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FEATURES OF BIOELEMENTS ACCUMULATION BY FUNGI L. EDODES DURING CULTIVATION IN SUBSTRATES ENRICHED WITH Cr, Se, Ge, Fe CITRATES

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Abstract. The features of citrates Cr, Se, Ge, Fe biotransformation during cultivation of Lentinula Edodes and their influence on the accumulation of some bioelements were investigated in order to predict the biological value, therapeutic and prophylactic properties of shiitake mushrooms. The strain Lentinula Edodes 3790, Mycelia, Belgium, was used for the study. The substrate for L. Edodes was prepared from oak sawdust, cereal bran, sunflower husk, secondary products of soybean, flax, or hemp processing, gypsum, and chalk. The addition of Cr, Se, Ge, Fe citrates into the substrate was carried out by moistening it with appropriate solutions until the substrates reached the content of Cr, Se, Ge citrates of 0.3, 1.0, and 3.0 mg/kg, and Fe citrate of 20.0, 50.0, and 100.0 mg/kg. The content of bioelements was determined by optical emission spectroscopy with inductively coupled plasma. According to the results of the study, enrichment of the substrate with citrates Cr, Se, Ge, Fe leads to their accumulation by shiitake mushrooms and also affects the trace element composition of the mushrooms in total, but modification of the trace element composition of the substrate does not lead to the accumulation of bioelements and toxicants above the level of the maximum permissible concentration. The addition of iron citrate 20 mg/kg, selenium 0.3 mg/kg and germanium 0.3 mg/kg to the substrates increases the chromium content in mushrooms in a much higher amount than when the substrate is enriched with chromium citrate. The addition of 0.3 mg/kg of chromium citrate provides a significant increase in the content of Germanium in mushrooms, and the enrichment of the substrate by 0.3 mg/kg of selenium citrate provides the accumulation of Germanium at the same level as when germanium citrate is added. Thus, the system of substrate-shiitake mushroom is a complex system where the Le Chatelier principle is observed, synergism and atognism of micronutrients are observed at the formation of the micronutrient composition of the mushroom fruiting body depending on the substrate composition. The investigation of these processes makes it possible to predictably modify the trace element composition of the mushroom, enriching it with one or more biologically active trace elements. The addition of predictable amounts of trace elements to the substrate allows L. Edodes to synthesise a balanced raw material base enriched with bioavailable trace elements for the creation of functional foods.

Key words: L. Edodes, fortification, Chromium, Selenium, trace element composition, functional foods.

Introduction. Formulation of the problem

The production of raw materials and finished products with high biological value and affordable for the average consumer has always been a challenge for the agri-food sector. The production of cultivated mushrooms to expand the domestic market and export potential deserves attention. Mushrooms are a valuable source of essential micro and macronutrients, have excellent sensory characteristics, besides, Ukraine has significant prerequisites for their cultivation and production of substrates [1].

Shiitake (Lentinula Edodes) is one of the most cultured and consumed mushrooms around the world

due to its high gastronomic and positive physiological properties. Shiitake mushrooms contain essential macro and micronutrients as well as many bioactive compounds, including polysaccharides, antioxidants, dietary fiber, and ergosterol. Bioactive compounds can be helpful for maintaining the good health of the users and preventing them from diseases. Due to the presence of many bioactive compounds shiitake mushroom can simultaneously act as an antimicrobial, antiviral, anticancer/antitumor, antidiabetic, antihyperlipidemic, anticholesterol, antioxidative, antiaging, hepatoprotective, and immunomodulatory agent [2-3].

The drugs and other medicinal products obtained from L. edodes are used in the treatment of various cancers due to their immunostimulating, anti-inflammatory, and anti-oxidant effects [4-7].

The polysaccharides present in the fruiting bodies of L. edodes enhance the immune function and eliminate the side effects of chemo- and radiotherapy, besides exhibiting anti-cancer, anti-viral, and antibacterial properties. The compounds responsible for the biological activity of L. edodes are the polysaccharides belonging to the group of β-D-glucans, which include lentinan [8-12].

