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Elemental Composition and the Intensity of Chemical Elements Accumulation in the Fruits of Sea Buckthorn (*Hippiophae rhamnoides* L.)

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Abstract

An elemental composition inherent in the ripe fruits of the Siberian sea buckthorn (*Hippophae rhamnoides* L. ssp. *mongolica* Rousi) endemically growing in West Siberia was studied. By means of X-ray fluorescence analysis with the use of synchrotron radiation, a quantitative content of K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, Pb in the fruits of sea buckthorn and in the soil, as well as biological absorption coefficients for these elements were determined. It has been found that sea buckthorn fruits accumulate titanium, niobium, chromium, zirconium at relatively high quantities with respect to averaged over-ground phytomass, whereas they accumulate manganese, cobalt, vanadium, calcium, lead, strontium, copper, rubidium, scandium, and zinc at relatively low quantities. It has been established that the fruits of sea buckthorn do not concentrate toxic chemical elements such as lead and arsenic.

Key words: elemental composition, synchrotron radiation, sea buckthorn fruits

INTRODUCTION

The fruits of sea buckthorn (*Hippophae rhamnoides* L.) contain a rich complex of organic compounds those exhibit biological activity [1]. A high therapeutic and prophylactic efficacy inherent in preparations obtained from the sea buckthorn is associated with a synergism of the hydrophilic and lipophilic components thereof [2]. Therewith, the biological activity of many components (enzymes, vitamins, hormones, pigments and others) is caused, to all appearance, by mineral macro and microelements entering into the composition [3, 4]. For example, complex compounds between the microelements and polyphenols of the buckthorn affect the resistance of an organism against viral and bacterial infections [5].

In this regard, it seems relevant to investigate, alongside with the organic components, also the mineral components of the complex of sea buckthorn active substances. Therewith a method is preferred that allows simultaneously determining the quantitative content of all the elements in a single sample of the material.

The technique of X-ray fluorescence analysis with the use of synchrotron radiation (SR-XRF) meets the mentioned requirements. With the help of this technique, one could quantitatively determine the content of elements from

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potassium to uranium within in a wide range of concentration values, regardless of the ratio between them in the sample. Owing to a high intensity of the synchrotron radiation there could be elemental analysis performed for the samples having the mass from 1 mg with the detection limit of 10^{-7} g/g and with the reproducibility of 5–20 % [6, 7]. This is especially valuable in the course of studying the sea-buckthorn with a high ecological and genetic variability of the chemical composition [1, 8].

The qualitative analysis of sea buckthorn fruits revealed the presence of sulphur, silicon, magnesium, calcium, iron, manganese, molybdenum, titanium, boron, aluminum, vanadium, copper, zinc, lead, nickel and cobalt therein [9–11]. At the same time, the quantitative mineral composition of the fruits of the Siberian sea buckthorn (*Hippophae rhamnoides* L. ssp. *mongolica*) was studied in a fragmentary manner and without taking into account any local features of concentrating the elements in the soil.

The authors of [12] determined the content of potassium and magnesium in the fruits of the Siberian sea buckthorn introduced in central Russia. The authors of [11] reported the content of copper, zinc, manganese and cobalt in the fruits of wild sea buckthorn Eastern Siberia. There is also some information concerning the mineral composition of the fruits of a wild Chinese variety (*Hippophae rhamnoides* L. ssp. *sinensis*) [13, 14] and Indian variety (*Hippophae rhamnoides* L. ssp. *turkestanica*) [15] of the sea buckthorn. However, no detailed studies concerning the mineral composition of the fruits of the Siberian forms of *H. rhamnoides* grown under endemic soil and climatic conditions of the south of West Siberia were performed until now.

There is no information available also concerning the extent of the accumulation of separate elements in the sea buckthorn fruits; although this information is particularly important in the case of using sea buckthorn cultivated on the ore dumps with a high content of heavy metals [16]. In addition, it is unknown to what extent the Siberian sea buckthorn fruits could satisfy the requirements of a human organism for essential mineral elements.

