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SPECIFICITIES OF CHANGES IN THE CONCENTRATIONS OF HEAVY METALS IN MILK THISTLE (*SILYBUM MARIANUM* (L.) GAERTN.)

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Aim. To investigate the content of heavy metals in the seeds of milk thistle *(Silybum marianum* (L.) Gaertn.*)* and in the product of its processing – oil. To reveal possible changes in the concentration of heavy metals in the years of cultivating this plant. **Methods.** The field and spectrometric methods were applied; the results were processed using the technologies, common for agriculture, plant production and statistics. **Results.** The content of heavy metals in the seeds of milk thistle was defined. It was determined that their concentration exceeds maximal acceptable coefficient for the medicinal plant material. It was established that in 2013 the seeds of milk thistle accumulated several times more Ba, Ca, Fe, Mg, Mo, Sr, V compared to the indices of 2014, while Al, Co, Ag were found only in the samples of the seeds, harvested in 2013. **Conclusions.** As the regulatory documents regarding many relevant elements, which belong to heavy metals, do not specify maximal acceptable coefficients for the content of the latter in the medicinal plant material, it is reasonable to conduct complex studies, involving the specialists of relevant fields, in order to stipulate this gradation. In the Kyiv region the milk thistle plants accumulate a large amount of such heavy metals as cadmium, manganese, strontium, and zinc, which exceeds maximal acceptable degrees of accumulation and testifies to partial pollution of region soils. It was established that the concentration of the main part of heavy metals in the product of processing milk thistle – oil – is decreased to the value under the threshold of analytic devices. This fact proves the prospects of growing milk thistle for industrial processing purposes.

Keywords: milk thistle, heavy metals, biomaterial.

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INTRODUCTION

The role of chemical elements with the valence of two and more is important for the living organism, as they ensure the activity of the enzymes, in particular, proteins – the catalysts of biochemical reactions. Small amounts of these elements are required for plants and all the living beings. At the same time the increased concentration of such elements is harmful for living organisms. Therefore, the study of the biological effect of heavy metals is presently an urgent trend of modern science.

Trophic chains promote the increase in the concentration and accumulation of heavy metals (HM) in the consuming organisms which has negative effect both on animals and people. The economic activity of humans leads to environment contamination with heavy metals and undesired chemical substances. For instance, the wastes of energy and transport systems are a considerable source of such heavy metals as Pb, Mn, Fe and others, as well as such substances as benzopyrene, oxides of nitrogen and carbon, *etc.*, entering the atmosphere.

The monitoring of the level of HM concentration in the environment and especially in the food products and feeds is of great relevance, as it allows taking timely measures to preserve the purity of environment and health of people. It is also very important to have information about the content of HM in biomaterial and other components of herbal medicines.

The aim of the studies was to determine the content of HM in the seeds of milk thistle *(S. marianum* (L.) Gaertn.) and the product of its processing – oil, as well as to reveal possible changes in their concentration in the years of cultivating *S. marianum* plants to elaborate corresponding sanitary and hygienic and economic-organizational measures to provide the required level of ecologic safety and reasonable use of the yield, harvested from this plant.

MATERIALS AND METHODS

The studies were conducted in the Institute of Horticulture of NAAS and the Institute of Physiology and Genetics, NAS of Ukraine in 2012–2014. Field experiments were conducted in the conditions of the permanent study area at the Laboratory of ornamental, medicinal and essential-oil plants of the Institute of Horticulture of NAAS. The scheme of the experiment presupposed crop rotation in a short-term field rotation and included the following variants: purple echinacea (*Echinacea purpurea*)–wild chamomile (*Matricaria recutita*)–clary sage (*Salvia sclarea* L.). The cultivation of these crops was planned without any fertilizers or pesticides. The experiments were conducted on four-rowed 2-meter-long fields. 40 seeds were sown manually in each row at the distance of about 5 cm. Crop protection involved manual weeding the rows as required.

