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Content of macro and microelements in the plants of *Artemisia annua* L., *A. ludoviciana* Nutt. and *A. austriaca* L.

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The purpose of this study was to investigate mineral composition of the plants species Artemisia annua L., A. ludoviciana Nutt. and A. austriaca L. Methods. Determination of the elemental composition of plant material was carried out by the X ray fluorescence method. **Results.** The content of mineral elements in plants depends on their individual ability to absorb elements from the soil and accumulate them in the roots, leaves and flowers. Plant samples of three species of wormwood were grown and studied during the flowering phase under conditions of introduction in M. M. Gryshko National Botanical Garden of National Academy of Sciences of Ukraine (NBG) during 2019–2022. The qualitative and quantitative content of different macro and microelements in the soil and plants were investigated. It was shown that aerial parts of the inves tigated plants accumulate the most important elements for the plants life, such as – K, Fe, Cu, Zn and Mn. Mesoelements Ca and S are present in sufficient quantities also. Elements Nb, Y, Ti, V, Cr were detected in soil, but were not determined in plants. Only A. annua plants contains Ni and Se, while A. ludoviciana and A. annua plants contain Pb. The amount of toxic elements in plants did not exceed the maximum permissible concentrations for vegetable raw materials and food products. Conclusion. Content of the main macro and microelements was determined in the plants A. annua, A. ludoviciana and A. austriaca growing in NBG. The tendency of plants A. ludoviciana to accumulate high concentrations of iron in the roots and aerial part was observed. The obtained data will be useful for forecasting and evaluating the results of introduction of new promising species of the genus Artemisia, in breeding of new varieties of wormwood, to determine their pharmacologi cal properties and to make a decision about the feasibility of using them in herbal tea and food products.

Keywords: introduction; Artemisia annua; A. austriaca; A. ludoviciana; macro and microelements; pharmacognosy; herbal tea.

Introduction

The biochemical analysis of both introduced and well-known medicinal and aromatic plants is an important task [1, 2]. This is because of

http://orcid.org/0000 0001 6656 4640 Jamal Rakhmetov http://orcid.org/0000 0001 7260 3263 Mariia Shanaida http://orcid.org/0000 0003 1070 6739 OlenaVergun http://orcid.org/0000 0003 2924 1580 Ludmyla Svydenko https://orcid.org/0000 0002 4043 9240 Volodymyr Voitsekhivskyi https://orcid.org/0000 0003 3568 0985 the natural complexes of substances are wide used in many classes of drugs. Plants require at least 14 mineral elements for their life [3– 5]. These include macronutrients nitrogen, phosphorus, potassium, calcium, magnesium and sulphur, as well as micronutrients chlorine, boron, iron, manganese, copper, zinc, nickel and molybdenum [6]. Crop production is often limited by low phytoavailability of essential mineral elements and/or the presence of excessive concentrations of potentially toxic mineral elements, such as Na, Cl, B, Fe, Mn and Al. Micronutrients are involved in all metabolic and cellular functions [7, 8].

The genus *Artemisia* L. is one of the largest in the *Asteraceae* Dumort family. The genus has a wide Holarctic range, grows throughout the Northern Hemisphere. The two major

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centers of the genus diversity are in Eurasia and western North America [9].

There are more than 500 species of wormwood in the world flora. About 180 species of sagebrush are common in the steppes and deserts of Northern Kazakhstan, Central Asia, and the Transcaucasia. Several species of wormwood are widespread and used as medicinal plants in India – Artemisia absinthium Linn., A. maritima Linn., A. vestita Wall. ex DC., A. vulgaris Linn. var. nilagirica Clarke [10]. There are 30 species of this genus growing in Ukraine, and they belong to 6 sections and 3 subgenera [11, 12].

Silver wormwood (A. ludoviciana Nutt.) is native to North America. The range includes most of Canada and east of the Rocky Mountains in the United States, except Alabama, Florida, and West Virginia. It is considered an invasive weed in some parts of the country, especially in the east. It's a perennial herbaceous decorative plant up to 70 cm tall, with branched, densely leafy stems. Silvery leaves, with ears at the base, have the ability to grow quickly (Fig. 1a). Spreading by rhizomes, A. ludoviciana can form dense colonies that give a distinctive silver-green accent to large plantings on sunny sites with dry soil. Its stems and foliage are covered with woolly gray or white hairs. A. ludoviciana subsp. mexicana has been traditionally used for the treatment of digestive ailments such as gastritis [13]. Flower baskets are small, brown. The flowering of plants lasts from the end of August to mid-October in the NBG. Wormwood likes sunny places. In such conditions, its silver color becomes more intense [14]. The plant does not tolerate heavy and wet soils, the roots rot. The soil needs to be light and permeable. The plant is considered exceptionally drought-resistant; watering should be done only in case of prolonged drought. The plant is frost-resistant, winters under the conditions of the Forest-Steppe of Ukraine without shelter. Plants are used as medicinal plants [15] and when creating mixed borders, grassy borders, including clipped ones, rockeries.



