ACTA Acta Sci. Pol. Hortorum Cultus, 16(1) 2017, 3–10

www.acta.media.pl ISSN 1644-0692

ORIGINAL PAPER

Accepted: 13.06.2016

INFLUENCE OF HABITAT CONDITIONS ON CHEMICAL COMPOSITION AND CONTENT OF ISOTOPES IN SEA BUCKTHORN (*Hippophae rhamnoides* L.) LEAVES

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ABSTRACT

The numerous publications on the positive impact of sea buckthorn and its products on human health concern mainly the fruits and seeds. However, there is little data relating to the properties of sea buckthorn leaves. The main objective of this study was to evaluate the chemical composition and isotope content in the leaves of sea buckthorn *Hippophae rhamnoides* L. depending on where its existence. The habitat conditions significantly differentiated the studies traits. Mean crude protein content in the leaves reached 235.0 $g \cdot kg^{-1}$ d. m. The most proteins contain plants growing on native soil in the Dolna Odra Power Station. The ash content, fat, fiber and total carbohydrates was significantly higher in the leaves of sea buckthorn growing on sandy soil reclaimed from using ash from coal with NPK 60 – 70 – 70 + sludge. Their average concentration was: 49.2, 63.0, 128.1, 587.4 $g \cdot kg^{-1}$ d. m., respectively. The concentration of micronutrients was varied and depended significantly from the place of living plants. A higher level of $\delta^{15}N$ found in sea buckthorn grown on land reclaimed and fertilized with NPK. The conditions living of sea buckthorn didn't differentiate $\delta^{13}C$ value. It cannot be unequivocally determined whether the results of the study on the influence of habitat conditions on chemical and isotope composition of sea buckthorn leaves are permanent. Therefore, it is necessary to continue the research.

Key words: *Hippophae rhamnoides* L., isotopes, micronutrients, nutritional value, ^{15}N, ^{13}C

INTRODUCTION

Currently, more and more focus is put on the problem of the influence of food eaten on an organism's health. Now, more than ever, the attention is placed on buying possibly least processed products not containing artificial additives or preservatives. This fact forced food manufacturers to seek new natural preservatives and additives, which would also serve as antioxidants and anti-pathogenic agents [Niesteruk et al. 2013]. Sea buckthorn is a valued medicinal plant, both cultivated and growing naturally, mainly in Siberia, Central-East Asia, China and on the Baltic Sea coast in Poland. It is a source of valuable products such as leaves, fruit, oils and juice, which are used in food, pharmaceutical and cosmetic industries [Zeb and Malook 2009, Suryakumar and Grupta 2011]. Bioactive components of food influence many mechanisms synergistically, they regulate metabolism in human organism without eliciting any side effects. Appropriate protein intake in par-

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ticularly important for correct organism's maintenance. Shortage of protein in everyday diet results in decreased muscle mass as well as heart, liver, intestines and nervous system disorders. The antioxidants, vitamins, phenols, carotenoids, tannins and other chemicals contained in fruit are considered as agents protecting humans against wide range of cancers [Grey et al. 2010, Piłat et al. 2012]. Also other parts of the plant are good sources of phenols. The phenols contained in leaves, such as phenol acids, flavonoids and tannins exhibit antioxidant and anti-pathogenic properties [Kaur and Arora 2009]. It has been proven that they mitigate stress, help in wound healing and protect against radiation. Consuming these compounds might therefore be an important strategy in inhibiting or delaying pathological conditions and preventing numerous diseases [Upadhyay et al. 2010]. Like other plants [Kobus-Moryson et al. 2014], sea buckthorn may contain undesirable substances related to contamination from environmental pollution. Food contamination may be a result of plants' accumulation tendencies, cultivation site, atmospheric conditions or yield period. Although the chemical composition of sea buckthorn leaves was thoroughly examined [Sabir et al. 2005, Tiitinet et al. 2006], it was revealed that it varies depending on the plants' origin, climate or components extraction method [Zeb and Malook 2009]. The increase of the interest in food beneficial for human health [Brandt and Molgaard 2001], imposes search for advanced methods of examining food products. The ways of food authenticity assessment include inter alia chromatography, enzymatic, genetic and isotope methods [Stój 2011]. Isotope methods have become increasingly important in verification of origin and authenticity of food, including the food declared as organic [Bateman et al. 2007, Wlazły and Targoński 2014]. The isotope methods are ones of the most advanced in detection of food adulterations. They rely on measurement of the amount and ratios of basic isotopes of naturally occurring elements: carbon, hydrogen, oxygen as well as nitrogen and sulphur and their comparison with standard values. With the commercialization of sea buckthorn's value-added products, the species has attracted the attention of researchers in many fields. The studies however, focus mainly on nutritious value of fruit and seeds and there is shortage of the information regarding buckthorn leaves [Sharma et al. 2014].

