *V. corymbosum cv. Bluejay shoots*. There was a moderate positive correlation between the mobile form of chrome content in soil and in plant samples of *V. uliginosum shoots*.

It is believed that if plants grow on the same type of soil, the difference in the composition of elements is due to differences in the mechanisms of absorption and metabolic rate of nutrients [14]. Minerals as nutrients are involved in the etiology and pathogenesis of several diseases. In our investigation, the most interest in Ca, Cu, Mn, Zn, Ni, Cr, Co, Pb, and Cd in our investigation is their bioavailability for plants from the soil and bioavailability for mammals from the foods. Most of the trace elements studied can be extracted by boiled water, albeit in different quantities [10, 15]. This makes them potentially effective in food or pharmaceuticals, especially of plant origin.

Low lead concentrations were found in all samples tested - no more than 0.10, which corresponds to European standards [16]. Safe for human health lead and cadmium accumulation levels in *Vaccinium* marked by other researchers [7, 10]. Our results indicated a significantly lower or the same average Pb and Cd content in berries compared to study in other regions of Europe by other authors [7, 17]. Thus, the potential for the influence of plant raw materials on the body is associated with its quality, particularly the content of elements. Total metal concentration in soils is not a useful predictor of bioavailability or soluble concentration of the metal [18]**.** Thus it is connected with the quality of harvested plant raw material as well as its potential of organism exposure and increased health risk [15].

4. Conclusions

Thus, our studies reveal those investigated species of *V. corymbosum* and *V. uliginosum* and cultivars contain essential Ca, Mn, Zn, Cu, Ni, Co, Cr, and two nonessential Cd and Pb. Generally, the same species' shoots and fruits differ in their elemental composition, such as highbush blueberry cultivars. Highbush blueberry varieties may be used to compensate for the deficiency of mineral deficiency conditions in humans, particularly in relation to Cu, Zn, and Mn (fruits and shoots). The element composition of *Vaccinium corymbosum* shoots and fruits has proven to be promising tools because they have a low content of toxic elements such as Pb and Cd and a good level of essential Ca, Mn, Zn, and Cu.

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Conflicts of Interest

The authors declare no conflict of interest.

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