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ENRICHMENT OF EDIBLE MUSHROOM BIOMASS WITH COMPOUNDS OF GERMANIUM, SELENIUM, AND MOLYBDENUM

Introduction. *Today, it is well known that mushrooms (pileated fungi, macromycetes) are not only a traditional food, but also an inexhaustible source of substances that have a wide range of pharmacological applications. Some species of mushrooms are considered a source of physiologically important, so-called essential elements, such as Cu, Fe, Zn, Cr, Se, Mo, Mn, etc.*

Problem Statement. *The biotechnological approaches aiming at enriching the essential elements of the mineral composition of mushroom biomass that is cultivated on a liquid nutrient medium and the fruit bodies of valuable edible species cultivated in the surface culture are relevant and promising, given the mineral composition of macromycetes is rather specific and characterized by a certain selectivity of the accumulation of individual elements from soils / substrates (species-specificity of accumulation). Enrichment with essential elements potentially has to increase the medicinal properties, biological activity, and nutritional value of such mushroom supplements.*

Purpose. *To identify the bio-accumulative ability of Pleurotus ostreatus (Jacq.) P. Kumm. and P. eryngii (DC.) Quél. mycelial biomass with the compounds of Ge, Se, and Mo.*

Materials and Methods. *The content of Ge, Se, and Mo in the mycelial biomass of three strains of the Pleurotus genus has been studied by the inductively coupled plasma mass spectrometry (ICP-MS) method during cultivation on a liquid nutrient medium with the addition of germanium, selenium, and molybdenum compounds at a concentration of 10, 25, and 50 mg / l, respectively.*

Results. *All tested strains have shown a high bioaccumulative ability: for germanium, the coefficients of accumulation are within the range from two to three orders of magnitude (404—3577), for selenium, they vary from one to three orders of magnitude (19—2118), and for molybdenum, they range from one to two orders of magnitude (12—162).*

Conclusions. *The further development and implementation of mushroom supplements enriched with essential elements should include study of the bioavailability and efficacy of the preparations, as well as biomedical and clinical trials.*

Keywords: culinary-medicinal mushrooms, Pleurotus spp., bioaccumulation, and essential elements.

In the modern world, mushrooms are used in various ways that sometimes differ from the conventional ones. They are not only a traditional food product, but also an inexhaustible source of substances that have a wide range of applications in pharmacology. To date, over 130 therapeutic effects of higher fungi (including antitumor, hepatoprotective, antioxidant, antidiabetic, cardiovascular, antibacterial, antiviral, detoxifying, cholesterol-lowering, anti-obesity, anti-aging, and neuro-regenerating) have been described [1—3].

Some species of mushrooms are considered a source of physiologically important, so-called essential elements, such as Cu, Fe, Zn, Cr, Se, Mo, Mn and so on. Given the fact that the mineral composition of macromycetes is quite specific and there is a certain selectivity of the accumulation of individual elements from substrates (species specificity of accumulation), biotechnological developments aiming at enriching the mineral composition of the mushroom species cultivated on liquid medium and fruiting bodies at solid cultivation with essential elements are interesting and promising direction of research [4—9].

The purpose of the research was to determine the bioaccumulative capacity of mycelium biomass of valuable edible and medicinal mushroom species: *Pleurotus ostreatus (Jacq.) P. Kumm.* (Oyster mushroom) and *P. eryngii (DC.) Quél.* (King Oyster mushroom) in the case of adding compounds of selenium, molybdenum, and germanium to the culture medium during cultivation.

Pure cultures of *P. ostreatus* (strains 198 and 1796) and *P. eryngii* (strain 1863) from the collection of mushroom cultures of the Kholodny Institute of Botany of the NAS of Ukraine [10] were used for determining the levels of mineral element bioaccumulation. Mycelial discs having a diameter of 0.5 cm were transferred to a liquid sterile nutrient medium (yeast extract with addition of K_2SeO_4, $Na_2MoO_4 \cdot 2H_2O$, and GeO_2 at three concentrations of 10, 25, and 50 mg/l, and the reference medium without compounds added). The mycelial biomass was cultured without stirring in glass containers (200 ml) in a thermostat at a temperature of 25 ± 0.5 °C for 20 days, then separated from the culture medium. The experiment was repeated three times. The obtained mushroom biomass was dried at a temperature of 105 °C and analyzed by the inductively coupled plasma mass spectrometry technique using an ICP-MS analyzer *Element-2* (Germany), based on the method proposed by Ponomarenko with co-authors [11].