Therefore, the aim of the present study included the determination of chemical composition and isotope content in sea buckthorn leaves originating from various sites in the Szczecin Lowland.

MATERIAL AND METHODS

Plant material

The leaf samples were collected in June 2014 and 2015 year from wild sea buckthorn shrubs (*Hippophae rhamnoides* L. *ssp. rhamnoides*) growing in the Dolna Odra Power Station at Nowe Czarnowo, near Gryfino (53°42'N, 14°58'S) and banks of the river Odra in Szczecin (53°26'17"N , 14°32'32"E), Poland.

Selected plants growing in four different positions:
Site 1 – Sandy soil using reclaimed coal ash + NPK 60 – 70 – 70 + sewage sludge in a ratio 1:1; pH: 7.32. Organic carbon content 16.5 $g \cdot kg^{-1}$ d.m., organic matter 28.4 $g \cdot kg^{-1}$ d.m., N – 2.78 $g \cdot kg^{-1}$ d.m.
Site 2 – Sandy soil using reclaimed coal ash + bark of conifers + compost from sewage sludge. in a ratio 1:2:4, pH: 7.59. Organic carbon content 15.4 $g \cdot kg^{-1}$ d.m., organic matter 26.5 $g \cdot kg^{-1}$ d.m., N – 1.61 $g \cdot kg^{-1}$ d.m.
Site 3 – Sandy native soil; pH: 7.76. Organic carbon content 6.1 $g \cdot kg^{-1}$ d.m., organic matter 10.5 $g \cdot kg^{-1}$ d.m., N – 0.40 $g \cdot kg^{-1}$ d.m.
Site 4 – Sandy soil on the banks of the River Odra; pH: 7.49. Organic carbon content 12.9 $g \cdot kg^{-1}$ d.m., organic matter 22.2 $g \cdot kg^{-1}$ d.m., N – 0.86 $g \cdot kg^{-1}$ d.m.

Leaves to the analysis were collected from 5 bushes (1 shrub – 1 replication) from the outside of the bush, with half of their height. The leaves taken from the one year old shoots without any signs of ageing or mechanical damage.

Chemical analysis

The chemical composition of samples were determined according to the Association of Official Analytical Chemists [AOAC 2012] procedures: dry matter, by drying at 105°C to constant weight; ether extract, by Soxhlet extraction with diethyl ether; crude ash, by incineration in a muffle furnace at 580°C for 8 h; crude protein (N × 6.25), by Kjeldahl method using a Büchi Distillation Unit B-324 (Büchi Labortechnik AG, Switzerland), crude fiber was determined with a fiber analyzer ANKOM 220 (ANKOM Technology, USA); Total carbohydrates (%) was calculated as: 100 − % (moisture + crude protein + crude fat + ash + crude fiber). The fibre components were determined using the detergent method according to Van Soest et al. [1991] performed with a fibre analyzer ANKOM 220. Determination of neutral detergent fibre (NDF) was on an ash-free basis and included sodium dodecyl sulphate (Merc 822050). Determination of acid detergent fibre (ADF) included hexadecyl-trimethyl-ammonium bromide (Merc 102342), while acid detergent lignin (ADL) was determined by hydrolysis of ADF sample in 72% sulfuric acid. Hemicellulose was calculated as the difference between NDF and ADF, while cellulose – as the difference between ADF and ADL. All the tests were conducted on dry matter (d.m.).

The plant material obtained for the micro-compound analyses was mineralized in an Anton Paar 3000 microwave oven in mixture of HNO_3, HF and H_2O_2. The elements were determined using the ICP-MS method (Elan DRC II 6000 Perkin Elmer) with the internal standard.

