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Development Of Zinc-Enriched Medicinal And Food Plants

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ABSTRACT

The decrease in the content of heavy isotopes of hydrogen (D) and oxygen (^{17}O, ^{18}O) in water is accompanied by a change in metabolic processes in plant and animal organisms, which is explained by the isotopic kinetic effect. This phenomenon underlies the technology of accumulation of microelements in medicinal and non-medicinal plants used for soft correction of hypoelementoses. The new technology includes isotope management of plant development; new laser methods for end-to-end quality control of aqueous solutions for irrigation and hydroponics of plants; a set of methods for end-to-end control of plant raw material enrichment in a microelement; online control of biotoxicity of plant raw materials and medicines made from them. Thus, in our work, the possibility of the development of metal-modified plants with zinc content of 1.4 mg/g dry basis is shown.

Keywords: Zinc-deficient conditions, kinetic isotope effect, medicinal and food plants.

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INTRODUCTION

Zinc-deficient conditions of the urban population lead to a variety of disorders, including impaired functioning of the immune system, neurological diseases, gynecological diseases and sexual disorders[1]. Zinc-deficient conditions are caused by a combination of malnutrition (including alcohol abuse) and air and water pollution in urban environments[2]. An outbreak of zinc-deficient conditions is due to zinc metabolism, showing close values of daily intake and excretion of this element, and the normal zinc balance is close to zero, especially in men[3].

Zinc deficiency impairs the physiological processes in the human body, including suppressing the development of the musculoskeletal system, skin lesions and impaired wound healing[4]. Mild deficiency without laboratory confirmation may cause non-specific consequences, such as susceptibility to infection and growth retardation[5].

Acute zinc deficiency causes a decrease and (or) weakening of innate and acquired immunity, and chronic deficiency enhances inflammatory processes[6]. In chronic zinc deficiency, the production of pro-inflammatory cytokines increases, which affects the course of a large number of inflammatory diseases, including rheumatoid arthritis[4]. A relative zinc deficiency was observed in patients with malabsorption syndrome, liver disease, chronic renal failure, sickle cell anemia and other chronic diseases[7]. The main clinical problems caused by zinc deficiency in humans include growth retardation; cell-mediated immune dysfunction and cognitive impairment[8]. FDA (USA) has obligated to include zinc in the programs of total parenteral nutrition[1,5,9].

The need for correction of zinc deficiency is not immediately apparent during a physical examination, since a comprehensive analysis of the content of microelements has not found widespread use in the practice of physicians and family doctors[10]. In many countries, diagnosis and correction of zinc-deficient conditions is affordable for a limited population only[11]. In addition, the use of zinc-containing drugs is always accompanied by the threat of allergic reactions due to the presence of by-products of chemical synthesis, chelating agents and individual intolerance to complexon substances that provide bioavailability of zinc ions when taken orally[12].

To solve the problem of correcting zinc deficiency, various approaches can be implemented. The first approach is a balanced diet that takes into account the increase in zinc intake with food[2]. Such a nutritional approach is impracticable on a mass scale because of high financial costs of controlling the digestible forms of zinc in a specific diet menu. In addition, it is impossible to cure systemic zinc deficiency by the nutritional way[2, 3]. Another way is the intake of synthesized zinc complexes with organic ligands, which are strictly necessary, since the intake of dissolved inorganic zinc salts is accompanied by hydrolysis and emetic effect[3]. Actually, zinc sulfate ($ZnSO_4$) when taken orally, is an old officinal emetic. However, zinc chelate compounds are always chemically synthesized products with side effects caused by both chelate agents and impurities in the form of by-products of the organic synthesis[13].

This work considers a new option for obtaining a zinc-enriched preparation – non-toxic, free from synthetic organic substances. For its implementation, it is necessary to grow medicinal and non-medicinal zinc-enriched plants[14]. The increase in the zinc content in a plant compared to the standard content for species, which is determined by biogeochemical factors, has some limitations both in terms of the Nernst's Distribution law and because of the limit values of the natural accumulating capacity of plants due to the concentration of dissolved chelate agents and their sorption at phase interfaces.