The composition of soils was analyzed in the Laboratory of Agrochemistry, the Institute of Horticulture of NAAS. The content of humus in the arable layer (0−40 cm) was 2.3 %, easily hydrolyzed nitrogen (according to Turin and Kononova) − 78.4−98.0 mg/kg, mobile forms of phosphorus (according to Kirsanov) – 93.2−180.9 mg/kg, exchange potassium (according to Kirsanov) – 106.1−202.8 mg/kg. The reaction of their soil solution is acid (pH – 5.3–5.8 and 5.5–6.1, respectively).

The content of HM in the raw material, seeds and oil was studied in the Institute of Physiology and Genetics, NAS of Ukraine. The composition of elements in the test samples was defined by ICP-MS method using the emission mass-spectrometer Agilent 7700×. The samples were dried until the dry mass and reduced to ashes in hydrogen nitrate (ACS) using the microwave sample preparation system Milestone Start D. The extract obtained was brought up to 50 ml with water of class 1 (18 multiples of median), purified at Scholar-UV NexUp 1000 system (Human Corporation, Korea).

The results of experiments were statistically processed by standard methods [1] using, the professional software package for statistical analysis Statistica 8.0.

RESULTS AND DISCUSSION

Milk thistle (*S. marianum* (L.) Gaertn.) has been used in non-conventional and traditional medicine for over a thousand years to treat various diseases. Therefore, it is important to know the compliance of the raw material of this plant with the requirements to its application and processing for medicine. The studies were aimed at the determination of the composition of chemical elements in the objects and the definition of the specificities of their accumulation in conditions of growing in the Kyiv region.

The results of the analysis of HM content in the seeds of *S. marianum* for two years of its cultivation (2013−2014) and in the product of its processing – oil, according to [2], are presented in the Table.

The analysis of the Table data demonstrates that during two vegetative periods there are on average 1,983.75 mg/kg of magnesium compounds in the milk thistle seeds; they are 185.74 mg/kg less in 2014 compared to 2013, and as for the oil of harvest 2014, the concentration of these compounds is 3.08 mg/kg.

Magnesium belongs to alkali metals. Its compounds participate in the photosynthesis processes, play a relevant role in the activation of enzymes, ensuring the consumption of phosphorus compounds by plants. The deficiency of magnesium in the soil inhibits the accumulation of sucrose in plants and leads to delayed blossoming of plants and the destruction of chlorophyll in leaves due to which they wither soon [3]. Normal conditions of magnesium nutrition are created on condition of systematic application of organic fertilizers, magnesium and lime materials.

Taking into consideration the fact that there has been no defined maximal acceptable coefficient (MAC) for magnesium in the herbal medicines, and the daily requirement of this element for an adult person is about 300–400 mg, it is evident that its quantitative content in the seeds and oil of milk thistle is insignificant, as the experiments were conducted without any fertilizers.

It was revealed that the concentration of certain chemical elements in herbal medicines in general and in milk thistle plants in particular, growing on the soil of the Kyiv region, exceeds the indices for MAC concentration, defined for raw material to be processed.

There is an excess in the concentration of cadmium compounds by 0.09 mg/kg in the milk thistle seeds on average for two years of cultivation, and as for the product of its processing – oil, the content of cadmium was in the MAC range (0.01 mg/kg below the norm).

Cadmium, one of the most toxic heavy metals, is referred to the second class of hazard – "highly hazardous substances". Similar to many other HM, it tends to accumulate in the organism, and its effective half-life is 10–15 years. By the age of 50 a person may have 30–50 mg of cadmium in the organism [4].