The used solutions and reagents (alkaline melts of Na_2O_2, $LiBO_2$ (ppm), concentrated HF, HCl, HNO_3, H_2SO_4, H_3PO_4 (ppm)) were further purified using the SUBBOILING system. Water with a resistance of 18.2 Mohm/cm was obtained using the DIRECT-03 system manufactured by *MILLIPORE* (USA). The samples were dissolved in a microwave (MX) oven *Ethos* manufactured by *Milestone* (Italy). The operating frequency of MX radiation was 2450 MHz, the maximum power was 1600 W. ^{115}Indium was used as internal standard, and standard samples of gabbro-essexite (SGD-1A; SGD-2) and gold ore tails (SGX-3) were used as external standard.

The morphological features of mycelial colonies were studied using light and scanning electron microscopes (SEM) by the method proposed by A.S. Bukhalo et al. [12]. The mycelium samples were fixed with OsO_4 vapor (1% solution) for 96 hours. Having been dried, they were coated with gold in a vacuum ion sprayer JEOL JFC-1100 (Japan) and studied with the use of SEM (JEOL JSM-6060 LA, Japan), at a magnification from × 100 to × 2000.

The concentrations of some macro- and microelements in the fruiting bodies of pileated fungi are known to significantly different from those of plants, which is caused, first of all, by differences in the nature of metabolism. In recent decades, publications in this field have highlighted the role of metal ions and individual rare earth elements in the physiology of macromycetes, the correlation between the ability to accumulate certain mineral elements, including toxic ones, and the species of fungi, the use of macromycetes as bio-indicators of anthropogenic contamination, and the unique bio-sorption properties that enables to consider the pileated mushrooms as sorbents, on the one hand, and, as an important source of essential and rare mineral elements for the human body, on the other hand [13—23].

The biological role of germanium (Ge) compounds is to enhance the processes of hematopoiesis in the bone marrow; they have antioxidant and antitumor effect.

Selenium (Se) is an essential element that, in addition to its pronounced antioxidant and antitumor effects, enhances immunity and promotes the proper functioning of the endocrine system. Some diseases, such as Keshan disease, thyroid dysfunction, and osteoarthritis are associated with selenium deficiency in the body. According to O.P. Perepelytsia [24], the optimal daily average dose of selenium for human ranges from 0.139 to 0.185 mg, while the daily intake of selenium at a dose of 1 mg/kg can cause chronic poisoning of human organism. In recent years, pharmacologists and biomedical specialists have focused their attention on this element. In terms of its physicochemical properties metalloid Se is similar to sulfur (S). Its biologically active form is represented by selenium-cysteine amino acid. Selenium is a part of the well-known multivitamin and multimineral supplement *Centrum* (*Pfizer*, USA) in at a rate of 55 mcg that makes up 79% of the daily dose for adults.

The biological role of molybdenum (Mo) is participation in metabolic processes, antioxidant effect, and the ability to accelerate the decomposition of purines and to remove uric acid from the body (the prevention of uratic arthritis). This metal is a part of many enzymes, participates in cellular respiration and synthesis of ascorbic acid, enhances the action of pituitary hormones, insulin, and prevents cholesterol deposition on the walls of vessels [24]. In general, given a high toxicity of germanium and selenium compounds, low concentrations of 10, 25, and 50 mg/l were used in the culture studies.

Preliminary study of the mineral composition of wild and cultivated species with valuable nutritional and medicinal properties is of great importance for determining the levels of possible enrichment of biomass with valuable macro- and microelements [25, 26]. In combination with a high content of vitamins, enzymes, and other biologically active substances, the enriched content of vital mineral elements should potentially enhance the pharmacological action of drug of a particular kind of medicinal macromycetes. The studies of elemental composition of 26 species of wild macromycetes from forest ecosystems with a preserved vegetation cover and a low anthropogenic load, collected in Kyiv, Zhytomyr, Volhynian, Rivne, Chernihiv, and Zakarpattia Oblasts have shown that not only fruit bodies of valuable edible and medicinal species *Boletus edulis* (up to 32 mg/kg of dry weight) and *Leccinum aurantiacum* (up to 24 mg/kg of dry weight) from different localities, but also the fruit bodies of *Macrolepiota procera* (up to 26 mg / kg of dry weight) and *Armillaria mellea* (up to 24 mg/kg of dry weight) have the highest concentrations of selenium. At the same time, it should be noted that the maximum content of molybdenum is reported only in mycorrhizal species from Boletaceae: *L. aurantiacum* (up to 30.16 mg/kg of dry weight), *Boletus badius* (up to 19.78 mg/kg of dry weight), and *B. edulis* (up to 15.48 mg/kg of dry weight), while the content of this element in the fruiting bodies of other ecological groups of macromycetes ranges 0.8—2.0 mg/kg of dry weight.