The purpose of this work consisted in a quantitative determination of the mineral composition inherent in the fruits of the Siberian sea buckthorn and revealing the patterns of the accumulation of the elements depending on the content thereof in the soil.

MATERIALS AND METHODS

The fruits of the four cultivars of sea buckthorn created at the Institute of Citology and genetics (ICG) of the SB RAS such as Zarnitsa, Zyryanka, Krasny Fakel and Sibirskiy Rumyanets those were selected basing on the gene pool of the Altai populations [17]. From three plants of each cultivar grown on the common plot, we chose average fruits samples in the phase of complete maturity (September 22, 2010). Whole fruits were dehydrated in a drying oven at 105 °C to attain constant mass to be ground using a propeller-type mill (10 000 min^{-1}) during 30 s. Further, the samples were sieved through a nylon sieve with a mesh size of 0.5×0.5 mm with using the sweepings in order to determine the elemental composition.

Where necessary, the results obtained were compared with the data available from the literature as calculated for the dry matter of the fruits.

Simultaneously with the fruits, we sampled soil with the mass of 300–500 g directly from under the plants under investigation, from the depth corresponding to the maximum density of rhizosphere (10–15 cm below the layer of organic ground litter, soil horizon A1–A2). The soil type was presented by gray forest soil, pH 5.5. The soil was dehydrated at 105 °C to attain constant mass. In order to avoid an abrasive-induced entering of the elements from the metal mill blade into the soil samples, the latter were sieved through a nylon sieve with a mesh size of 0.5×0.5 mm without additional grinding. The sweepings were used for the analysis.

The elemental composition of the fruits and of the soil was determined by means of SR-XRF technique. The measurements were carried out at the station of elemental analysis of the Siberian Center of Synchrotron and Terahertz Radiation, Budker Institute of Nuclear Physics of the SB RAS (Novosibirsk) basing on the electron storage ring VEPP-3 according to a technique described by the authors of [7].

From a homogeneous paste of the sample prepared for the analysis, were formed tab-

TABLE 1

Absolute concentration and the coefficient of biological absorption (CBA) of the elements belonging to group A for the soil and for the fruits of sea buckthorn

Elements	Earth's soil*, μg/g	Soil at ICG, μg/g	Phytomass CBA_{Fe}, %	Fruits		
				Content, μg/g	CBA, %	DHR**, %
Fe	40 000	23525±938	0.35	75.8±15.3	0.33±0.08	5
K	14 000	15462±907	100	13 780±935	89.0±6.2	6
Ca	15 000	13033±946	120	1216±106	9.7±1.7	2
Ti	5000	4623±276	0.02	4.8±1.9	0.11±0.05	n/d
Mn	1000	772±30	63	17.25±1.11	2.20±0.12	9
Zr	400	348±88	0.2	0.90±0.25	0.33±0.10	n/d
Sr	250	171±5	10	3.48±0.43	2.06±0.29	n/d

Note. n/d – the requirement is not determined.

* According to Bowen, 1966.

** The daily human's requirement (DHR) for the element man satisfied with 100 g of fresh fruits.

lets with the mass of 30 mg and 1.0 cm in diameter with the use of a specially designed moulding tool. A sample in the form of the tablet was packed in PTFE rings between two chemically pure films with the thickness of 5 μm. When the sample is irradiated with synchrotron radiation there occurs the characteristic fluorescence radiation that is decomposed into spectrum. The spectrum measurement time amounts to about 300 s.

The concentration values for the elements were determined with the use of the method of external standard. As the external standards those are the closest in composition to the samples under investigation, we used Russian standards: cereal grass mixture SORM1 GSO 8242–2003 for plant tissues and BIL-1 GSO 7126–94 for soil samples.

We studied the quantitative content of the following 22 chemical elements: K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, Pb .