Isotopes

The determination of carbon and nitrogen (weight percentage) and isotope content of both elements in dry, ground and homogenized sea buckthorn samples were performed with a Flash 1112 NC Elementary Analyser (Thermo Finnigan, Italy). Additionally, the isotope analyses there were performed with a DELTA plus mass spectrophotometer (Finnigan, Germany). Depending on the aim of the research, determination of the elements' mass participation or isotope composition of carbon and nitrogen, the analyses were carried out separately (different sample weights, standards, software and measuring techniques). Elements' mass participation was determined using gas chromatography in an elementary analyzer, whilst the isotope content was determined with a mass spectrophotometer intended for such use. Measurements of standards with a known element (mass participation of carbon and nitrogen) and isotope content (stable carbon and nitrogen isotopes) were conducted alternately with the samples. The final results were calculated with dedicated measurement software.

Statistical analysis

The statistical analysis was carried out using the Statistica 10 programme. In order to determine the significance of the differences in chemical composition of the analysed plant samples one way analysis of variance (ANOVA) was conducted, after assessing normality and homogeneity of variance. For the purpose of determining homogenous subsets of means the Duncan test was carried out at $P \leq 0.05$. Results are presented as mean ± standard deviation. All samples were analyzed in triplicate.

RESULTS AND DISCUSSION

According to Gonnella et al. [2004] dry mass content is an important trade value parameter for plants eaten fresh, which also determines their durability. According to these authors, best values are exhibited by plants with over 7% dry mass. Most of plant material wilts soon after collection. To maintain their high quality they should be preserved by drying, which involves water removal and enzyme deactivation. The content of active agents in correctly dried material does not change for a long time. The present research determined dry mass in dried sea buckthorn leaves with the gap at 948.2–953.8 $g \cdot kg^{-1}$. Leaves from the native position (site 3) had significantly most dry matter The highest significant dry matter content (953.8 $g \cdot kg^{-1}$ d.m.) was found in leaves of the plants growing on native position (site 3).

According to dietary guidelines daily protein intake should not exceed 0.8 $g \cdot kg^{-1}$ of body mass [Elango et al. 2012, Cichosz and Czeczot 2013]. The place of the plants growth had a significant influence on basic chemical composition of buckthorn's leaves (tab. 1). Mean protein content in the leaves was 235.0 $g \cdot kg^{-1}$ d.m. The highest significant protein content (262.6 $g \cdot kg^{-1}$ d.m.) was found in leaves of the plants growing on local soil around the Dolna Odra power plant and the lowest – in the plants growing on reclaimed soil, fertilised with NPK (193.2 $g \cdot kg^{-1}$ d.m.). Kashif and Ullah [2013] were studying selected medicinal plants and found the highest protein content in sea buckthorn leaves (120.35 $g \cdot kg^{-1}$ d.m.). Significantly lower values were found in leaves of neem (*Azadirachta indica*), pomegranate (*Punica* *granatum*) and basil (*Ocimum tenuiflorum*) (99.6, 36.9 and 51 g·kg^{-1} d.m., respectively).

Fat determines the energy content in plant material to much further extent than protein, total carbohydrates or fibre. It is a concentrated source of energy for all tissues except the brain, kidneys and red blood cells. The highest fat content was found in leaves of buckthorn plants growing on reclaimed soil, fertilized with NPK (site 1). Similarly, the content of ash, crude fibre and total carbohydrate was also highest in the plants growing at this site.

Dietary fibre is one of the natural components which modern populations lack in their diets. It is believed that fibre plays an important role in preventing metabolic civilization diseases and in their treatment. This includes inter alia obesity, constipation, coronary heart disease, type II diabetes and colon cancers [Zołoteńka-Synowiec 2013, Eskicioglu et al. 2015]. There are many structural forms of dietary fibre. They are important in the physiology of the alimentary canal as well as in food technology [Ötles and Cagindi 2006]. Plant material contains various amounts of fibre in various ratios of its fractions. Cereal grain, vegetables, legume seeds and herbs are rich sources of this component [Kudełka and Kosowska 2008, Nawirska-Olszańska et al. 2010].