The content of germanium in the fruiting bodies of macromycetes varies within 0.16—0.17 mg/kg of dry weight (0.099 mg/kg of dry weight, in cultivated species *Stropharia rugosoannulata* and wild species *Boletus pinicola;* 0.055 mg/kg of dry weight in *B. edulis*, and 0.04 mg/kg of dry weight in *Amanita muscaria*. The minimum value is found in *Boletus subtomentosus* and in *Russula cyanoxantha* (0.001 mg/kg of dry weight).

All laboratory strains of the *Pleurotus* species have been established to have an extremely bioaccumulative activity with respect to the studied mineral elements (especially, germanium and selenium). The degree of their accumulation fairly correlates with the added concentrations (Figs. 1—3, Table). To assess the degree of accumulation, the bioaccumulation factor (BAF) is used. It is defined as the ratio of the content of an individual element in the enriched biomass to the content of that element in the reference sample.

In the case of addition of 50 mg/l GeO_2 to the culture medium, the maximum accumulation of germanium is reported for *P. eryngii* (up to 798 mg/kg of dry weight), with the bioaccumulation factor reaching 3577. *P. ostreatus* (strains 198 and 1796)

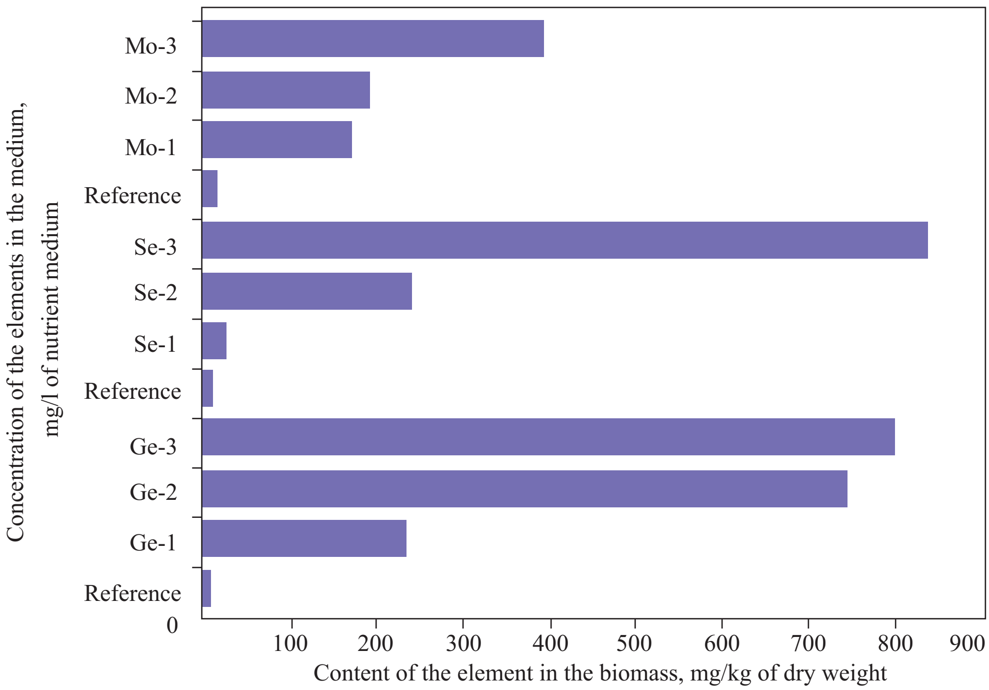


Fig. 1. Bioaccumulative activity of mycelial biomass of *Pleurotus ostreatus* (strain 198) with respect to mineral supplements of GeO_2, K_2SeO_4, and $Na_2MoO_4 \cdot 2H_2O$ at three concentrations: 10 mg per 1 l of nutrient medium (Ge-1, Se-1, and Mo-1); 25 mg/l (Ge-2, Se-2, and Mo-2); 50 mg/l (Ge-3, Se-3, and Mo-3)