It is possible to increase the assimilation capacity of a plant by changing the isotopic composition of the aqueous solution used for irrigation or used in hydroponics[15]. The innovativeness of the developed approach is as follows. Instead of conventional aqueous nutrient solutions, it is necessary to use a new liquid which has never been described before – water depleted in the content of heavy isotopes, which differs from normal water by a number of physical and chemical parameters, including freezing temperature, proton spin-spin relaxation time, self-diffusion coefficient[16,17]. The reaction of living systems to the depletion of the aqueous solution in the content of deuterium (D) and oxygen isotopes (^{17}O, ^{18}O) is accompanied by a change in metabolism[18-21]. One of the most significant consequences of such a change in the isotopic composition of water is a change in the constants of absorption/clearance rates of solutes[22]. This is the effect that allows overcoming thermodynamic limitations under nonequilibrium conditions by increasing the saturation of the medicinal plant with zinc compounds up to the content of 5 mg Zn per 1 g dry basis[15].

The purpose of the article is to consider and present the development of the fundamentals of the technology for producing new pharmaceutical products used for the correction of hypoelementoses.

ZINC CONTENT IN PLANT MATERIALS

It is known[23] that the concentration of chemical elements in plants can vary depending on the environment, taxonomic position, as well as the stage of the organism development. However, there is no proportional relationship between the content of elements in the Earth's crust and their absorption by plants. Therefore, concentrations in samples can be determined only using statistical laws[24]. The "geochemical selection" of elements is the most important factor in the formation of chelate complexes and strong organic compounds[25]. The range of zinc content in plants is 10-200 mg/kg. When zinc content in plants is 300-500 mg/kg dry matter, signs of their toxicity appear[26-28].

The content of certain microelements of medicinal and non-medicinal plants is a purely species characteristics[29]. In particular, the contents of zinc in plants of various families are different. For the herb of common nettle (herba *Urticae dioicae*, *Urticaceae* family) and the leaves of Gumweed (folia *Grindeliae robustae*, sunflower family (*Asteraceae*)) zinc content determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) is about 100 mg/kg, and the content of zinc in the herb of medicinal lavender (herba *Lavandulae officinalis*, mint family (*Lamiaceae*)) is only about 7 mg/kg (Figure 1)[30].

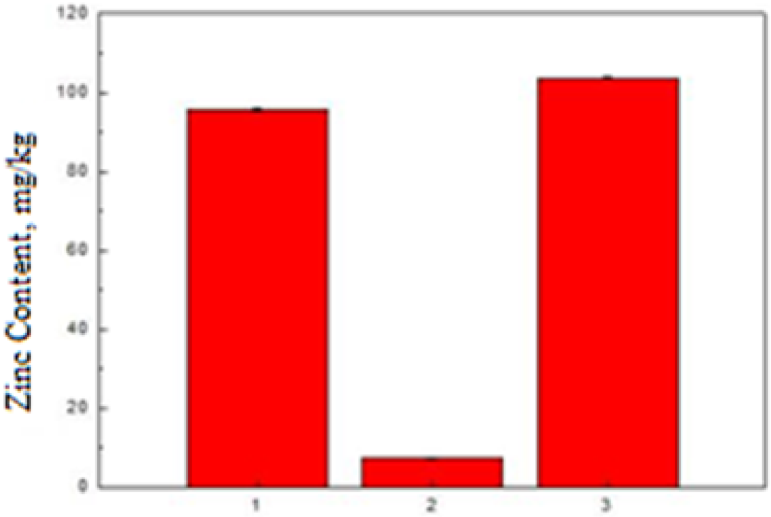

Figure 1: Zinc content in various medicinal plants growing in Transylvania. 1 – common nettle (*Urtica dioica*), 2 – gumweed (*Grindelia robusta*), 3 – medicinal lavender (*Lavandula officinalis*).

