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Using Synchrotron Radiation X-Ray Fluorescence Analysis in Botanical Research to Study the Elemental Composition of Altai Mountain Plants of the Family Fabaceae

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Abstract—Synchrotron radiation X-ray fluorescence analysis is used to study for the first time the elemental composition of above-ground organs of such plants of the family Fabaceae as *Oxytropis argentata*, *Astragalus tibetanus*, and *Caragana Bungei* growing in the Chuiskaya Basin of the Altai Mountains and soils from their habitats. A relationship is found between the accumulation of elements, the species of the plants, their life forms, and the growing conditions. The data obtained for the elemental composition of the vegetable specimens and soils can be included in databases.

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INTRODUCTION

Members of the family Fabaceae are of medicinal, food, forage, and decorative value. They are also capable of actively enriching soil with plant-available nitrogen in any association. Even rare species from the family Fabaceae can therefore be an important element of maintaining stability and biodiversity in biocenoses and act as highly nutritious forage for animals.

The highland zone in the Altai Republic occupies a large area that consists mainly of dry steppe, arid, and semiarid ecosystems. The vegetation of these ecosystems is traditionally used by the indigenous communities as pastures that are the basis of the forage reserve for cattle breeding [1]. It is therefore relevant to investigate such plants and their elemental composition to preserve and sustainably use the vegetable resources of the mountain ecosystem. Plants are capable of controlling their chemical composition through biological selectivity toward chemical elements. To evaluate the role of each chemical element in the life of plants, we must determine its gross content and local forms that participate in processes of transport, metabolism, and accumulation [2].

There is almost no information on the composition and concentration of macro- and microelements in members of the family Fabaceae that inhabit the intermountain Chuiskaya Basin in the southwestern Altai at a height of 1700–1900 m above sea level. In the Chuiskaya Basin, plants of the family are found in different life forms—shrubby and grassy. *Oxytropis argentata* (Pallas) Pers (silvery oxytrope) and *Astragalus tibetanus* Benth. Ex Bunge (Tibetan oxytrope) are perennial herbaceous plants and *Caragana Bungei* Ledeb. (Bunge pea shrub) is a shrub [3].

Various spectroscopic means are used to study the chemical composition of plants and soils. Many of them are characterized by a series of limitations and disadvantages, especially in terms of requirements for preparing samples and spatial resolution. Using special synchrotron units allows us to employ special properties of synchrotron radiation as a research tool in determining the minerals and pollutants in environmental specimens [4–6]. The analytical capabilities of multielement synchrotron radiation X-ray fluorescence analysis (SR XRFA) are successfully employed in analyzing vegetable materials, as is apparent from numerous studies in the fundamental and applied sciences [7–10]. The main problem in using XRFA is the need for a large number of different reference standards (RSes) adequate with respect to the composition of a given object during calibration [11]. As was noted by I.E. Vasil'eva and E.V. Shabanova in 2021, the total number of reference standards for plants is extremely small in comparison to, e.g., those for raw materials and products in metallurgy, despite the enormous variety of plant species [11]. The search for the optimum vegetable reference samples (standards) can therefore help solve the problem of using SR XRFA in botanical studies.

Table 1. GOST limits of detection C_{min} at a 23 keV energy of excitation for GSO SORM1 and BIL-1, ppm

Elements	SORM1		BIL-1	
	C_{min}	S_r, %[1]	C_{min}	S_r, %
K	27	10	206	6
Ca	13	11	106	8
Ti	3	26	31	5
V	0.1	19	8	8
Cr	1.9	64	6	6
Mn	0.9	9	8	4
Fe	0.6	8	5	4
Co	0.03	36	0.3	9
Ni	0.3	39	3	4
Cu	0.15	14	2	5
Zn	0.2	12	1	5
As	N/C[2]	–	1	3
Br	0.05	11	0.4	8
Rb	0.07	11	0.3	9
Sr	0.1	12	0.3	7
Y	0.6	29	0.4	12
Zr	0.5	58	0.3	16
Nb	1.4	40	0.3	9
Mo	0.07	10	0.1	14
Pb	0.05	35	0.5	14

[1] S_r is the relative standard deviation.
[2] N/C: The concentration of the chemical element is not certified.

The aim of this work was to identify characteristics of the composition and concentration of elements in the above-ground organs of plants of different species and life forms from the family Fabaceae, find species with a high concentrations of macro- and microelements, and determine the possibility of using vegetable samples as standards.

EXPERIMENTAL

Specimens of perennial herbaceous plants *Oxytropis argentata* (silvery oxytrope), *Astragalus tibetanus* (Tibetan oxytrope), and *Caragana Bungei* (Bunge pea shrub) served as our material. The plant specimens were collected in the Chuiskaya Steppe in the Kosh-Agach district of the Altai Republic in July 2020. *O. argentata* and *A. tibetanus* grew in a grain–oxytrope association in a complex steppe with salinification at a height of 1804 m above sea level (N 49.92669 E 88.84287); *C. Bungei*, in the vicinity of Kosh-Agach rural locality at a height of 1760 m above sea level (N 50.01888 E 88.64833). The leaves, stems, and reproductive organs of the plants and soils from the points of collecting the specimens were analyzed. An average specimen comprised five to ten individual plants. Soil specimens were taken from the root habitable layer (10–25 cm) and the plant envelopes.