Fig. 1. Plants of species of *Artemisia* **genus:** a – A. ludoviciana, b – A. austriaca, c – A. annua

Austrian wormwood (A. austriaca L.) is a perennial herbaceous whitish downy plant that grows throughout Ukraine (except the Carpathians and northern Polissia) on steppe slopes, outcrops and dry meadows as a weed in open places. Stems are almost erect, often curved, 15-70 cm high, slightly woody at the base, branched below the middle or from the base, with numerous shortened axillary branches under the inflorescence, infertile shoots departing from the rhizome, much shorter than the stems (see Fig. 1b).

Lower leaves are short-petioled or sessile, from 2-3-parted to twice pinnate dissected, sometimes with 2-3-parted segments of the 2nd order (except the lowest fan-shaped), ovate or oval, with more or less developed, separate or pinnate dissected ears at the base. The flowers are heterogeneous, in ovoid or almost spherical small, more or less drooping baskets, forming a paniculate inflorescence. The flowering of plant lasts from August to end of September. The plant has a pleasant aroma [16].

Annual wormwood (A. annua L.) is an annual herbaceous cross-pollinated plant. Pollination occurs with the help of wind or insects. Annual wormwood is an adventitious species distributed throughout Ukraine, as well as in Belarus, Moldova, the Caucasus, Central Asia, the northern part of Europe, and Eastern Siberia (from Altai to Transbaikalia). In the wild, it occurs in North America, Central Europe, the Mediterranean, Japan, China, Iran, and Mongolia (see Fig. 1c). A short-day plant, distinguished by its resistance to diseases and pests. Blooms in August-September. Annual wormwood is undemanding to soil conditions, it does not grow only in swampy places, it does not tolerate drought well, it is resistant to light frosts. The raw material of annual wormwood has a strong smell and contains from 0.2 to 0.6% essential oil of a light yellow color with a greenish tint and a pleasant floral-balsamic smell. The composition of the essential oil includes artemisiaketone, isoartemisiaketone, l-camphor, 1.8 cineole, pinene, borneol, camphene, cadinene, caryophyllene, alcohols, and acetic and butyric acids [17, 18].

The plant is not included in pharmacopoea. Extracts based on annual wormwood have been used for centuries in traditional Chinese medicine for the treatment of complex infectious diseases, as a remedy for stomach ailments and as an appetite stimulant. Skin diseases are treated with fresh juice. The plant has long been used in China for fever and liver diseases. Annual wormwood is a very effective remedy in the treatment of malaria [19–21]. Now there are new data on the antitumor activity of compounds isolated from annual wormwood [22].

Materials and methods

The studies were conducted during 2019-2022 at the plot "Aromatic plants" of the M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (NBG), located in the city of Kyiv on the border of the Forest-Steppe and Polissia zones of Ukraine [23]. The objects of the research were plants of species Artemisia ludoviciana Nutt., A. annua L. and A. austriaca L. grown from seeds of their own reproduction. The aerial parts of plants, roots and soil from the collection plots of aromatic plants were used. Plant materials for research were collected in the phenological phase of flowering. Healthy plants without visual signs of a negative effect of a harmful amount of any elements from soil or air were selected for the analysis.

Dust-cleaned aerial parts of the plants were crushed and dried to a constant weight. Analysis of soil and plant material samples required minimal sample preparation. After drying and grinding the sample, a weight of 50 mg was taken and pressed into a tablet, which is placed in the device for analysis. The elemental composition of plant raw materials of wormwood and soil was determined by the X-ray fluorescence method on an energy-dispersive X-ray energy spectrometer «ElvaX» [24, 25]. The method is based on measuring the intensity of X-ray fluorescence spectrum lines of atoms of a chemical element when they are excited by primary X-ray radiation, the source of which is an X-ray tube. Registration of analytical intensities was performed using a multichannel spectrometer with an energy dispersive semiconductor detector (Sip-i-n diode) with thermoelectronic cooling. Specialized software allows to build the most probable model of the spectrum, identify its analytical lines in the presence of a large number (15-30) of sample elements, determine the exact weight of the object and mass concentration of the element. The range of elements determined by this method is from sulfur (z = 16)to uranium (z = 92). How many samples were analysed? Analysis of each sample was performed in two replicates.

The results of research were evaluated by statistical methods using the Microsoft Excel 2010 program and a package of programs for statistical analysis in crop production «AGROS» [26].

Results and discussion

Plant objects are a promising source of macro- and microelements, and therefore can be used as preventive and therapeutic agents in the complex therapy of diseases associated with the deficiency of certain elements in the human body [27].

