UDC 581.192:582.573.76

DOI: 10.15372/CSD2019170

Elemental Composition of the Leaves and Rhizomes of *Hemerocallis Hybrida* hort.

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(Received April 01, 2019; revised June 10, 2019)

Abstract

The quantitative content of 22 chemical elements (As, Br, Co, Cr, Cu, Mn, Mo, Nb, Ni, Pb, Rb, Sc, Se, Sr, Ti, Zn, Zr, V, Y, Ca, Fe, K) in the vegetative organs of *Hemerocallis hybrida* was determined for the first time with the help of X-ray fluorescence analysis with synchrotron radiation (XRD). Comparative data on the elemental composition of leaves and rhizomes in the plants of varieties *Speak to me* and *Regal Air* in the forest-steppe zone of West Siberia are presented. The specific features of the content of 19 microelements and 3 macroelements in above-ground and underground organs were revealed. The concentrations of all elements in the leaves and rhizomes of *Speak to me* and *Regal Air* plants were at the maximum permissible level. The leaves of *Regal Air* were distinguished by the higher (by a factor of 1.5–7) content of molybdenum, bromine, chromium, rubidium, selenium. The concentrations of lead, strontium, yttrium, zirconium, niobium were 1.5–6 times higher in the leaves of *Speak to me*. Underground organs were found to be the richest in trace elements. It was shown that the total content of trace elements is 1.5–3 times higher in the organs of the *Regal Air* variety. The variety specificity of the quantitative content of chemical elements in vegetative organs is detected.

Keywords: leaf, rhizome, chemical elements, X-ray fluorescence analysis, *Hemerocallis hybrida*, West Siberia

INTRODUCTION

At present, special attention is paid to the investigation of the elemental composition of biological objects with the help of modern methods, including X-ray fluorescence analysis with synchrotron radiation (XFA SR) [1–3]. This method allows reliable determination of element content independently of the elements ratio in the material, without preliminary ashing of the samples [4, 5], which ensures precise representative data. The elemental composition of plants is species- and variety-specific, it depends on the ecological factors of the environment and serves as an indicator of their stable state [6–8].

It is known that day lilies (*Hemerocallis* L. genus, Hemerocallidaceae family) possess a range of biologically active (pectic, phenol) and reserve substances. Underground and ground organs of this plant are used in official and folk medicine to treat various diseases of liver, gall-bladder, tumours, rheumatism, dysuria [9–12]. The elemental composition of the vegetative organs of the representatives of this species is studied insufficiently. The concentrations of 14 elements in organs were determined only in *H. minor* Mill. [13–15]. The highest content of Ca, Fe, Mn, Br, Ba was detected in leaves; Ni, As, I, Pb – in stems; Ti, Cr, Sr – in rhizomes; Cu, Zn – in flowers. No data on the concentrations of micro- and

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macroelements in hybrid day lilies (*Hemerocallis hybrida* hort.) are available, which determines the relevance and novelty of this work.

The goal of the investigation is the quantitative determination of the elemental composition of leaves and rhizomes of hybrid day lilies growing under the conditions of the forest-steppe zone of West Siberia.

EXPERIMENTAL

Leaves and rhizomes of two varieties of perennial plants *H. hybrida* hort. – *Speak to me* and *Regal Air* were studied. The plants were taken from the Bioresource Scientific Collection of the CSBG SB RAS, "Collections of living plants growing outdoors and in greenhouse" UNU No. USU 440534. These plants possess a high adaptive potential over the economic-biological qualities [16]. The samples were grown at the collection site of the Laboratory of decorative plants introduction, CSBG SB RAS, under identical soil and climatic conditions. The raw material (leaves and rhizomes with roots) was collected in September 2014. That year was distinguished by a cool vegetation period with excess moisture, with cold early spring. At the same time, the soil was sampled by means of the envelope from the soil horizon A_0–A_1 at a depth down to 10–15 cm, corresponding to the position of the underground organs of each variety.