The microelement composition has not only interspecies differences, but can also accumulate unequally in different parts of the same plant. The phase of plant vegetation is also important. Example: different parts of common chicory (*Cichorium intybus*, dandelion family (*Asteraceae*)) (Table 1)[31].

Table 1. Zinc content in different parts of the plant based on the example of common chicory (*Cichorium intybus*)[31].

Part of the Plant	Zinc Content, mg/kg dry basis
Young Leaves	50.07±0.36
Young Shoots	33.59±0.17
Roots	34.48±0.35
Grass	57.29±0.54
Flowers	42.00±0.44
Leaves	97.71±0.62

The zinc content in the leaves of shrubby cinquefoil (*Pentaphylloides fruticosa L.*) determined by atomic absorption spectrometry (AAS) is presented in the range from 27 to 83 mg/kg depending on the specific location[26]. The zinc content in plants of the same family can be approximately the same, for example, in the aerial part of medicinal chamomile is 28.2 mg/kg dry matter and 30 mg/kg in the raw material of wild chamomile (AAS method)[32].

The State Pharmacopoeia of the Russian Federation currently contains particular articles on more than 100 plants[33]. The list of domestic medicinal plants significantly reduces the possibility of creating effective herbal medicines produced by the industry. In this regard, the search and practical application of new medicinal and non-medicinal plant materials is an urgent task[34]. There are many examples of commonly used non-medicinal plants, such as fireweed (*Chamaenerion angustifolium L.*), willowherb family (*Onagraceae*). The fireweed contains about 26 vital microelements involved in redox processes, hematopoiesis and vitamin activity in the human body[35]. Fermented tea from fireweed, unlike black tea, is not addictive and does not stain tooth enamel[36]. It grows mainly in North America, in the midland of the European part of Russia, in the Caucasus and the Far East[37]. In[38], the ICP-AES method was used to determine the elemental profile of fireweed (*Chamerion angustifolium L.*) growing in three regions of the Krasnoyarsk Territory. Table 2 shows that the zinc content varies depending on the specific growing region and part of the plant.

Table 2. The zinc content in leaves, stems, and flowers of fireweed from different growth areas in the Krasnoyarsk Territory (mg/kg dry raw materials) [38].

Part of the Plant	**Region of Growth**		
	Sayany Area	**North-Yenisey Area**	**Yemelianovo Area**
Leaves	56.93	57.91	84.89
Stems	34.02	43.83	79.99
Flowers	8.30	9.17	13.09

METHODS FOR DETERMINING Zn IN SAMPLES WITH A COMPLEX MATRIX

The selection of the analysis method is the main task in determining the content of chemical elements in a sample[39]. Several stages precede the determination of microelements in samples: sample preparation, analytical procedures (measurement), statistical processing and interpretation of the results.

There are many methods for the qualitative and quantitative determination of elements in objects, including those with a complex organic matrix[40]: atomic absorption spectrometry (flame (AAS), with electrothermal atomization (ETAAS); optical emission spectrometry with inductively coupled plasma (ICP-OES) or plasma atomic emission spectrometry (ICP-AES); mass spectrometry with inductively coupled plasma (ICP-MS); X-ray fluorescence spectrometry (XRF); flame photometry; neutron activation analysis; gamma resonance (Mössbauer) spectrometry; spectrophotometric method; electrochemical methods (inversion voltammetry, ionometry, polarography; chromatographic methods (HPLC, HPLC-MS system, etc.) and other methods[41].