The soil conditions did not influence the amount of hemicelluloses, which in sea buckthorn leaves ranged from 103.2–111.7 g·kg^{-1} d.m. (tab. 2). Similarly to fibre, the highest concentration of neutral-detergent fraction (NDF), acid-detergent fraction (ADF), acid-detergent lignin (ADL) and cellulose concentrations were found in the buckthorn plants growing on reclaimed soil fertilized with NPK. Compared to buckthorn with the lowest values of these components (growing by the river Odra), the amount of NDF was higher by 17%, ADF by 24%, ADL by 22% and cellulose (CEL) by 31%. The obtained results indicate that contents of ADF, ADL and cellulose in Sea buckthorn leaves were several times higher and hemicelluloses content was lower than in medical fenugreek (*Trigonella foenum-graecum* L.)

Table 1. Chemical composition of sea buckthorn leaves (g·kg^{-1} d.m.), mean from 2014–2015

Position*	Dry matter	Crude ash	Crude protein	Crude fat	Crude fibre	Total carbohydrates
1	948.2 ±0.6^{c}	50.9 ±0.6^{a}	193.2 ±1.0^{d}	65.6 ±0.9^{a}	136.9 ±0.9^{a}	618.0 ±1.3^{a}
2	951.4 ±0.4^{ab}	49.2 ±0.2^{ab}	247.5 ±0.5^{b}	63.7 ±0.5^{b}	131.5 ±2.3^{b}	571.9 ±2.0^{c}
3	953.8 ±1.8^{a}	47.9 ±0.3^{b}	262.6 ±0.0^{a}	60.8 ±0.2^{c}	130.8 ±0.9^{b}	558.7 ±1.2^{d}
4	950.8 ±0.6^{bc}	48.8 ±1.5^{ab}	236.9 ±1.1^{c}	61.8 ±0.9^{c}	113.3 ±2.4^{b}	600.9 ±2.8^{b}

* 1–4 position of living of sea buckthorn, as in methodology; mean values with the same letter in each column are not significantly different at P ≤ 0.05; total carbohydrates = dry matter – (crude protein + crude fat + crude fibre + total ash)

Table 2. Fibre fractions of tested sea buckthorn leaves (g·kg^{-1} d.m.), mean from 2014–2015

Position*	NDF	ADF	ADL	HCEL	CEL
1	318.9 ±2.1^{a}	207.3 ±2.7^{a}	62.5 ±0.2^{a}	111.7 ±4.8^{a}	144.8 ±2.5^{a}
2	310.9 ±1.9^{a}	194.4 ±1.2^{b}	60.7 ±0.5^{b}	116.5 ±3.1^{a}	133.7 ±0.6^{b}
3	297.7 ±7.4^{b}	194.5 ±2.4^{b}	59.5 ±0.5^{b}	103.2 ±5.1^{a}	134.9 ±1.8^{b}
4	271.1 ±2.9^{c}	166.8 ±2.8^{c}	56.5 ±0.5^{c}	104.0 ±5.6^{a}	110.3 ±2.2^{c}

*1–4 position of living of sea buckthorn, as in methodology, NDF – neutral detergent fibre, ADF –acid detergent fibre, ADL – acid detergent lignin, HCEL – Hemicellulose, CEL – Cellulose, Mean values with the same letter in each column are not significantly different at P ≤ 0.05

(2.35 g·100 g^{-1} d.m., 0.38 g·100 g^{-1} d.m., 1.46 g·100 g^{-1} d.m., 32.8 g·100 g^{-1} d.m., respectively [Kochhar 2006] Leaves of fenugreek are popular in Asia, dried and powdered seeds are valued as spice. In many countries leaves of fenugreek are a popular medicinal agent [Król-Kogus and Krauze Baranowska 2011].

The present study revealed that differential content of macro-compounds (tab. 3) is probably a result of soil conditions and plants' variable abilities to take up and accumulate these minerals. This in turn directly influences the value of products derived from sea buckthorn. Mineral compound are vital for correct functioning of a human organism. Both prolonged shortage and excess of minerals can contribute to some metabolic civilisation diseases [Bolesławska et al. 2009] Iron, zinc and copper are micro-compounds essential for correct development and functioning of every organism, especially during childhood, when growth and development are particularly intense [Szczepaniak et al. 2002, Staniek et al. 2006].