The concentration of iron compounds in the seeds exceeds MAC considerably – by 24.76 mg/kg on average for two years of cultivation, and during 2013 their accumulated content exceeded that for 2014 by about 5 mg/kg. After the thermal processing of the raw ma-

The accumulation of heavy metals (HM) and other elements by milk thistle plants

Metal	HM concentration, mg/kg				MAC* of HM in the productive part of plants, mg/kg
	In the seeds			In oil yield of 2014	
	2013	2014	Average for two years		
Aluminum	3.68 ± 0.002	< 0.00	3.68 ± 0.002	< 0.00	20
Barium	3.18 ± 0.004	2.53 ± 0.008	2.86 ± 0.006	0.060 ± 0.003	0.7
Beryllium	< 0.00	< 0.00	< 0.00	< 0.00	n.i.**
Bismuth	< 0.00	< 0.00	< 0.00	< 0.00	n.i.
Cadmium	0.10 ± 0.002	0.13 ± 0.007	0.12 ± 0.005	0.02 ± 0.006	0.03
Calcium	1284.19 ± 0.02	1262.98 ± 0.005	1273.59 ± 0.004	< 0.00	n.i.
Cesium	0.0003 ± 0.12	0.0014 ± 0.031	0.0009 ± 0.076	< 0.00	n.i.
Chromium	0.034 ± 0.005	0.004 ± 0.23	0.019 ± 0.118	0.020 ± 0.094	0.2
Cobalt	0.018 ± 0.009	< 0.00	0.018 ± 0.009	< 0.00	0.1
Copper	6.70 ± 0.003	8.94 ± 0.004	7.82 ± 0.004	< 0.00	1.0
Iron	27.52 ± 0.003	22.59 ± 0.004	25.06 ± 0.004	< 0.00	0.3
Lead	< 0.00	< 0.00	< 0.00	< 0.00	0.3
Lithium	< 0.00	< 0.00	< 0.00	< 0.00	n.i.
Magnesium	2076.62 ± 0.4	1890.88 ± 0.3	1983.75 ± 0.4	3.08 ± 0.014	n.i.
Manganese	11.52 ± 0.4	11.99 ± 0.002	11.76 ± 0.02	< 0.00	2.1
Molybdenum	0.14 ± 0.2	0.11 ± 0.009	0.125 ± 0.006	< 0.00	0.12
Nickel	< 0.00	< 0.00	< 0.00	< 0.00	0.5
Potassium	2637.11 ± 0.1	2984.73 ± 0.9	2810.92 ± 0.5	10.73 ± 0.03	n.i.
Rubidium	1.43 ± 0.06	2.78 ± 0.03	2.11 ± 0.005	0.05 ± 0.124	n.i.
Silver	0.003 ± 0.03	< 0.00	0.003 ± 0.003	< 0.00	n.i.
Sodium	148.44 ± 0.22	143.00 ± 0.22	145.50 ± 0.22	5.00 ± 0.06	–
Strontium	29.10 ± 0.1	9.77 ± 0.03	19.44 ± 0.02	< 0.00	1.0
Thallium	< 0.00	< 0.00	< 0.00	< 0.00	n.i.
Vanadium	0.062 ± 0.006	0.004 ± 0.23	0.033 ± 0.118	0.142 ± 0.009	n.i.
Zinc	19.35 ± 0.3	25.12 ± 0.2	22.24 ± 0.03	< 0.00	10.0

Note. *Maximum acceptable content; ** no information.

terial for oil, the concentration of this element decreased to the value, smaller that the threshold of analytic devices.

In the living organisms iron is an important microelement, catalyzing the processes of oxygen exchange (breathing). The main intracellular depot of iron is globular protein complex – ferritin. Iron deficiency manifests itself as a disease: chlorosis for plants and anemia for animals; its excessive accumulation causes toxic effect. The overdosing with iron stimulates the production of free radicals, inhibits the antioxidant system of the organism and is likely to promote the development of atherosclerosis [5].

The content of manganese in the test plants exceeds MAC by over 17 %. Manganese is one of the most active microelement which occurs almost in all the plants and living organisms. It improves the processes of blood formation. However, the excess of its compounds leads to the toxic effect on the human organism. In case of significant poisoning with this element there is nervous system impairment with a remarkable manganese parkinsonism syndrome.