The main amount of macro- and microelements enters the human body with food products [29]. It should be taken into account that excessive intake of even essential, i.e., vital minerals, can cause poisoning. To establish the possibility of using these species of Artemisia in the food and pharmaceutical industry, it is necessary to determine the quantitative and qualitative elemental composition of plant raw materials. In general, as a result of the research, 21 elements were determined in the soil (Table) and 17 elements were determined in the roots and above-ground mass raw materials of plants (Figs. 2-5), namely: macro elements – K, Ca, S, Cl and microelements – Fe, Cu, Zn, Sr, Mn, Ni, Co, Cr, Zr, Br, Pb, Rb, and Se, of which K, Ca, Fe, Cu, Zn, Mn are essential. Elements Nb, Y, Ti, V, Cr were detected in soil, but were not determined in plants.

A certain regularity was revealed in the distribution of these elements in the soil-rootplant system.

The following elements are listed in descending order of concentration in soil: Fe >Ca > K > S > Ti > Cl > Zr > Co > Mn > V > Sr > Rb>Ni > Cr > Zn > Cu > Nb > Y > Br > Pb > Se.

The content of Fe in the soil was quite high, but it entered the roots and grass in moderate amounts.

The quantitative content of the main elements in the solt, µg, g									
Macro elements	S	CL	К	Са					
	3875.66	2242.60	12561.71	20583.37					
	± 913.50	± 175.1	±767.49	± 840.70					
Micro elements	Fe	Zn	Sr	Си	Br	Mn	Rb	Pb	
	25637.42	34.79	67.08	25.12	12.55	449.59	64.69	19.95	
	± 264.37	± 4.15	± 2.40	± 3.77	± 1.72	± 38.21	± 3.34	± 2.10	
Ultra	Se	Со	Cr	Ti	Zr	Nb	Y	Ni	V
trace	2.84	536.38	46.12	2809.43	567.94	24.04	22.44	60.87	70.86
elements	± 0.85	± 31.16	± 9.37	± 175.10	± 8.12	± 2.03	± 1.84	± 8.30	± 22.16



Table







Fig. 3. The content of microelements Fe, Zn and Sr in the roots and grass of plants *A. austriaca*, *A. ludoviciana* and *A. annua*

It is known from the literature that about 1% of iron reaches the roots of plants from the soil, and 0,25-0,3% enters the grass [30, 31]. We found that the largest amount of iron

was accumulated in the above-ground mass of A. *ludoviciana* (more than 300 µg), which can serve as a source of iron in the human body when consuming the plant with herbal tea.

A. annua plants deserve more attention due to the rather attractive content of elements Zn, which play a significant role in hematopoiesis, have a positive effect on the condition of nails, hair, etc., which is consistent with the data of other researchers [32-34].



Fig. 4. The content of microelements Cr, Mn, Br and Rb in the roots and grass of plants A. austriaca, A. ludoviciana and A. annua

It was established that sulfur, entering the plant through the roots, is mainly accumulated in the grass, while most of manganese remains in the roots, which delay the further movement of the element into the leaves and inflorescences A. ludoviciana.

Selenium can bind heavy metals in living organisms by the formation of insoluble stable complexes and thus protect them [35]. The ability of plants to accumulate selenium can be used for the purpose of phytoremediation of contaminated selenium compounds of soils, as well as for the purpose correction of its deficiency in human nutrition and with trace element diseases.

We found that the elements Ni and Se were determined only in the aerial part of *A. annua* plants. Apparently, most wormwood plants do not absorb these elements from the soil, which was confirmed in our previous studies of plants *Artemisia argyi* H.Lév. & Vaniot [36].

Element Pb was found only in the roots of *A. annua* and the grass *A. ludoviciana* in amounts that did not exceed the maximum permissible standards [37].

Plants adapted to specific soil and climatic conditions largely depends on the balance of plant nutrition. Co is part of vitamin B12 and a number of enzymes and proteins involved in plant metabolism. Deficiency of Co in the plant may appear if there is a serious limitation of its availability from the soil. Conversely, high Co content is toxic to plants. High Co levels result in pale leaf color, vein discoloration and leaf loss, and can cause iron deficiency in plants [38]. We found that the highest concentration of Co in all studied plants was observed in the roots, while the content of Co was lower in the leaves and inflorescences.

Cu – is an important metal for the vital activity of the human body, but it becomes toxic in high doses. However, the presence of sufficient amounts of Zn and Mn in plants largely regulates the assimilation of Cu at a safe level [39].

Cu is not a nutrient commonly deficient in agricultural land, except in cases of uncontrolled use of Cu-based fungicides. Therefore, accurate calculation and control of Cu content can improve yield by reducing Cu toxicity [40].