Elements content was determined by means of XFA SR, based on the interaction of the substance with the high-energy electromagnetic radiation. Analysis of the elemental composition of the samples was carried out at the station of elemental analysis of the Shared Equipment Centre at Budker Institute of Nuclear Physics, SB RAS (VEPP-3 storage ring) [4]. The major characteristics of the station and the methods involved in the experiment were described in [17, 18]. The samples were prepared from air-dry plant raw material (1 g). The material was triturated preliminarily in an agate mortar into powder, then it was pressed in a specially made press mold at a pressure of 100–150 kg/cm^2 into a tablet 30 mg in mass, 1 cm in diameter, using the hydraulic press. Then the sample in the form of a tablet was packed in fluoroplastic rings between two fluoroplastic films 5 μm thick. Measurements were carried out with the excitation energy of 23 keV, the time of spectrum measurement was 300–500 s for each sample. The recorded fluorescence spectra were processed using the AXIL software package intended for processing complicated spectra. The concentrations of elements were determined by means of the external standard. The major requirement implied by the application of this method is the similarity of the chemical compositions of the matrices of the sample under investigation and the standard sample, as well as the levels of element content in them. Russian certified standards for plant material GSO SORM1 (grass-cereals mixture) [19] were chosen as the standard reference samples that were most close in composition to the samples under study The gross content of chemical elements was determined by summing up the data over every element in the sample in accordance with the procedures indicated in [1–3]. The bioconcentration factor (BCF) was calculated as a ratio of element content in the dry mass of leaves and rhizomes to the content of this element in soil [2, 20, 21]. The limit of element detection and the relative standard deviation for these experimental conditions (excitation energy 23 keV) were determined in 20 parallel measurements of the standard SORM1 sample. The statistical analysis of the data was carried out using the Statistica 6.1 and Microsoft Office Excel 2007 software packages.

RESULTS AND DISCUSSION

Results of the comparative analysis of the content of three macroelements (K, Ca, Fe) and 19 microelements (As, Br, Co, Cr, Cu, Mn, Mo, Nb, Ni, Pb, Rb, Sc, Se, Sr, Ti, Zn, Zr, V, Y) in soil, leaves and rhizomes from the experimental ground and in reference samples are listed in Tables 1, 2. The content is chemical elements in soil samples from the site of growth of the two *H. hybrida* hort. varieties in CSBG is presented in comparison with the literature data [8]. One can see (see Table 1) that the experimental samples only weakly differ from each other in the gross content of elements, and for some elements (Ti, Co, Y, Se, Sc, Nb, Mo, Pb) they are quite comparable with each other.

Macroelements

Non-equipollent distribution of macroelements (K, Ca, Fe) was detected in the leaves (see Table 2). Potassium, as a mobile strong-intake element, well penetrates into the leaves, and its concentration in the *Regal Air* variety is twice as high as that in the *Speak to me* variety. Unlike for *Speak to me*, the concentration is 1.5 times higher in the leaves of *Regal Air* than in rhizomes. Calcium is actively accumulated by the leaves of both varieties, which is indicated by a 2-fold excess of its concentration in leaves in comparison with rhizomes for both varieties (see Table 2). It is also necessary to stress a comparable content of calcium in the leaves of both varieties (12 742–13 101 mg/kg). Iron, as the medium-capture element, plays a significant part in photosynthesis in plants. Nevertheless, its concentration in underground organs in comparison with top organs is 6 times higher in the case of *Regal Air* and 1.5 times higher for *Speak to me*. This prevalence of iron accumulation in roots is observed for the majority of plants [22], and this is necessary for the normal growth and development of plants.

TABLE 1

Gross content of chemical elements in soil samples from the site of *Hemerocallis hybrida* hort. growth at CSBG, mg/kg

Element	Variety		Soil [8]
	Regal Air	*Speak to me*	
As	1.5±0.1	2.9±0.3	1.7
Br	4.5±0.5	9.0±0.9	10.0
Ca	13 242.0±107.4	14 012.0±104.1	15 000.0
Co	11.0±0.9	12.0±1.0	18.0
Cr	40.0±2.0	69.0±3.5	83.0
Cu	17.0±1.0	27.0±1.6	47.0
Fe	21 218.0±106.1	21 213.0±102.1	40 000.0
K	13 615.0±68.1	14 011.0±70.0	14 000.0
Mn	705.0±35.3	764.0±37.2	1000.0
Mo	0.4±0.0	0.3±0.0	1.1
Nb	12.0±3.6	13.0±3.9	20.0
Ni	32.0±3.2	38.0±3.8	58.0
Pb	96.0±9.6	97.0±9.7	16.0
Rb	59.0±7.1	74.0±8.9	35.0
Sc	5.0±0.3	5.0±0.3	10.0
Se	0.2±0.0	0.2±0.0	0.1
Sr	174.0±23.1	175.0±22.9	340.0
Ti	4009.0±200.5	4001.0±360.0	4500.0
V	67.0±3.4	88.0±9.1	90.0
Y	20.0±3.0	21.0±3.2	20.0
Zn	41.0±2.9	67.0±4.7	83.0
Zr	304.0±60.8	368.0±73.6	170.0

Note. The gross content is given as a mean value ± standard error of the mean.