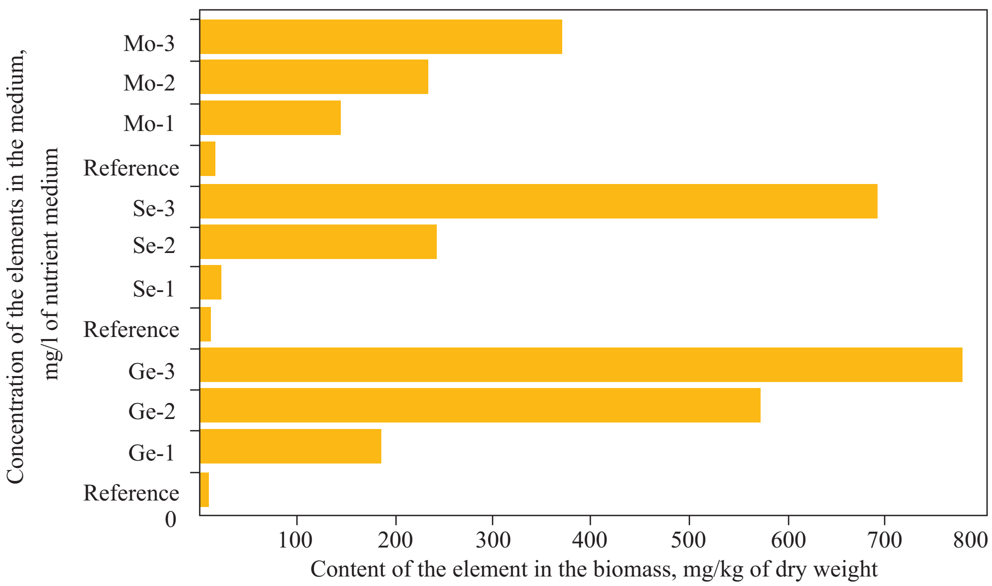


Fig. 2. Bioaccumulative activity of mycelial biomass of *Pleurotus ostreatus* (strain 1976) with respect to mineral supplements of GeO_2, K_2SeO_4, and $Na_2MoO_4 \cdot 2H_2O$ at three concentrations: 10 mg per 1 l of nutrient medium (Ge-1, Se-1, and Mo-1); 25 mg/l (Ge-2, Se-2, and Mo-2); 50 mg/l (Ge-3, Se-3, and Mo-3)

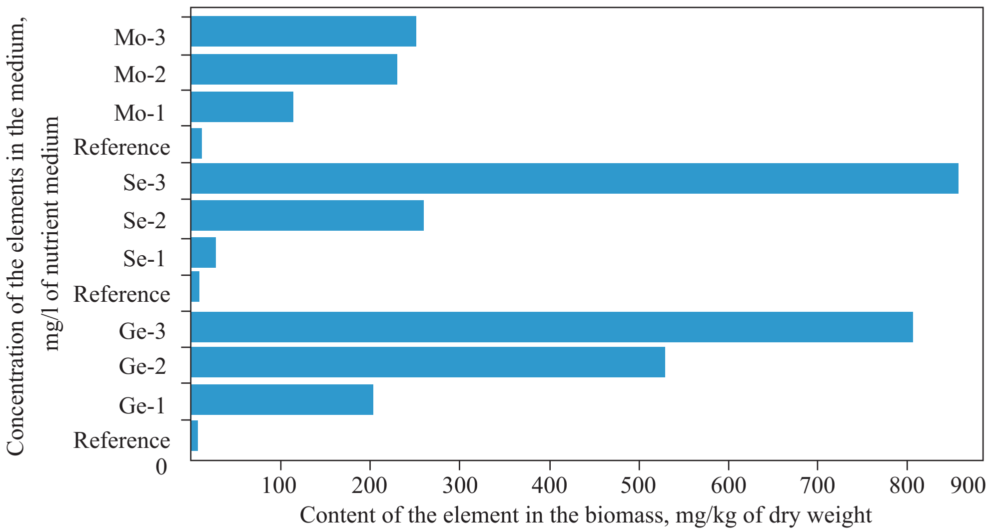


Fig. 3. Bioaccumulative activity of mycelial biomass of *Pleurotus eryngii* with respect to mineral supplements of GeO_2, K_2SeO_4, and $Na_2MoO_4{\cdot}2H_2O$ at three concentrations: 10 mg per 1 l of nutrient medium (Ge-1, Se-1, and Mo-1); 25 mg/l (Ge-2, Se-2, and Mo-2); 50 mg/l (Ge-3, Se-3, and Mo-3)

has a slightly lower accumulation, namely, 788 mg/kg of dry weight (BAF is 1452) and 770 mg/kg of dry weight (BAF is 1726), respectively.