Analysis methods must comply with the following validation parameters: selectivity, detection limit, as well as high information content and reliability of the obtained results that can provide proper metrological characteristics. Modern instrumental methods suitable for analyzing low concentrations of the elemental composition of materials (for example, ICP-OES, ICP-MS) with appropriate sample preparation give acceptable metrological parameters, including accuracy and precision also in cases of complex multicomponent composition of the organic sample matrix[42]. For zinc determination all of the above methods can be used. The main methods for quantitative determination of the analyzed element are: 1. Calibration Graph Method. 2. Addition Method. 3. Bracketing Technique[43]. Since the d-orbital ($3d^{10}$ $4s^0$) of zinc is filled, its content in biological objects is most often determined by the AAS method[13,43]. In the atomic absorption spectrometry, when analyzing the content of an element to be determined, a calibration curve constructed using standard samples is commonly used.

The correct choice of a method requires understanding of its basic principles, capabilities and limitations, as well as knowledge of the requirements for the analysis to be done: the required sensitivity of element determination, the working range of the concentrations to be determined, the number of samples to be analyzed, the quality of the data obtained, etc.[41]. It should be noted that ETAAS is characterized by a lower speed of analysis compared to AAS. In addition, the list of elements determined in the graphite furnace is somewhat shorter than in the case of atomic absorption in flame. However, a better sensitivity of the ETAAS method and small sample volumes used for the implementation of this method significantly expand the capabilities of the atomic absorption.

The most important criteria for selecting analysis method are: detection limit; analytical interference; performance; expenses; simplicity of operation; quality of results; working range of analytical concentrations; availability of proven techniques.

The detection limits (Table 3)[44] of specific elements determine the effectiveness of a particular method. In some cases, pre-concentration of the test sample may be required.

Table 3. Element detection limits by methods of spectral analysis[44].

AAS, μg/l	**ETASS, μg/l**	**ICP-OES, μg/l**	**ICP-MS, μg/l**	**XRF, ppm**
1.5	0.02	0.2	0.0003	0.1

THE TECHNIQUE OF OBTAINING ZINC-ENRICHED MEDICINAL AND FOOD PLANTS

The technique for obtaining zinc-enriched medicinal and spice/food plants consists in the following key elements. The first stage of the technique is the selection of solvent – water depleted in the content of deuterium and oxygen isotopes (^{17}O and ^{18}O)[14,15,45]. The second stage is the preparation of zinc salt solution with a chelate agent. For effective enrichment of the plant and avoiding suppression of its growth it is critical to determine the content of the zinc ion complex with an organic ligand at a given pH, the concentration of free zinc ions (aquacomplexes) taking into account the dissociation constant of the chelate complex. The third stage of the technique includes selecting food/medicinal plant, selecting the duration of incubation with chelated zinc compound in water, selecting exposure conditions (irrigation or hydroponics) under given lighting conditions. The key point for the successful implementation of the technique is the quality control of water, final zinc-containing solution, the biogeochemical control of medicinal plant materials at all stages of zinc enrichment and subsequent preparation for the user. The selection of a medicinal plant is also critical in terms of bioavailability of zinc extracted from a zinc-enriched plant[46–50].

As a model of a plant that accumulates the Zn microelement, a medicinal plant – basket plant (*Callisiae fragrans*) and a vegetable plant – bell pepper (*Capsicum annuum L.*) are used[51–55].

The SLAP standard characterizes the lightest natural water on Earth: the concentration of deuterium D/H in this water is 89 ppm, oxygen-18 $^{18}O/^{16}O$ – 1894 ppm. It is shown that deuterium-depleted water has pronounced properties of plant growth stimulator[56]. It accelerates metabolic processes – both physical-chemical and biological, which leads to various physiological effects[21,22].

Therefore, in studies of metal-modified plants, water with different D/H ratios is used to obtain zinc-modified plants, for example, water with a low deuterium content (D/H <90 ppm)[14,15].

The quantitative and volume particle size distribution for an ensemble of particles (size spectra) is recorded by the method of low-angle laser light scattering (LALLS)[57]. For the first time, we have characterized the deuterium-dependent Tyndall effect for homogeneous solutions and for the detection of a low-angle indicatrix of laser radiation scattering from a single dispersed particle. We have also developed a counter for measuring particle diameters (Particle sizer)[45,58].