The intensity of the absorption of an individual chemical element X by a living organism is determined by the value of biological absorption coefficient CBA_X [18] calculated as

TABLE 2

Absolute concentration and the coefficient of biological absorption (CBA) of the elements belonging to group B for the soil and for the fruits of sea buckthorn

Elements	Earth's soil*, μg/g	Soil at ICG, μg/g	Phytomass CBA_{Fe}, %	Fruits		
				Content, μg/g	CBA, %	DHR**, %
Zn	90	45.5±0.9	111	18.8±1.9	41.5±4.9	2
V	90	87.3±9.4	1.8	0.09±0.02	0.11±0.02	11
Cr	70	65.5±6.9	0.3	1.0±0.8	1.35±1.00	20
Ni	50	42.5±2.4	6.0	1.5±0.09	3.62±0.29	n/d
Rb	35	60.0±3.3	57	10.8±0.3	18.1±1.1	n/d
Cu	30	20.0±1.1	47	2.8±0.15	14.0±1.6	3
Y	30	23.3±1.2	–	0.39±0.04	1.71±0.28	n/d
Pb	12	11.3±0.1	23	0.25±0.09	2.13±0.78	n/d
Nb	10	11.0±1.3	0.2	0.11±0.02	1.00±0.01	n/d

Note. n/d – the requirement is not determined.

* According to Bowen, 1966.

** The daily human's requirement (DHR) for the element man satisfied with 100 g of fresh fruits.

TABLE 3

Absolute concentration and the coefficient of biological absorption (CBA) of the elements belonging to group C for the soil and for the fruits of sea buckthorn

Elements	Earth's soil*, μg/g	Soil at ICG, μg/g	Phytomass CBA_{Fe}, %	Fruits		
				Content, μg/g	CBA, %	DHR**, %
Br	10	4.38±0.61	150	6.85±1.30	164±35	n/d
Co	8.0	9.75±0.25	6.3	0.031±0.027[a]	0.31±0.21	1
Sc	7.0	4.75±1.65	0.11	0.003***	0.04	n/d
As	6.0	6.28±0.46	3.3	0.13***	2.1	n/d
Mo	1.2	0.56±0.13	75	0.25±0.02	52.0±11.1	4
Se	0.4	1.53±0.62	50	0.20±0.03	29.8±17.5	3

Note. n/d – the requirement is not determined.

* According to Bowen, 1966.

**The daily human's requirement for the element man satisfied with 100 g of fresh fruits.

*** The concentration of the element at the limit of detection.

a ratio between the concentration values in the biological object and in the soil: $CBA_X = C_X/C_{X/s}$, where C_X is the concentration of the element X in the material of the object; $C_{X/s}$ is the concentration of the element X in the soil.

The daily human's organism requirements for the trace elements are presented according to the recommendations concerning the official standards of physiological requirements for energy and nutrients for different groups of the population of the Russian Federation [19].

RESULTS AND DISCUSSION

The results of the analysis of the elemental composition for the soil within the experimental plot of the ICG of the SB RAS and that for sea buckthorn fruits are presented in Tables 1–3. It is evident that gray forest soil within the experimental plot of the ICG of the SB RAS is rather similar in elemental composition thereof to the average earth's soil [20, 21], so the comparison of our data with the concentration of the elements in the average earth's soil and the above-ground phytomass [21] is quite correct.

The Clarke values of different elements in the lithosphere are several millions times different, so, for convenience, the elements are divided into the three groups according to the value of this parameter. The A group is presented by the elements with the concentration in the earth's ground greater than 100 μg/g of dry matter in the soil; the B group is presented by the elements with the concentration from 10 to 100 μg/g; whereas the C group is presented by the elements with the concentration thereof lower than 10 μg/g.

Elements of group A

Iron is contained in the fruits in a relatively high concentration (more than 75 μg/g), which is caused by a significant role of this element in performing the main functions of plant organisms [3]. Despite a low absorption coefficient from the soil (0.35 %), the sea buckthorn plants, to all appearance, maintain a necessary level of iron concentration in different tissues, including the fruit pulp. This is indicated also by similar values of CBA_{Fe}, both for the fruits of sea buckthorn and for the average phytomass thereof (see Table 1). A similar iron concentration (69 μg/g) was found for the fruits of *H. rh. sinensis* [13]. As it is known, the absorption intensity of this element from the soil depends on the soil acidity level [3], so the high iron content in the fruits of the Himalayan forms of *H. rh. turkestanica* (427–800 μg/g) and *H. rh. salicifolia* (441 μg/g) [15], to all appearance, could be caused by the acidity of the soil of mountain valleys (2600–3200 m). A more accurate answer require for studies in more detail. 100 g of fresh sea buckthorn fruits could satisfy the daily human's requirement for iron by 5 %.