Among the studied strains of *Pleurotus* species, the *P. eryngii* biomass most actively accumulates selenium, at the maximum added concentration, its content increases to 847 mg/kg of dry weight (BAF is 763). At the same time, the BAF for selenium in *P. ostreatus*-198 is higher (2118), and the biomass content reaches 826 mg/kg of dry weight.

Molybdenum is less actively accumulated in the biomasses of all tested strains. The highest levels have been recorded in *P. ostreatus* samples (up to 387 mg/kg of dry weight, in strain 198, and up to 361 mg/kg of dry weight, in strain 1796). The maximum molybdenum bioaccumulation factors for the tested strains range within 35—162.

The morphological features of the mycelium have been studied by scanning electron microscopy using the compounds of the essential elements studied. In particular, it has been found that an increase in the concentration of potassium selenite (K_2SeO_4) results in morphological changes in the texture of the colony, thickening of the hyphal walls, anastomosis formation, interlacing and fusion of hyphae, which testifies to the

Table

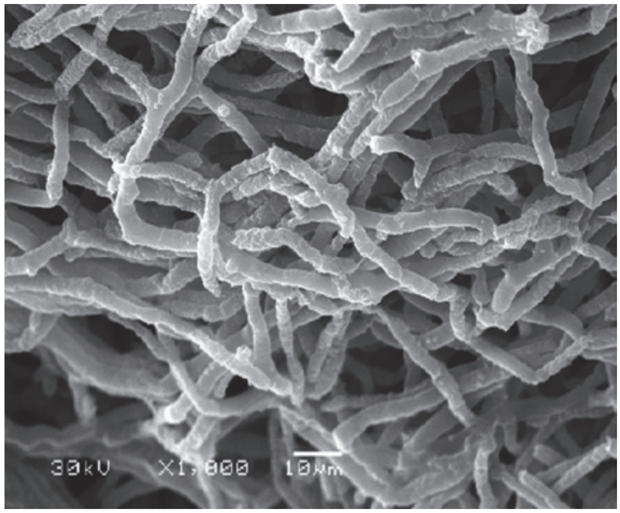
Content of Germanium. Selenium. and Molybdenum in Mycelial Biomass and BAF of *Pleurotus* Species

Element*	Content in the biomass, mg/kg	BAF
	Pleurotus ostreatus 198	
Germanium:		
reference	0.543 ± 0.027	
Ge-1	222.84 ± 13.37	410.4
Ge-2	731.13 ± 36.56	1346.5
Ge-3	788.33 ± 39.42	1451.8
Selenium:		
reference	0.39 ± 0.02	
Se-1	19.06 ± 0.95	48.9
Se-2	230.85 ± 11.54	591.9
Se-3	826.15 ± 41.31	2118.3
Molybdenum:		
reference	6.40 ± 0.32	
Mo-1	162.87 ± 8.14	25.5
Mo-2	181.74 ± 9.09	28.4
Mo-3	387.2 ± 19.36	60.5
	Pleurotus ostreatus 1796	
Germanium:		
reference	0.446 ± 0.022	
Ge-1	180.32 ± 9.02	404.3
Ge-2	564.35 ± 28.22	1265.4
Ge-3	769.60 ± 38.48	1725.6