At the second and third stages of the technique, the content of elements in solutions and medicinal plant materials is controlled before/after enrichment and before/after preparation for the final dosage form (AAS with graphite atomization and XRF) (Table 4).

Table 4. Zinc content (X ±Sr) in the fruits and seeds of various plants. (n=3, P=0.95)

Type of plant raw material	**Zinc Content, µg/g**	
	XRF	**AAS**
Seeds of Dill (*semina Anethi graveolentis*)	68.0±0.6	74±15
Peeled Seeds of Common Pumpkin (*semina Cucurbitae pepo*)	160.0±0.8	101±20
Seeds of Field Mint (*semina Menthae aravensis* L.)	62.0±0.1	67±13
Seeds of Field Mint (semina *Menthae arvensis* L.)	57.0±0.6	63±13

The solvent, solutions of zinc chelates and aqueous extracts of plant materials before and after zinc enrichment are checked for biotoxicity. For this purpose, a unicellular biosensor based on free-living infusoria (*Spirostomum ambiguum*) has been developed[59].

As an example of the technique application, let us consider the results of basket plant enrichment in an essential element – zinc. Having selected water with an optimal ratio of hydrogen isotopologues that provides the required isotopic kinetic effect, we estimated its particle size distribution by low-angle laser light scattering (LALLS) and/or using our own development – the counter for measuring particle diameters (Particle sizer). The solvent and zinc-glycinate solution were characterized by a bioassay method – Spirotox. As a result, the toxicity of the samples was not identified[15].

At the first stage, we analyzed the leaves of the plant, the shoots of which had been exposed to deionized water without zinc. The leaves selected for the analysis were soft dried and homogenized (crushed) in an agate mortar[45]. The content of the essential element in the homogenizate samples (AAS and XRF methods using the IAEA reference sample) did not exceed 0.02 µg/g. Then, the shoots of the basket plant were incubated in solutions of zinc (II) glycinate – *bis(glycinato-N,O)zinc* – in deionized water and in deuterium-depleted water. Twelve days later, it was found that the zinc content in the leaves of the plant increased by an order of magnitude in the water with a natural ratio of isotopes (([D]/[H] = 142 ppm) and by two orders in DDW ([D]/[H] = 12 ppm). Moreover, the accumulation factors of zinc in the leaves of the plant for two types of water were$(\bar{x} \pm s_r)$: (40 ± 10) and (160 ± 10), respectively, Table 5.

Table 5. Coefficient of Zinc accumulation in *Callisia fragrantis* L. leaves after 12 days root incubation in zinc glycinate solutions in deionized water (DW) ([D]/[H] = 142 ppm) and in deuterium depleted water (DDW) ([D]/[H] = 12 ppm)

Element	**Accumulation Factor**	
	DW	**DDW**
Zn	40 ± 10	160 ± 10
Mn	1 ± 0.3	0.7 ± 0.2

Thus, a change in the isotopic composition of water led to the production of a modified plant that accumulates the essential element. The obtained zinc-enriched plant material can be considered as an analogue of synthetic zinc medicines.

CONCLUSION

The development of metal-enriched medicinal plants is an important step in pharmacy. In this work, innovative developments were considered and combined for the first time in order to develop the fundamentals of the technique for saturating a medicinal/food plant with an essential microelement: isotope-based control of plant development; new laser methods for end-to-end quality control of aqueous solutions for irrigation and hydroponics of plants; a new set of methods for end-to-end control of plant raw material enrichment in Zn microelement; online control of biotoxicity of plant raw materials and medicines made from them.

Zinc-enriched plant material, being a natural analogue of artificial mixtures of zinc-containing active pharmaceutical preparations and auxiliary substances, will allow for a soft correction of hypoelementoses without causing unwanted reactions of the human body.

CONTRIBUTIONS OF AUTHORS

All the author has contributed equally.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

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