Microelements

As a rule, underground organs of *H. hybrida* are most reach in microelements (As, Br, Co, Cr, Cu, Mn, Mo, Nb, Ni, Pb, Rb, Sc, Se, Sr, Ti, Zn, Zr, V, Y) (see Table 2). For both varieties, the rhizomes are characterized by the high concentrations (23.0–181.0 mg/kg) of such vitally important elements as manganese, titanium, chromium, zinc, and low concentrations (0.1–3.6 mg/kg) of molybdenum, yttrium, rubidium, and bromine. Higher concentrations of the elements in rhizomes were determined for *Regal Air* variety (see Table 2). The same content of microelements – cobalt, vanadium, arsenic, molybdenum, scandium – was detected in the organs of *Speak to me* in general, the rhizomes of *H. hybrida* well accumulate Mn, Cu, Zn, Cr, Ti, their concentrations being 2–6 times higher than in the leaves (141.0–181.9 mg/kg). For some elements, the concentrations were below the detection limit (0.01 mg/kg): for Se, Sc in rhizomes and for Y, Se in the leaves of *Regal Air*, for Pb, Se, Sc in rhizomes and for Se, Sc in the leaves of *Speak to me* (see Table 2).

One can see in Table 2 that the concentrations of the majority of the studied elements were 1.5–8 times (*Regal Air*) and 1.5–3 times (*Speak to me*) lower in leaves than in rhizomes. An exception is Br, Sr, Mo, as their concentrations are 1.4–4 times higher in leaves than in rhizomes of both varieties. Non-equipollent distribution of Pb, Zr, Nb over plant organs was detected: the content of these elements is higher in the leaves of *Speak to me* variety, while for *Regal Air*, quite contrary, it is higher in rhizomes. For example, zirconium concentration (45 mg/kg) is 6 times higher in the leaves of *Speak to me* than in *Regal Air*. Higher (by a factor of 1.5–7) molybdenum, bromine, chromium, rubidium, selenium content was detected in the leaves of *Regal Air*. The concentrations of lead, nickel, manganese, strontium, vanadium, yttrium, zirconium, niobium were 1.5–6 times higher in the leaves of *Speak to me*. Equal content of cobalt, copper, arsenic, scandium was determined in the leaves of both varieties. The content of dangerous toxicants for plants and humans in the organs of *H. hybrida* was: lead 1.0–2.0 mg/kg, arsenic 0.02–0.09, which did not exceed the MPC level for tea (Pb – 10 mg/kg, As – 1.0 mg/kg), which was taken as a standard [23].

It was established by comparing the level of total accumulation of microelements that their

TABLE 2

Element content in the leaves and rhizomes of *Hemerocallis hybrida* hort. plants, mg/kg of air-dry mass