Calcium is accumulated by the Siberian sea buckthorn fruits rather poorly: the CBA_{Ca} for the sea buckthorn fruits is lower than 10 % (whereas this value is equal to 120 % for the phytomass). The concentration of the element in the fruits of Chinese and Siberian varieties of the buckthorn is identical (about 1200 μg/g) [13], whereas for the fruits of the Himalayan varieties of *H. rh. turkestanica* and *H. rh. salicifolia* this parameter is much higher amounting to 2310–2550 and 2700 μg/g, respectively [15]. 100 g of fresh sea buckthorn could satisfy daily human's requirement for calcium by 2 %.

Potassium readily penetrates into all the tissues, including buckthorn fruit from the soil solution, and the concentration values thereof in the soil and in the sea buckthorn are almost identical. In addition, they are comparable to each other and the concentration of this element in the Siberian sea buckthorn fruits growing in Siberia, under the conditions of introduction thereof in the Central Russia (12 000–14 000 μg/g) [12] and the concentration in the fruits of the Himalayan *H. rh. salicifolia* (about 12 000 μg/g) [15]. Another level of potassium is found in the juice of the fruits of other varieties such as *H. rh. sinensis* (up to 20 000 μg/g) [13] and *H. rh. turkestanica* (9000–10 000 μg/g) [15], which could be explained by the difference in the concentration thereof in soil. As it is well known, potassium represents one of the most mobile elements in the biosphere that can easily penetrate in all the plant tissues in the form of free ions [21]. For this reason, the CBA_K values for the fruits and above-ground phytomass are almost the same. The daily human's requirement for potassium could be satisfied with 100 g of fresh fruits by 6 %.

Titanium. Despite a relatively high content of titanium in soil (4600 μg/g), the concentration of the element in plants is negligible. However, the intensity of its accumulation by the fruits of sea buckthorn is six times higher than the intensity of its accumulation by the phytomass. The biological role and daily human's requirement for titanium have not been established until now.

Manganese. Despite the proximity thereof to iron in many chemical properties [22], this element is absorbed by the above-ground phytomass much more intensely than iron (63 and 0.35 %, respectively). However, the supply of manganese into sea buckthorn fruits is very poor: for fruits the CBA_{Mn} = 2.2 %. The concentration of manganese we determined for the fruits of Siberian sea buckthorn *H. rh. mongolica* (17.3 μg/g) is comparable with the data reported in [11] (13.8 μg/g) and [13, 14] (12.7–16.7 μg/g for *H. rh. sinensis*). The daily human's requirement for manganese can be satisfied with 100 g of fresh fruits by 9 %.

Zirconium. Buckthorn fruits contain zirconium in the concentration amounting to 0.9 μg/g. Despite such a low absolute content, the biological uptake of this element by sea buckthorn fruits is 1.5 times higher than the average value for the phytomass, which indicates an increased accumulation of the element by the buckthorn. No other details concerning the content of zirconium in the sea buckthorn are available, and its biological role was not revealed.

Strontium according to the chemical properties thereof is very similar to calcium, but its biological absorption by the sea buckthorn above-ground phytomass is 12 times lower and that for the fruits is 60 times lower as compared with the absorption of calcium (for the fruits CBA_{Sr} = 2.06 %). The strontium concentration values inherent in the fruits of *H. rh. mongolica* and *H. rh. sinensis* are close to each other amounting to 3.5 and 2.0–4.3 μg/g, respectively [13, 14]. The daily human's requirement for this element is not determined.