Weighed amounts of air-dried vegetable feedstock and soils (1 g each) were ground in an agate mortar. Samples were then pressed in the form of tablets ~1 cm in diameter and weighing 30 mg. The elements were determined via synchrotron radiation X-ray fluorescence analysis (SR XRFA) on the elemental analysis station of the VEPP-3 collector at the Budker Institute of Nuclear Physics' Siberian Center of Synchrotron and Terahertz Radiation. The samples were measured at a 23 keV energy of exciting radiation. Each measurement took 300 to 500 s for the weighed amounts of vegetable matter and soil. The main characteristics of the experimental station and instructional aspects of the work were presented in [12–14].

The obtained spectra were processed in the AXIL program for fluorescence spectra. The shape of each line was described using its Gauss curve. The spectra were calculated using nonlinear least squares. The concentration of elements was determined according to external standards. Russian standards of grass–grain mixtures (GSO SORM1) and Baikal bottom silt (BIL-1) were used as reference standards [15]. Limits of detection C_{min} and relative standard deviation S_r were calculated by measuring ten parallel measurements of a SORM1 reference standard in three replicates and a BIL-1 reference standard in five (Table 1). Table 1 shows the values of C_{min} vary, depending on the element and reference standard that is used. Overall, a reduction in the limits of detection upon moving from light (K) to heavier elements (Pb) is noticeable.

RESULTS AND DISCUSSION

The concentrations of at least 20 elements were found by studying the above-ground organs of plants of three species and soil from their points of collection (Tables 2 and 3).

A comparative analysis of the soils found excess concentrations of Ca (62284 ppm) and Br (18 ppm) at the point of collecting the grassy plants and As (50 ppm) at that of collecting *C. Bungei*. The concentrations of the remaining elements varied little (Table 2).

Our study of the concentration of macro- and microelements in the above-ground organs of the given plants showed that for most elements, the maximum concentrations did not exceed the upper limits of the ranges of concentrations of these elements presented in the literature [16, 17]. The concentration of K in the grassy plants grew in the series leaves > stems > beans. In contrast, the concentration of Ca fell (Table 3). A reverse trend was observed in the shrubs: the concentration of Ca in stems was higher than in leaves, while the concentration of K remained the same. Overall, a higher concentration of microelements (K + Ca) was found in the leaves of *O. argentata* (38769 ppm), and a lower one in the stems of *A. tibetanus* (16478 ppm).

The total concentration of microelements was greatest in the stems of *C. Bungei* and beans of *A. tibetanus* (590–588 ppm); the smallest, in the leaves of *C. Bungei* (376 ppm). The leaves of *C. Bungei* were distinguished by an increased concentration of Mn (77 ppm); the stems, by elevated concentrations of Fe (371 ppm), Br, and Cr (6.2 ppm). The leaves of *O. argentata* mainly accumulate Sr (205 ppm); the stems of *A. tibetanus*, Zn (46 ppm), Ni (11 ppm), and Cu (8 ppm). The concentration of Rb is two to three times higher in the leaves and stems of the grassy plants, relative to the shrub. An elevated concentration of Mo in the above-ground organs of the grassy plants was noted (up to 2.4 ppm in the leaves and stems of *O. argentata* and 2.6 ppm in the beans of *A. tibetanus*, due probably to the availability of Mo to plants on carbonate soils) and confirmed in the literature [16]. The concentration of Mo in the leaves and stems of *C. Bungei* is two orders of magnitude lower than that of *O. argentata*.

A shift in the ratios of the concentrations of some elements was noted. The value of Fe/Mn changed from 2.3 in the leaves of the shrubs to 6–7 in the grasses. The value of Ca/Sr was above 100 in all samples, which is quite normal. However, it was greatest in the *C. Bungei* shrub at 255. The value of K/Rb varied from 340–660 in the above-ground organs of the grasses and up to 3344 in the shrubs, due probably the different species and growing conditions.

Table 2. Concentration of elements in the soil from the points of collecting the plants in the Altai Mountains (K, Ca, Fe are given in mg/g of dry weight; the remaining elements, in mg/kg)

Elements	Points of collecting the specimens		Maximum allowable concentration (MAC)* [16], Clarke** [17]
	1[1]	2	
K	14 ± 1[2]	17 ± 1	25**
Ca	38 ± 3	62 ± 5	30**
Ti	3253 ± 163	2514 ± 126	5000*
V	94 ± 5	59 ± 3	100**
Cr	49 ± 2	30 ± 2	200**
Mn	675 ± 34	639 ± 32	1500*
Fe	29 ± 1	24 ± 1	38**
Co	12 ± 1	11 ± 1	5*
Ni	25 ± 3	29 ± 3	85*
Cu	26 ± 2	19 ± 1	55*
Zn	52 ± 4	70 ± 5	100*
As	50 ± 4	4.7 ± 0.4	2*
Br	1.0 ± 0.1	18.2 ± 1.8	5**
Rb	59 ± 7	77 ± 9	60**
Sr	160 ± 21	236 ± 31	300**
Y	18 ± 3	21 ± 3	50**
Zr	80 ± 16	120 ± 24	300**
Nb	5 ± 2	11 ± 3	10**
Mo	0.3 ± 0.1	0.3 ± 0.1	2**
Pb	13 ± 1	18 ± 2	32*

[1] Habitat of the plants: (1) *Caragana Bungei*; (2) *Oxytropis argentata* and *Astragalus tibetanus*.
[2] Average value ± standard deviation.