The interdependence of iron and copper is based mainly on copper participation in iron metabolism. Prolonged copper shortage favors certain cancers, atherosclerosis or aortic aneurysm, but it also causes changes in bones leading to osteoporosis. There are hypotheses showing negative role of copper shortage in atherosclerosis development [Tissato et al. 2010]. Leaves of the plants growing in natural conditions (by the river Odra) were found to contain increased content of Cu and Fe compared to the plants growing in different environments. Mean Cu concentration (9.9 mg·kg^{-1} d.m.) was higher than in leafy vegetables [Asaolu et al. 2012]: *Basella alba* L. (0.14 mg·100 g^{-1} d.m.) and *Amaranthus hybridus* (0.08 mg·100 g^{-1} d.m). More copper than these buckthorn leaves contained only *Hibiscus sabdariffa* (3.14 mg·100 g^{-1} d.m). Iron content (350 mg·kg^{-1}, on average) was similar to that in: *Indian spinach* (34.47 mg·100 g^{-1}), *Hibiscus sabdariffa* (21.84 mg·100 g^{-1}) and *Amaranthus hybridus* (39.04 mg·100 g^{-1}). Some of the trace elements, such as strontium (Sr), exhibit close chemical resemblance to calcium. In concentrations higher than those physiologically required they exert pharmacological effects on bones. There is some unequivocal evidence that strontium (Sr) in its stable form affects bone metabolism on tissue and cell level in various ways through stimulating bone formation and inhibiting bone resorption [Marie et al. 2001]. Molybdenum (Mo) is an important element in human diet, with a range between 150 and 500 mg daily being considered appropriate and safe for an adult person [Vyskocil and Viau 1999]. Molybdenum content in food products, including plants, is mainly influenced by the soil properties and plant species. High Mo content is usually found in legumes and leafy plants, lower Mo content – in root crops. The present research found that Zn, Sr and Mo content in sea buckthorn leaves was: 73.2, 14.9, 15.3 mg·kg^{-1} d.m., respectively. Compared to other sites, significantly higher content of these elements was found in leaves of the plants grown on reclaimed soil supplemented with coal ash, conifer bark and compost from sewage sludge. In leaves of sea buckthorn growing in North-West Himalayas the mean content of Zn ranges from 0.09–0.64 mg·kg^{-1} [Sharma et al. 2014]. Inappropriate supply of Mn or disrupted absorption processes and/or retention may be a reason for development of certain diseases. Still however, the knowledge about

Table 3. Contents of micronutrients in sea buckthorn leaves (mg·kg^{-1} d.m.), mean from 2014–2015

Position*	Cu	Fe	Sr	Mo	Zn	Mn	Ni
1	10.6 ±0.0^{ab}	268.9 ±29.6^{b}	5.80 ±0.7^{d}	18.9 ±0.0^{a}	65.4 ±5.9^{b}	17.9 ±1.5^{c}	17.9 ±4.5^{a}
2	9.45 ±1.5^{ab}	399.9 ±5.4^{a}	26.8 ±0.7^{a}	19.4 ±0.7^{a}	94.1 ±5.2^{a}	33.1 ±3.7^{b}	17.9 ±0.0^{a}
3	8.40 ±0.0^{b}	276.6 ±26.9^{b}	18.9 ±0.0^{b}	4.19 ±0.0^{b}	58.7 ±0.1^{b}	43.6 ±0.7^{a}	6.29 ±0.0^{b}
4	11.1 ±0.7^{a}	458.1 ±42.7^{a}	8.41 ±10.4^{c}	18.9 ±10.4^{a}	74.7 ±17.8^{ab}	42.6 ±0.7^{a}	6.31 ±0.0^{b}

*1–4 position of living of sea buckthorn, as in methodology; mean values with the same letter in each column are not significantly different at $P \leq 0.05$

Mn role in preventing certain diseases is insufficient and therefore little is known about Mn influence on a human organism [Zabłocka-Słowińska and Grajeta 2012]. In the present research found 34.3 mg·kg^{-1} d.m. of Mn in leaves. Twice as much Mn (143% more) was found in sea buckthorn growing on sandy local soil around the Dolna Odra power plant than in those from reclaimed soil fertilised with NPK. According to Asaolu et al. [2012] green vegetables from Nigeria contain between 2.54–10.06 mg·100 g^{-1}. Whilst Sharma et al. [2014] report that Mn in leaves of sea buckthorn cultivated in North-West Himalayas ranges from 0.20 to 0.56 mg·kg^{-1}. Significant differences in Mn and Zn content between the present and other studies [Asaolu et al. 2012, Sharma et al. 2014] probably are caused by the habitat differences and they confirm that the environment that plants grow in influence sea buckthorn chemical composition [Zeb and Malook 2009]. Nickel is one of the metals that are essential for human health in low concentrations, but in higher – it can pose a health threat [Duda-Chodak and Błaszczyk 2008]. The results of previous research on animals indicate that poisoning symptoms would occur in humans on over 250 mg daily intake of dissolved nickel [Nielsen 1993]. Reclaimed sites clearly favored Ni accumulation in buckthorn leaves, which contained twice as much Ni (184% more) that leaves of buckthorn growing on local soil by the river Odra. Moderate Ni content in buckthorn leaves was 12.1 mg·kg^{-1} d.m. According to Bosiacki and Roszyk [2012] leafy vegetables (lettuce, cabbage, parsley and leek) mean Ni content ranged from 2.46–3.21 mg·kg^{-1} d.m, depending on a method of mineralization.