According to the results of our studies, the concentration of Cu in *A. ludoviciana* and *A. austriaca* plants was lower in leaves than in roots, while in *A. annua* the Cu content increased from the root to the inflorescence, i.e., the plant is able to accumulate the element in the green mass.

Transfer of elements from the soil to plants is an important link in the chain of events that lead to the entry of metals into human and animal food. Only a small proportion of zirconium is available for plant uptake. Zr is less soluble than other metals and tends to exist as insoluble particles. The main route by



Fig. 5. The content of microelements Co, Cu and Zr in the roots and grass of plants A. austriaca, A. ludoviciana and A. annua

which Zr is absorbed by plants is soil-root transfer [41].

The following elements are listed in descending order of concentration in plant roots and leaves:

A. austriaca, roots: S >K >Ca >Fe >Sr >Zn >Cu >Mn >Zr >Br >Rb;

leaves: K >S >Ca >Fe >Sr >Zn >Co >Mn >Cu >Zr >Br >Rb >Cr;

A. annua, roots: K >S >Ca >Fe >Sr >Zn >Co >Mn >Cu >Zr >Br >Rb >Cr;

leaves: K >Ca >S >Cl >Fe >Zn >Sr >Cu >Mn >Br >Rb >Co >Zr >Ni >Se;

A. ludoviciana, roots: K >Ca >S >Fe >Sr >Zn >Co >Cu >Zr >Mn >Rb;

leaves: Ca >K >S >Fe >Cl >Zn >Sr >Co >Zr >Cu >Mn >Cr >Rb >Br > Pb.

Pb was determined in the soil in small amounts not exceeding maximum permissible level. Among plants, this element was determined only in the aerial mass of A. *ludoviciana*, but its concentration was 10 times lower than in the soil. Nickel and selenium were detected only in the aerial mass of A. *annua* plants.

Conclusion

The content of the main macro- and microelements was determined for the plants *Artemisia ludoviciana*, *A. annua* and *A. austriaca* under the conditions of introduction into the Forest Steppes of Ukraine. The peculiarities of the accumulation of elements by roots, leaves and flowers of plants during their transportation from the soil to the aerial part were clarified. Among the studied plants, *A. annua* and *A. ludoviciana* stands out due to its rich mineral composition. The obtained results can be used in the breeding of new varieties, for determination of pharmacological properties and decision-making on the expediency of their use in food products and for the production of chamazulene.

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Мета. Визначення кількісного та якісного елементно го складу рослин видів Artemisia annua L., A. ludoviciana Nutt. та *A. austriaca* L. за умов інтродукції в Національ ному ботанічному саду імені М. М. Гришка НАН України. Методи. Елементний склад рослинної сировини визна чали рентгенофлуоресцентним методом. Результати. Вміст мінеральних елементів у рослинах залежить від їхньої індивідуальної здатності поглинати елементи з ґрунту та накопичувати їх у коренях і надземній части ні. Зразки рослин трьох видів полину, вирощених у НБС, досліджували протягом 2019-2022 рр. у фазі цвітіння. Визначено якісний і кількісний вміст 21 макро та мікро елемента в ґрунті та 17 – в досліджуваних рослинах, а також деякі особливості міграції цих елементів у сис темі ґрунт – корінь – трава. Встановлено, що надземна частина досліджуваних видів містить найважливіші для життя рослин елементи – К, Fe, Cu, Zn i Mn. Мезоелемен ти Са і S присутні в достатній кількості. Елементи Nb, Y, Ті, V, Cr виявлено лише у ґрунті. Ni та Se містили тільки рослини A. annua, тоді як Pb – A. annua та A. ludoviciana. A. ludoviciana продемонструвала схильність до нако

пичення заліза в надземній масі. Кількість токсичних елементів у рослинах не перевищувала гранично допус тимих концентрацій для рослинної сировини та продук тів харчування. Висновки. Визначено вміст основних макро та мікроелементів у ґрунті та рослинах A. annua, A. ludoviciana й A. austriaca за умов інтродукції в НБС імені М. М. Гришка, а також особливості накопичення елементів окремими органами рослин під час їх транс портування з ґрунту в надземну частину. Встановлено схильність рослин *A. ludoviciana* до накопичення висо ких концентрацій заліза в коренях і надземній частині, а також можливість накопичення Ni та Se рослинами A. annua. Результати досліджень будуть корисними для прогнозування й оцінки інтродукції нових перспектив них видів роду Artemisia, селекції у процесі створення нових сортів полинів, визначення їхніх фармакологіч них властивостей і прийняття рішень щодо доцільності їх використання у чаях та харчових продуктах.

Ключові слова: інтродукція; Artemisia annua; A. austriaca; A. ludoviciana; мікроелементи; макроелемен ти; фармакогнозія; фіточай.

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