Элемент	*Regal Air*		*Speak to me*	
	leaves	rhizomes	leaves	rhizomes
K	**16 752.0±345.6**	10 599.0±318.0	8153.0±244.6	**11 544.0±346.6**
Ca	**13 101.0±786.0**	6763.0±405.8	**12 742.0±764.5**	6749.0±404.9
Fe	233.0±18.6	**1547.0±123.8**	566.0±45.6	924.0±73.9
Ti	20.0±0.4	**181.0±9.1**	79.0±7.0	**141.0±13.0**
V	0.3±0.0	1.6±0.3	0.7±0.1	1.0±0.1
Cr	11.1±4.4	35.0±1.8	6.7±3.0	23.0±12.0
Mn	40.0±2.0	**80.0±4.0**	50.0±4.0	51.0±4.1
Co	0.2±0.1	0.6±0.2	0.3±0.0	0.3±0.0
Ni	2.7±0.5	3.9±0.8	3.2±0.6	3.5±0.7
Cu	3.7±0.2	10.4±0.5	4.0±0.1	5.7±0.2
Zn	19.0±1.9	27.0±2.7	17.0±1.7	29.0±2.9
Br	3.6±0.3	0.8±0.1	2.0±0.2	0.5±0.1
Rb	6.0±0.3	6.9±0.8	4.5±0.2	6.0±0.1
Sr	**63.0±3.8**	41.0±2.5	**69.0±3.5**	38.0±0.4
Y	–	2.0±0.2	2.0±0.2	1.0±0.0
Zr	7.0±2.8	40.0±8.0	45.0±9.0	17.0±3.0
Se	0.02±0.01	–	–	–
Sc	–	–	–	–
As	0.02±0.01	0.04±0.01	0.02±0.01	0.1±0.0
Nb	2.0±0.8	2.9±0.9	**8.0±4.0**	1.8±0.2
Mo	0.7±0.1	0.2±0.1	0.1±0.0	0.1±0.0
Pb	1.0±0.1	1.6±0.2	2.0±0.7	–
The sum of microelements	120.5	452.5	61.1	301.0

Notes. 1. For designations, see Table 1. 2. Dash means that element concentration is below the detection limit (0.01 mg/kg).

content in underground organs is 3.5 times higher in *Regal Air* and 4.5 times higher in *Speak to me* than in top organs. In general, in the rhizomes of both varieties, the total content of microelements is 2–3.5 times higher than in leaves. The total content of microelements both in leaves and in rhizomes is 1.5–2 times higher in *Regal Air* (in leaves: 120.54 mg/kg, in rhizomes: 452.49 mg/kg) than in *Speak to me* (61.06 and 301.01 mg/kg, respectively).

An individual parameter characterizing the ability of plants to concentrate chemical elements is BCF [21]. The following groups are distinguished in plant classification according to BCF value [20]: 1 – very strong accumulation (100 > BCF > 10), 2 – strong accumulation (10 > BCF > 1), 3 – weak accumulation and medium intake (1 > BCF > 0.1), 4 – weak intake (0.1 > BCF > 0.01), 5 – very weak intake (0.01 > BCF > 0.001). The leaves and rhizomes of *H. hybrida* accumulate the majority of elements but with different BCF values (Table 3). The elements of weak intake in the organs of both varieties (BCF = 0.011–0.092) are Fe, V, Mn, Co, Y, Zr, Pb. Such elements as Ca, Cu, Zn, Sr, Nb belong to the third group of weak and medium intake (BCF = 0.989–0.138). Unambiguity of a very weak intake of Sc is expressed by the organs of *H. hybrida* (BCF = 0.002). However, the accumulation of elements in the organs occurs nonuniformly even within the same variety. Strong accumulation of K and Mo (BCF = 0.989–1.750) is expressed in the leaves of *Regal Air* variety, with the weak accumulation of these elements in its rhizomes and in the leaves of *Speak to me* (BCF = 0.333–0.824). According to [21], element concentrating with BCF close to 1 and >1 may be considered for Mo (1.750), K (1.230), Ca (0.989), Br (0.800) in the leaves and Cr (0.875), K (0.777) in the rhizomes of *Regal Air*, as well as Ca in the leaves (0.909) and K in the rhizomes (0.824) of *Speak to me*. Accumulation of

TABLE 3

Biological concentrating factor (BCF) of chemical elements in the vegetative organs of *Hemerocallis hybrida*

Element	*Regal Air*		*Speak to me*	
	leaves	rhizomes	leaves	rhizomes
K	1.230	0.777	0.582	0.824
Ca	0.989	0.511	0.909	0.482
Fe	0.011	0.073	0.027	0.044
Ti	0.004	0.045	0.019	0.035
V	0.004	0.024	0.008	0.011
Cr	0.228	0.875	0.097	0.333
Mn	0.057	0.113	0.065	0.068
Co	0.018	0.055	0.025	0.025
Ni	0.084	0.122	0.026	0.092
Cu	0.218	0.612	0.148	0.211
Zn	0.463	0.658	0.410	0.707
Br	0.800	0.178	0.222	0.055
Rb	0.102	0.117	0.061	0.081
Sr	0.362	0.236	0.394	0.217
Y	–	0.100	0.095	0.048
Zr	0.023	0.132	0.122	0.046
Se	0.125	–	–	0.062
Sc	0.002	–	0.002	0.002
As	0.013	0.027	0.007	0.031
Nb	0.167	0.242	0.615	0.138
Mo	1.750	0.500	0.333	0.333
Pb	0.010	0.016	0.021	–

Note. Dash means the value is below the detection limit.