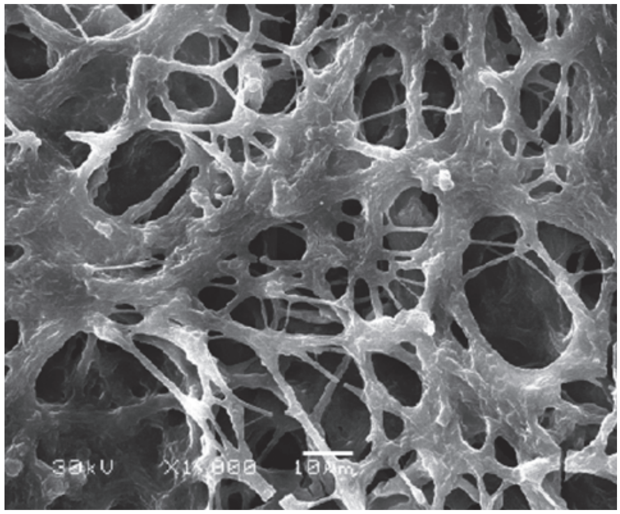
End of table

Element*	Content in the biomass, mg/kg	BAF
Selenium:		
reference	0.488 ± 0.024	
Se-1	16.0 ± 0.8	32.8
Se-2	232.94 ± 11.65	477.3
Se-3	684.92 ± 34.25	1403.5
Molybdenum:		
reference	10.23 ± 0.51	
Mo-1	131.27 ± 6.56	12.8
Mo-2	221.93 ± 11.10	21.7
Mo-3	361.19 ± 18.06	35.3
	Pleurotus eryngii 1863	
Germanium:		
reference	0.223 ± 0.01	
Ge-1	194.92 ± 9.75	874.0
Ge-2	520.04 ± 26.0	2332.0
Ge-3	797.67 ± 39.88	3577.0
Selenium:		
reference	1.110 ± 0.056	
Se-1	21.07 ± 1.05	19.0
Se-2	250.65 ± 12.53	225.8
Se-3	847.24 ± 42.36	763.3
Molybdenum:		
reference	1.481 ± 0.074	
Mo-1	104.25 ± 5.21	70.4
Mo-2	221.02 ± 11.05	149.2
Mo-3	240.36 ± 12.02	162.3

**Note*: addition of germanium, selenium, and molybdenum compounds to the nutrient medium at three concentrations: 10 mg per 1 l of nutrient medium (Ge-1, Se-1, Mo-1); 25 mg/l (Ge-2, Se-2, Mo-2); and 50 mg/l (Ge-3, Se-3, Mo-3).



a



b

Fig. 4. Anastomoses and fusion of *Pleurotus ostreatus* hyphae (strain 198) (SEM, × 1000) when K_2SeO_4 is added to the nutrient medium, at different concentrations: *a* — 10 mg/l; *b* — 50 mg/l

adverse effect of increased concentrations on the physiological condition and mycelium growth process (Fig. 4).

The obtained data have confirmed the results published by M.C.S. da Silva et al. [9] concerning certain toxicity of sodium selenite at concentrations above 25.4 mg per 1 kg of medium to fungi, which manifests itself in changes of their macro- and micromorphology, a decrease in hyphae size, and a slowdown in the rate of growth processes and biomass increase. Thus, while developing nutritional supplements of mushroom biomass enriched with essential elements, it is necessary to keep balance between the optimal content of these elements in the biomass and the physiologically good condition of the mycelium itself.

In combination with a high content of proteins, carbohydrates, vitamins, enzymes, other biologically active substances, a well-balanced content of vital mineral elements should potentially enhance the pharmacological action of preparations of a certain species of medicinal mushroom. Undoubtedly, further stages of development and implementation of domestic biological supplements based on mushroom products should include studies of the bioavailability and efficacy of such food supplements, as well as biomedical and clinical trials.

Hence, the study of the mineral composition of wild and cultivated species with valuable nutritional and medicinal properties is of great importance for determining the possible and safe levels of biomass enrichment with valuable macro and microelements.

The studies have shown a high bioaccumulation capacity of certain strains of *P. ostreatus* and *P. eryngii* with respect to mineral supplements of selenium, germanium, and molybdenum. The sorption coefficients are 10^2—10^3, for germanium, 10—10^3, for selenium, and 10—10^2, for molybdenum. Consequently, the enrichment of mushroom biomass with cultivated valuable medicinal and edible species having a balanced content of essential elements should potentially enhance their pharmacological action.

The development and implementation of enriched biological supplements based on mushroom products should include studies of the bioavailability and efficacy of such preparations, as well as biomedical and clinical trials.

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ЗБАГАЧЕННЯ БІОМАСИ ЦІННИХ ЇСТІВНИХ ГРИБІВ СПОЛУКАМИ ГЕРМАНІЮ, СЕЛЕНУ І МОЛІБДЕНУ

Вступ. На сьогодні загальновідомо, що шапинкові гриби (макроміцети) є не лише традиційним продуктом харчування, а й невичерпним джерелом речовин, які мають широкий спектр застосування у фармакології. Деякі види грибів розглядають як джерело фізіологічно важливих, так званих ессенціальних елементів, зокрема Cu, Fe, Zn, Cr, Se, Mo, Mn тощо.