The concentration of strontium compounds in the samples of milk thistle seeds, selected in 2013, exceeded maximum acceptable levels more than 29-fold, and on average for two years of cultivation – 19-fold, but these compounds were not found in oil – the product of processing seeds. It is likely that under the impact of cold pressing (centrifugation) the strontium compounds reacted to produce an unreadable form and thus did not penetrate oil (Table).

Strontium enters plant organisms from soil, and those of animals and humans – from food and water, *etc*. Soluble compounds of strontium are easily absorbed in the intestines (10–60 %), but the figure for low soluble ones is smaller – about 1 %. Strontium is accumulated in the bone tissue of the organism of animals and humans. Thus, it is an osteotropic element [6].

The value of strontium microelement in the activity of animals and plants is low. However, it is always present in the organism as a constant support and partial replacement for cadmium. The increased content of strontium in the human organism leads to bone tissue impairment, the increase in bone fragility and fast dental decay. The liver and blood are next targets.

Zinc compounds in plants activate the effect of a number of enzymes, are a part of enzymatic systems, which participate in breathing, the synthesis of proteins and auxins, enhance the resistance of plants to stresses, and play a relevant role in regulating growth processes. The relevance of zinc for the growth of plants is closely related to its participation in the nitrogen exchange. Due to the effect of zinc there is improved synthesis of sugars and starch, the increase in the total content of carbohydrates, protein substances, ascorbic acid and chlorophyll. Zinc excess in the nutrition of plants is rather rare. High content of zinc in soil decreases the ability of plants to consume copper compounds.

However, high concentration of zinc compounds was found in the test samples of milk thistle seeds, especially in the seeds, harvested in 2014. On average for two years of cultivation the content of zinc exceeded maximum acceptable levels by about 45 %, whereas it was not found in the product of processing, oil.

Such elements as aluminum, cobalt, silver were found within the range of maximum acceptable concentration in the seeds, harvested in 2013, and their concentration in the seeds, grown in 2014, decreased to the value below the threshold of analytic devices.

The results of the analysis demonstrate that chemical compounds of barium, calcium, iron, magnesium, molybdenum, strontium, vanadium were accumulated in the seeds, harvested in 2013, in much higher concentrations compared to 2014, exceeding the maximum acceptable coefficient (if the information about MAC for them was available). It can be explained by the fact that the predecessor of milk thistle – plants of purple echinacea (*E. purpurea*) purified soil, having absorbed chemical compounds during their vegetation period.

The results of analysis of oil, made of milk thistle seeds, allow the assumption that the processing of raw material has considerable effect on HM concentration in the final product – the former mainly remain in the press cake.

Therefore, HM are natural components of the Earth crust, and are usually present in all the ecological matrices. Nonetheless, HM concentration in some ecosystems increased several times due to anthropogenic activity. The contamination of environment with heavy metals is getting global, setting even higher hazard (mainly due to toxicological risks) for human health [7]. At the same time heavy metals are required components of all the living organisms, they manifest their toxicity only being in high concentrations.

Therefore, the soils of Kyiv region are quite suitable for the cultivation of medicinal plants, as HM concentration, accumulated by them during their vegetation, is generally within the limits of the maximum acceptable level. The number of elements, exceeding the mentioned concentration, should be regulated with agroecological methods, which should be improved and developed.

CONCLUSIONS

As the regulatory documents regarding many relevant elements, which belong to heavy metals, do not specify maximal acceptable levels for the content of the latter in the herbal medicines, it is reasonable to conduct complex studies, involving the specialists of relevant fields, in order to stipulate this gradation.

In the Kyiv region the milk thistle plants accumulate large amounts of such heavy metals as cadmium, manganese, strontium, and zinc, which exceed maximal acceptable level; it testifies to partial contamination of region soils.