Elements of group B

Zinc is characterized by a high intensity of accumulation comparing to the other elements of this group. The fruits of Siberian *H. rh. mongolica* contain 18.8 μg/g thereof, which is comparable with the results reported in [11] (16.5 μg/g). The fruits of variety *H. rh. sinensis*, *H. rh. turkestanica* and *H. rh. salicifolia* contain zinc in a somewhat lesser amount: 6.6–12.5 μg/g [13–15]. The daily human's requirement for zinc can be satisfied with 100 g of fresh fruits by 2 %.

Vanadium is characterized by a low-intensity absorption by plants: $CBA_V < 2$ %. The fruits of sea buckthorn absorb the element to a particularly low extent, and the absolute content of vanadium in the dry matter of the fruits amounts to about 0.1 μg/g. A similar result was obtained by the authors of [13] for *H. rh. sinensis.* The daily human's requirement for vanadium can be satisfied with 100 g of fresh fruits by 11%.

Chromium. Rather low chromium concentration values (1.0 μg/g in the fruits of *H. rh. mongolica* and 1.7 μg/g in the fruits of *H. rh. sinensis* [13]) are caused by a general pattern of pure accumulating this element in plants [18, 21]. However, the fruits of sea buckthorn contain the chromium 4.5 times greater than it is inherent the average phytomass, which indicates the ability of the fruits to accumulate the mentioned element. The daily human's requirement for chromium can be satisfied with 100 g of fresh fruits by 20 %.

Nickel. The concentration of this element in the fruits of *H. rh. mongolica* (1.5 μg/g) is close to that of *H. rh. sinensis* (2.2 μg/g) [13]. The fruits of sea buckthorn absorb this element 2–3 times weaker than the phytomass. The daily human's requirement for nickel has not been officially established.

Copper. The copper concentration we found (2.8 μg/g) is in a good agreement with the data for *H. rh. mongolica* (3.7 μg/g) [11] being comparable with those for Chinese varieties of *H. rh. sinensis* (3.1 μg/g) [13] and the Himalayan varieties of *H. rh. turkestanica* and *H. rh. salicifolia* (3.1–4.7 μg/g) [15]. The daily human's requirement for copper can be satisfied with 100 g of fresh fruits by 3 %.

Rubidium. The absorption of this element is quite intensive, which is caused by a high mobility of this alkali metal; for the phytomass the CBA_{Rb} amounts up to 60 %, for the fruits of *H. rh. mongolica* this value is equal to 18 %. the daily human's requirement for rubidium is not determined.

Yttrium is present in the soil at the concentration values close to those for the copper and rubidium. This element is characterized by a poor biological absorption and a low concentration in sea buckthorn fruits (1.7 % and 0.39 μg/g, respectively). The biological role of yttrium was not been investigated.

Lead is accumulated by the phytomass with a moderate intensity ($CBA_{Pb} = 23$ %), whereas the fruits of the Siberian sea buckthorn accumulate this element in an order of magnitude lower amounts ($CBA_{Pb} \sim 2$ %). The absolute concentration of lead found in the dry matter of fruits is equal to 0.25 μg/g. The data obtained by Chinese researchers are contradictory, which, to all appearance, could be explained by soil features inherent in different provinces of China: the fruits of *H. rh. sinensis* contain from 0.1 (Shaanxi Province) [14] to 6.0 μg/g of lead (Shanxi Province) [13]. The daily human's requirement for the lead is not determined.

Niobium is very poorly absorbed by the phytomass, the $CBA_{Nb}= 0.2$ %. However, the fruits of sea buckthorn five times exceed the phytomass in this parameter. The biological role of niobium is not investigated until now.

Elements of group C

Bromine is accumulated by the phytomass very actively ($CBA_{Br} = 150$ %). The fruits of *H. rh. mongolica* accumulate this element intensely as well ($CBA_{Br} = 164$ %). The daily human's requirement for bromine was not established.