Every plant has a unique composition of naturally occurring carbon, nitrogen, hydrogen and oxygen isotopes, which is influenced by physical and biochemical conditions as well as climate conditions [Ogrinc et al. 2003] soil condition or the amount of fertiliser [Georgi et al. 2005]. Leaves of sea buckthorn growing on reclaimed soils fertilized with NPK had less $\delta^{15}N$ (tab. 4), which probably resulted from higher nitrogen content in soil. According to Bateman et al. [2007] lettuce cultivated in conventional conditions had less $\delta^{15}N$ than lettuce fertilized organically. Organically fertilized lettuce was found to contain 7.6‰ of $\delta^{15}N$, whilst that fertilized chemically – 2.9‰. Higher $\delta^{15}N$ content in vegetables from organically fertilized soils are confirmed by Nakano et al. [2003].

Although not confirmed statistically, the habitat conditions did not alter $\delta^{13}C$ content in sea buckthorn leaves (tab. 4) that in all of the examined cases were very similar, as confirmed in the literature [Nakano et al. 2003]. The examined plants contained on average 3.3% N in dry mass, which is consistent with the results of N content assessment in sea buckthorn leaves obtained in the present study (N × 6.25) and in the studies of other authors [Sharma et al. 2014]. Percentage content of nitrogen in the examined samples was slightly higher in those from reclaimed soils. Sea buckthorn growing on reclaimed soils had also slightly narrower C:N ratio than that growing in natural sites.

Table 4. Values of $\delta^{15}N$ and $\delta^{13}C$, content N (%) and ratio C:N in sea buckthorn leaves, mean from 2014–2015

Position*	$\delta^{15}N$(‰)**	$\delta^{15}C$(‰)***	% N****	C:N*****
1	3.19	-27.85	3.46	12.77
2	1.17	-28.48	3.47	12.77
3	-0.43	-27.15	3.12	13.76
4	-0.26	-28.63	3.15	13.77

* 1–4 position of living of sea buckthorn, as in methodology, ** nitrogen isotopic composition, *** carbon isotopic composition, **** the percentage of nitrogen in the measured sample, ***** the ratio of the percentage of carbon to nitrogen in the samples

Conclusion

For a healthy and conscious-eating consumer one of the most important diet elements is a product that would be natural, possibly least processed and uncontaminated. The quality of plant products depends not only on species characteristics but also on habitat and agricultural technology.

1. The mean protein content in sea buckthorn leaves was 235.0 g·kg^{-1} d.m.

2. The content of ash, fat, fibre and total carbohydrates was significantly higher in leaves of sea buckthorn growing on sandy reclaimed soil fertilized with NPK (site 1). Mean content was: 49.2, 63.0, 128.1, 587.4 g·kg^{-1} d.m., respectively.

3. Similarly to fibre, the highest concentration of NDF, ADF, ADL and cellulose concentrations were found in the sea buckthorn plants growing on reclaimed soil fertilized with NPK.

4. The content of micro-compounds varied significantly, which is probably influenced by habitat conditions and plants' variable abilities to take up and accumulate these minerals in biomass.

5. Higher $\delta^{15}N$ was found in sea buckthorn cultivated on reclaimed soils fertilized with NPK. The values of $\delta^{13}C$ were similar in all of the examined samples of sea buckthorn.

6. It cannot be unequivocally determined whether the results of the study on the influence of habitat conditions on chemical and isotope composition of sea buckthorn leaves are permanent. Therefore, it is necessary to continue the research.

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