The studies revealed that the concentration of most heavy metals decreases in the product of processing of milk thistle – oil – down to the value below the threshold of analytic devices. It testifies to the prospects of cultivating the mentioned herbal medicines in the Kyiv region for industrial processing purposes.

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Особливості змін концентрацій важких металів у розторопші плямистої (*Silybum marianum* (L.) Gaertn.)

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Мета. Дослідити вміст важких металів у насінні розторопші плямистої (*Silybum marianum* (L.) Gaertn.) та в продукті її переробки – олії. Виявити можливі зміни концентрації важких металів за роками вирощування культури. **Методи.** Використано польовий і спектрометричний методи, результати обробляли загальноприйнятими методиками у землеробстві, рослинництві та статистиці. **Результати.** Визначено вміст важких металів у насінні розторопші плямистої. Виявлено, що їхня концентрація перевищує гранично допустимий коефіцієнт для сировини лікарських рослин. Встановлено, що у 2013 р. у насінні розторопші накопичилось у декілька разів більше Ba, Ca, Fe, Mg, Mo, Sr, V порівняно з показниками 2014 р., а Al, Co, Ag виявлено лише у зразках насіння врожаю 2013 року. **Висновки.** Оскільки у нормативно-правових документах для багатьох важливих елементів, які належать до важких металів, відсутні гранично допустимі коефіцієнти для їхнього вмісту в біосировині лікарських рослин, то актуальним є проведення комплексних досліджень із залученням фахівців відповідних профілів для встановлення подібної градації. Рослини розторопші плямистої за умов Київської області у великій кількості акумулюють такі важкі метали, як кадмій, залізо, марганець, стронцій, цинк, що перевищує гранично допустимі рівні накопичення і свідчить про часткове забруднення ґрунтів регіону. Встановлено, що в продукті переробки розторопші плямистої – олії − концентрація основної частини важких металів знижується до величини, меншої від порогу чутливості аналітичних приладів. Це підтверджує перспективність вирощування розторопші плямистої для промислової переробки.

Ключові слова: розторопша плямиста, важкі метали, біосировина.

Особенности изменения концентраций тяжелых металлов у расторопши пятнистой (*Silybum marianum* (L.) Gaertn.)

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Цель. Исследовать содержание тяжелых металлов в семенах расторопши пятнистой (*Silybum marianum* (L.) Gaertn.) и в продукте ее переработки − масле. Выявить возможные изменения концентрации тяжелых металлов по годам выращивания культуры. **Методы.** Использованы полевой и спектрометрический методы, результаты обрабатывали с помощью методик, общепринятых в земледелии, растениеводстве и статистике. **Результаты.** Определено содержание тяжелых металлов в семенах расторопши пятнистой. Выявлено, что их концентрация превышает предельно допустимый коэффициент для сырья лекарственных растений. Установлено, что в 2013 году в семенах расторопши накопилось в несколько раз больше Ba, Ca, Fe, Mg, Mo, Sr, V по сравнению с показателями 2014 года, а Al, Co, Ag обнаружены в образцах семян только урожая 2013 года. **Выводы.** Поскольку в нормативно-правовых документах для многих важных элементов, относящихся к тяжелым металлам, отсутствуют пре-

дельно допустимые коэффициенты их содержания в биосырье лекарственных растений, то актуальным является проведение комплексных исследований с привлечением специалистов соответствующих профилей для установления подобной градации. Растения расторопши пятнистой в условиях Киевской области в большом количестве аккумулируют такие тяжелые металлы, как кадмий, железо, марганец, стронций, цинк, что превышает предельно допустимые уровни и свидетельствует о частичном загрязнения почв региона. В результате исследований установлено, что в продукте переработки расторопши пятнистой – масле − концентрация основной части тяжелых металлов снижается до величины, меньшей порога чувствительности аналитических приборов. Это подтверждает перспективность выращивания расторопши пятнистой для промышленной переработки.

Ключевые слова: расторопша пятнистая, тяжелые металлы, биосырье.

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