It is known that in the process of their growth, fungi absorb nutrients and trace elements from the environment. The concentrations of some macro- and microelements in the fruiting bodies of fungi differ significantly from those in plants, which is primarily due to differences in metabolism. Some elements can promote the growth of fungi of a certain species, while others, on the contrary, can inhibit their growth. Publications of recent decades have highlighted the role of bioelements in the physiology of macromycetes, the correlation between the ability to accumulate certain mineral elements, including toxic ones, and the species affiliation of fungi. Macromycetes can be used as bioindicators of anthropogenic environmental pollution. The unique biosorption properties of fungi allow to consider them as sorbents on the one hand, and as an important source of essential and rare mineral elements for the human body as well. In addition, mushrooms can be considered a kind of 'bio-laboratory' that can quickly convert inorganic forms of elements into bio-organic ones, making them favourable for human absorption.

This property opens up the possibility of purposefully modifying the trace element composition of fungi, including L. edodes, enriching them with essential bioelements by introducing compounds of these trace elements into the wooden substrate in certain non-toxic concentrations [13-14].

Analysis of recent research and publications

It is known that mushrooms absorb and accumulate various chemical elements, but the effectiveness of this process depends on the species of mushroom, the concentration of the element, and the chemical form in which it is bound [14-16]. Enrichment of mushrooms with trace elements necessary for vital activity can significantly increase the nutritional and pharmaceutical value of the final product and expand the scope of its application.

Most of the essential bioelements, including Cr, Se, Ge, and Fe, are promising for L. edodes fortification.

The biological role of germanium (Ge) compounds is to enhance haematopoiesis in the bone marrow, antioxidant and antitumour effects [14].

Selenium (Se) is an essential element that, in addition to its pronounced antioxidant and antitumour effects, boosts immunity and contributes to the normal functioning of the endocrine system [14].

Iron (Fe) is one of the most important trace elements that is part of more than 100 enzymes of the human body, participates in respiration, hematopoiesis, immunobiological processes, redox reactions, etc. Insufficient iron supply to the body can provoke iron deficiency anemia [17].

Recent studies indicate the important role of trivalent chromium in trace element homeostasis of the body. Chromium, being part of the corresponding enzymes, acts as a regulator of the assimilation and transformation of sugars in the body. Chromium enhances the action of insulin, a hormone that is critical important for the metabolism and storage of carbohydrates, fats and proteins in the body. Lack of chromium, or its poor absorption, is one of the reasons for the development of diabetes [3,18].

Mushrooms that are purposefully enriched with various trace elements can be used as functional food products [19]. There are a number of studies that consider the fortification of edible mushrooms with bioelements.

The authors of [20-21] investigated the enrichment of medicinal mushrooms with inorganic Lithium and Selenium salts.

Paper [22] examines the effect of enriching the substrate for growing edible mushrooms with calcium on the accumulation of this element in the fruiting body of mushrooms.

Muszyńska et al. have investigated accumulation Cu, Zn, or Se in *Lentinula edodes* as potential anti-inflammatory materialIt was found that addition of Cu, Zn, or Se enhanced the anti-inflammatory properties of L. edodes mycelial extracts, suggesting that the mycelium of L. edodes may be used as a potential component in natural anti-inflammatory dietary supplement [23].

Авторами роботи [24] *L. edodes* biomass was obtained on liquid Oddoux medium enriched with Se(IV) in the form of selenitetriglycerides. The culture medium was supplemented with 25 and 50 mg/L of Se(IV) (0.5 and 1 mL of selenitetriglicerides per liter of the medium), respectively. The selenium-enriched mushroom was found to be safe based on cytotoxicity tests. The mean quantity of selenium in the serum of

calves fed with selenium-enriched L. edodes mycelium was significantly higher than that of control calves.

In [14], a study was conducted to determine the bioaccumulative capacity of the mycelial biomass of edible and medicinal mushroom species – Pleurotus ostreatus and Pleurotus eryngii – when Selenium, Molybdenum and Germanium compounds were added to the nutrient medium during their cultivation (K_2SeO_4, $Na_2MoO_4 \cdot 2H_2O$ та GeO_2). Studies have shown a high bioaccumulation capacity of certain strains of P. ostreatus and P. eryngii for mineral additives of Selenium, Germanium and Molybdenum. Sorption coefficients for Ge were 10^2–10^3, for Se – 10–10^3, and for Mo –10–10^2.

Nunes Regiane et al. have studied how Strains of Lentinula edodes (Berk.) were grown on artificial logs composed of eucalyptus sawdust, and were subjected to cold shock in water containing sodium selenite (Na_2SeO_3) at concentrations of up to 1.28 mM. The content of Se in the mushrooms increased linearly with increasing amounts of Na_2SeO_3 although above 0.96 mM, mushroom formation was inhibited. Concentrations greater than 17 mg Se 100/g of dried mushrooms were observed after treatment with 0.64 mM Na_2SeO_3 [25].