Проблематика. Зважаючи на те, що мінеральний склад макроміцетів є досить специфічним і їм властива певна вибірковість накопичення окремих елементів з ґрунтів/субстратів (видоспецифічність накопичення), актуальними і перспективними є біотехнологічні розробки, спрямовані на збагачення ессенціальними елементами мінерального складу грибної біомаси, що культивується на рідкому живильному середовищі, та плодових тіл цінних їстівних видів, які вирощують у поверхневій культурі. Збагачення мінерального складу потенційно має підвищувати лікарські властивості, біологічну активність і харчову цінність грибних добавок.

Мета. Визначення біоакумуляційної здатності міцеліальної біомаси *Pleurotus ostreatus* (Jacq.) P. Kumm. і *P. eryngii* (DC.) Quél. щодо сполук Ge, Se, and Mo.

Матеріали й методи. Методом мас-спектрометрії з індукційно зв’язаною плазмою (ICP-MS) досліджували вміст Ge, Se і Mo в міцеліальній біомасі трьох штамів видів роду *Pleurotus* при культивуванні на рідкому поживному середовищі із додаванням сполук германію, селену і молібдену у концентраціях 10, 25 і 50 мг/л відповідно.

Результати. Всі досліджені штами продемонстрували високу біоакумуляційну здатність: для германію коефіцієнти акумуляції знаходилися у межах двох-трьох порядків (від 404 до 3577), селену — від одного до трьох порядків (від 19 до 2118), а молібдену від одного до двох порядків величин (від 12 до 162).

Висновки. Подальші етапи розробки та впровадження збагачених есенціальними елементами грибних додатків мають включати дослідження біодоступності та ефективності таких препаратів, медико-біологічні та клінічні випробування.

Keywords: culinary-medicinal mushrooms, *Pleurotus spp*., bioaccumulation, and essential elements.

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ОБОГАЩЕНИЕ БИОМАССЫ ЦЕННЫХ СЪЕДОБНЫХ ГРИБОВ СОЕДИНЕНИЯМИ ГЕРМАНИЯ, СЕЛЕНА И МОЛИБДЕНА

Введение. Сегодня общеизвестно, что шляпочные грибы (макромицеты) являются не только традиционным продуктом питания, а и неисчерпаемым источником веществ, имеющих широкий спектр применения в фармакологии. Некоторые виды грибов рассматриваются как источник физиологически важных, так называемых эссенциальных, элементов, в частности Cu, Fe, Zn, Cr, Se, Mo, Mn и др.

Проблематика. Учитывая то, что минеральный состав макромицетов достаточно специфичен и им свойственна определенная избирательность накопления отдельных элементов из почвы/субстратов (видоспецифичность накопления), актуальными и перспективными являются биотехнологические разработки, направленные на обогащение эссенциальными элементами состава грибной биомассы, культивируемой на жидкой питательной среде и плодовых тел ценных съедобных видов, выращиваемых в поверхностной культуре. Обогащение минерального состава потенциально должно повышать лекарственные свойства, биологическую активность и пищевую ценность таких грибных добавок.

Цель. Определение биоаккумуляционной способности мицелиальной биомассы *Pleurotus ostreatus* (Jacq.) P.Kumm. и *P. eryngii* (DC.) Quél. относительно соединений Ge, Se и Mo.

Материалы и методы. Методом масс-спектрометрии с индукционно связанной плазмой (ICP-MS) исследовали содержание Ge, Se и Mo в мицелиальной биомассе трех штаммов видов рода *Pleurotus* при культивировании на жидкой питательной среде с добавлением соединений германия, селена и молибдена в концентрациях 10, 25 и 50 мг/л соответственно.

Результаты. Все исследованные штаммы продемонстрировали высокую биоаккумуляционную активность: для германия коэффициенты аккумуляции находились в пределах двух-трех порядков (от 404 до 3577), селена — от одного до трех порядков (от 19 до 2118), а молибдена — от одного до двух порядков величин (от 12 до 162).

Выводы. Дальнейшие этапы разработки и внедрения обогащенных эссенциальными элементами грибных пищевых добавок должны включать исследования биодоступности и эффективности таких препаратов, медико-биологические и клинические испытания.

Ключевые слова: съедобные и лекарственные грибы, *Pleurotus spp*., аккумуляция, эссенциальные элементы.