K in leaves is higher than in the rhizomes of *Regal Air*, while the situation is quite the contrary with *Speak to me*. Other microelements may be related to deconcentrators; their accumulation was within the BCF range (0.057–0.658) and lower. Lower BCF was characteristic of the organs of *H. hybrida* for the following elements: V (0.004–0.024), As (0.007–0.031), Ti (0.004–0.045), Sc (0.002), Pb (0.010–0.021) Fe (0.011–0.073), Zr (0.023–0.132), Y (0.048–0.100), Co (0.018–0.055).

CONCLUSION

The elemental composition and the quantitative content of 22 chemical elements were investigated by means of XFA SR for the first time in the leaves and rhizomes of two varieties of *H. hybrida* – *Speak to me* and *Regal Air*, growing under the conditions of the forest-steppe zone of West Siberia. It was demonstrated that so essential and vitally necessary elements as nickel, chromium, molybdenum belonging to the elements of weak and medium biological intake are present in the organs of *H. hybrida* in the sufficient amount with respect to the minimal significance boundary. The content of lead and arsenic in the organs of the studied samples is substantially lower than the maximum admissible level (0.02–2.0 mg/kg). The biological role of these elements is minor [8], but they exhibit toxicity in the case of high concentrations in polluted technogenic areas [24]. Equal concentrations in the organs of *H. hybrida* were determined for such elements of weak biological intake as vanadium and selenium. It is known that the lack of these elements in the organism leads to a decrease in immunity and liver function, a disorder in cholesterol exchange and enzyme activities [6, 8].

The concentrations of each element in the organs of *H. hybrida* are characterized by individuality and variety-specificity. Though foliar accumulation of microelements in organs is expressed in the plants [2], the concentrations of the majority of the elements are 1.5 times higher in rhizomes than in leaves. Exceptions are lead, nickel, yttrium, zirconium, niobium for *Speak to me* variety. For both varieties, the concentrations of bromine, calcium and strontium are 1.5–4 times higher in leaves than in rhizomes, which is the evidence of the specificity of element distribution over the organs of plants. The maximal amounts of Mn, Ti, Sr were accumulated in rhizomes, while Zr distribution was non-equipollent: for *Speak to me* the concentration of this element was higher in leaves (by a factor of 2.5), while for *Regal Air* in was higher in rhizomes (by a factor of 5.7). In general, it may be assumed that the distribution of chemical elements in the organs of *H. hybrida* has acropetal and more rarely basipetal direction, which is characteristic of the majority of plants [2]. The data on the content of scandium, niobium, selenium in the organs of H. hybrida as stressed by the authors of [8] provide evidence of the specificity of soil and climatic conditions in the forest-steppe zone of West Siberia. The total content of 19 microelements in the underground organs of *H. hybrida* is 3.5–4.5 times higher than in the top organs. The highest Br content was determined in the leaves of both varieties, while the highest Ti and Cr content was determined in the rhizomes, which is in agreement with the data reported in [14, 15] for *H. minor*.

Non-equipollent distribution of macroelements was confirmed by the comparative data on their content in vegetative organs. So vitally essential elements as Ca, K, Fe, which provide normal natural development, were detected in rather large amounts in the organs of *H. hybrida*. The top part is rich in calcium, while the underground part is rich in iron; potassium occupies an intermediate position, its content is higher in the leaves of *Regal Air* and in the rhizomes of *Speak to me*. In general, the leaves and rhizomes of adapted *H. hybrida* varieties may serve as a source of raw material in phytoterapeutic herbal mixtures.

Acknowledgements

The authors thank the Institute of Nuclear Physics, SB RAS, for assess to the infrastructure of the Shared Equipment Centre STsSTI based on VEPP-3 with the financial support from the Ministry of Education of Science of Russia.

The work was carried out within the State Assignment to the Central Siberian Botanic Garden SB RAS, project No. AAAA-A17-1170126100053-9 “Revelation of the routes of adaptation of plants to contrast habitation conditions at the levels of population and organism”.

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