Cobalt. The cobalt concentration revealed in the fruits *H. rh. mongolica* is extremely low (0.031 μg/g) being at the lower limit of detection by SR XRF. The author of [11] revealed the concentration of this element in the fruits of the sea buckthorn to be equal to 0.082 μg/g. According to [14], for the fruits of *H. rh. sinensis* (Shaanxi Province, China) the cobalt content is much larger amounting to 0.34 μg/g. The daily human's requirement for cobalt could be satisfied with 100 g of the fresh fruits of Siberian sea buckthorn by 1 %.

Molybdenum is present in the Siberian sea buckthorn fruits in an amount of 0.25 μg/g. According to [13, 14], the fruits of *H. rh. sinensis* contain the concentration thereof 2–40 times higher. The daily human's requirement for molybdenum could be satisfied with 100 g of the fresh fruits of Siberian sea buckthorn by 4 %.

Selenium. The selenium concentration values revealed in the fruits of *H. rh. mongolica* (0.25 μg/g) and in the phytomass (0.2 μg/g) are almost identical. At the same time, according to the data obtained by Chinese researchers, the fruits of *H. rh. sinensis* contains from 10.2 (Shaanxi Province) [14] to 94.3 μg/g (Shanxi Province) [13]. Such a high concentration of selenium in the wild sea buckthorn in China, to all appearance, could be caused by great fluctuations in the soil content thereof and the fact requires for further studying. As much as 100 g of the fresh fruits of the Siberian sea buckthorn could satisfy the daily human's requirement for selenium by 3 %.

Arsenic and **scandium** are characterized by of low intensity absorption by the plants. The concentration of elements in the fruits is so small that it is at the limit of SR-XRF detection, so the results obtained should be considered tentative. The daily human's requirement for arsenic and scandium is not determined.

CONCLUSIONS

According to the results of the studies performed, the fruits of Siberian sea buckthorn exhibit a poor absorption of the majority of the elements under investigation from the soil. This is true for manganese (CBA for fruits 30 times lower than that for phytomass), cobalt (more than 20 times), vanadium (by 18 times), calcium (13 times), lead (11 times), strontium (4.8 times), copper (3.4 times), rubidium (3.2 times), scandium (2.8 times) and zinc (2.7 times). The causes for such low capturing the elements by the tissue of the fruits are not clear due to the fact that a number of the representatives of this group (manganese, calcium, and zinc, copper) are vital for plant organisms [3].

The other part of the elements is characterised by the comparable values of this parameter inherent in the fruits and the phytomass. This can be explained by a universal and strictly limited need of all the plant tissues in essential elements (potassium, iron, nickel, molybdenum, bromine, selenium), as well as by a common barrier function of plant tissues in the course of the forced penetration of such elements as arsenic [3] those are hazardous or insignificant from the phytophysiological standpoint of elements such as arsenic.

It is rather interesting that some of the chemical elements (titanium, niobium, chromium, and zirconium) exhibit a relatively high accumulation level with respect to the Siberian sea buckthorn fruits. As our further studies demonstrated, the ability of the selective accumulation of these elements is inherent not only in the fruits, but also in the other organs of the species *Hippophae rhamnoides* L. ssp. *mongolica* under investigation [23]. However, it can be stated that the fruits of sea buckthorn do not concentrate toxic chemical elements such as lead and arsenic.

The human being's requirement for essential chemical elements varies to a great extent, so the evaluation thereof could be only tentative [24]. However, the fruits of sea buckthorn can serve as a naturally occurring food source of a number of chemical elements in an easily digestible biogenic nutrient form.

Thus, for the first time we revealed an absolute content and biological absorption coefficients for the complex of 22 chemical elements such as K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, Pb in the fruits of sea buckthorn (*Hippophae rhamnoides* L. ssp. *mongolica* Rousi) growing in the endemic climatic zone of Siberia. Species-specific features were found with respect to the intensity of the accumulation of individual elements in the Siberian sea buckthorn fruits. These features are exhibited both as excess (titanium, niobium, chromium, and zirconium) and deficiency (manganese, cobalt, vanadium, calcium, lead, strontium, copper, rubidium, scandium, zinc) in the biological absorption coefficients thereof as compared to the above-ground phytomass.

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