CONCLUSIONS

Reliable data on the concentration of 20 elements in the above-ground organs of *O. argentata*, *A. tibetanus*, and *C. Bungei* and soils from their habitats in the Chuiskaya Basin were obtained for the first time. SR XRFA can be used to determine elements in plants and soils. It allows new analytical data to be obtained that is confirmed according to Russian reference standards for grass–grain mixtures and Baikal bottom silt. A relationship was noted between the accumulation of elements and the species of the plants, life form, organs, and growing conditions. The data obtained on

Table 3. Concentration of elements in the above-ground organs of plants from the family Fabaceae in the Altai Mountains (ppm in air-dried weight)

Element	*Oxytropis argentata*			*Astragalus tibetanus*			*Caragana Bungei*	
	leaves	beans	stems	leaves	beans	stems	leaves	stems
K	7729 ± 386[1]	13870 ± 693	8020 ± 401	5982 ± 299	14810 ± 740	5387 ± 269	16003 ± 800	15944 ±797
Ca	31041 ± 2483	4800 ± 384	11057 ± 885	24080 ± 1926	9849 ± 788	11091 ± 887	17837 ± 1427	21347 ± 1708
Ti	4 ± 0	9 ± 1	24 ± 1	4 ± 0	22 ± 1	10 ± 1	5 ± 0	24 ± 1
V	0.4 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
Cr	3.4 ± 0.2	1.6 ± 0.1	3.9 ± 0.2	1.5 ± 0.1	1.0 ± 0.1	3.2 ± 0.2	1.5 ± 0.1	6.3 ± 0.3
Mn	43 ± 2	18 ± 1	26 ± 1.3	36 ± 2	27 ± 1	21 ± 1	77 ± 3.9	36 ± 2
Fe	260 ± 13	177 ± 9	300 ± 15.0	258 ± 13	381 ± 19	218 ± 11	178 ± 8.9	371 ± 19
Co	0.1 ± 0.0	BLD[2]	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Ni	4.4 ± 0.4	14.6 ± 1.5	5.8 ± 0.6	5.0 ± 0.5	12.1 ± 1.2	11.4 ± 1.1	2.1 ± 0.2	6.6 ± 0.7
Cu	4.2 ± 0.3	4.7 ± 0.3	3.6 ± 0.2	5.8 ± 0.3	5.7 ± 0.3	8.1 ± 0.5	5.3 ± 0.3	5.3 ± 0.3
Zn	16 ± 1	31 ± 2	17 ± 1	38 ± 3	44 ± 3	46 ± 3	23 ± 2	28 ± 2
As	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
Br	2.5 ± 0.2	1.0 ± 0.1	1.6 ± 0.2	1.2 ± 0.1	1.3 ± 0.1	2.0 ± 0.2	2.2 ± 0.2	6.2 ± 0.6
Rb	19 ± 2	31 ± 4	18 ± 2	11 ± 1	22 ± 3	16 ± 2	6 ± 1	5 ± 1
Sr	205 ± 27	34 ± 4	113 ± 14.7	130 ± 17	56 ± 7.3	95 ± 12	70 ± 9.1	91 ± 12
Y	2.1 ± 0.3	0.3 ± 0.0	2.0 ± 0.3	0.6 ± 0.1	2.0 ± 0.3	NDA[3]	0.2 ± 0.0	0.7 ± 0.1
Zr	10.4 ± 2.1	3.3 ± 0.7	9.0 ± 1.8	5.0 ± 1.0	7.1 ± 1.4	3.3 ± 0.7	3.7 ± 0.7	7.0 ± 1.4
Nb	1.6 ± 0.5	0.3 ± 0.1	1.6 ± 0.5	0.7 ± 0.2	2.7 ± 0.8	0.5 ± 0.1	0.2 ± 0.1	1.5 ± 0.4
Mo	2.3 ± 0.7	2.1 ± 1	2.4 ± 0.7	0.9 ± 0.3	2.6 ± 0.8	1.2 ± 0.4	BLD	0.1 ± 0.0
Pb	1.0 ± 0.1	0.8 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.6 ± 0.2	0.9 ± 0.1	1.7 ± 0.2

[1] Average value ± standard deviation.
[2] BLD: The concentration of the element is below the limit of detection (0.01 ppm).
[3] NDA: No data available.

the elemental composition of vegetable specimens and soils can be included into databases.

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

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