The peculiarities of substrate enrichment with bioelements (Mg, Zn, Cu, Fe) and detection of their content in Agaricus bisporus were studied in [26].

The aim of this study [27] was to compare the effects of three Fe forms ($FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, or FeHBED) in three concentrations (5, 10, or 50 mM) for three mushroom species (Pleurotus eryngii, P. ostreatus, or Pholiota nameko) on their chemical composition, phenolic compounds, and organic acid production. The most effective metal accumulation of all the investigated species was for the 50 mM addition. $FeCl_3 \cdot 6H_2O$ was the most favorable additive for P. eryngii and P. nameko (up to 145 and 185% Fe more than in the control, respectively) and FeHBED for P. ostreatus (up to 108% Fe more than in control). Additionally, P. nameko showed the highest Fe accumulation among studied species (89.2 ± 7.51 mg·kg^{-1} DW).

Therefore, a number of studies have been devoted to the investigation of the macro- and micronutrient composition of edible mushrooms, including L. Edodes, which also consider their enrichment with bioelements by modifying substrates for growing mushrooms. As a rule, researchers analyse the content of biotransformed micro- and macronutrients added to the substrate in the mycelium and body of the fungus, while their effect on the accumulation of other biological elements has not been studied. Of particular interest is also the enrichment of L. Edodes with such essential trace elements as Cr, Se, Ge, Fe in organic form and the study of their effect on the micro- and macronutrient composition of this fungus.

The purpose of the study is to investigate the peculiarities of biotransformation of Cr, Se, Ge, Fe citrates during the cultivation of Lentinula Edodes and their influence on the accumulation of some bioelements to predict the biological value, therapeutic and prophylactic properties of shiitake mushrooms and the possibility of creating functional foods based on them.

Objectives:

– preparation of substrates enriched with Cr, Se, Ge, Fe citrates and cultivation of L. Edodes;
– determination of the content of some micro- and macroelements in the edible part of L. Edodes by inductively coupled plasma optical emission spectroscopy;
– analysis of the features of Cr, Se, Ge, Fe, other micro- and macroelements and some toxicants accumulation in L. Edodes.

Research materials and methods

The strain of Lentinula Edodes 3790, Mycelia, Belgium, was used for the study. The substrate for L. Edodes was prepared from oak sawdust 66.99%, cereal bran 15.53%, sunflower husk 9.79%, secondary products of soybean processing 2.92%, flax or hemp 2.87%, gypsum and chalk 0.95% (in wet weight), which is identical to the preparation of a substrate for industrial mushroom cultivation. Disinfectant heat treatment of the substrate was carried out at a temperature of 100°C for 22 hours.

To moisten the substrate, tap water was used, into which the calculated amounts of citrates Cr (initial concentration 600 mg/l), Se (initial concentration 300 mg/l), Ge (initial concentration 600 mg/l), Fe (initial concentration 2 g/l) were previously added. Each series of trace element enrichment of the substrate was carried out in five replicates at three different concentrations on wood substrate blocks weighing 4.8 kg (Table 1).

The content of trace elements in the substrate was chosen on the basis of primary studies of the fungicidal effect of chromium citrate solutions on Lentinula edodes culture and the approximate quantitative ratio between trace elements in shiitake mushrooms [3].

Table 1 – Trace element content in enriched wood substrates

Trace element citrate, mg/l	Element content, mg/kg of substrate			Element content in 1 block of substrate, mg/4.8 kg			Volume of primary solution added to water for moistening, ml		
Cr, 600	0.3	1.0	3.0	1.44	4.8	14.4	2.4	8.0	24.0
Se, 300	0.3	1.0	3.0	1.44	4.8	14.4	4.8	16.0	48.0
Ge, 600	0.3	1.0	3.0	1.44	4.8	14.4	2.4	16.0	48.0
Fe, 2000	20	50	100	96	240	480	48.0	120.0	240.0

Lentinula Edodes 3790 strain (Mycelia, Belgium) was added to the enriched moistened substrate, after which the mushrooms were matured for three months under appropriate conditions. Control samples were prepared in parallel.

After harvesting, the fruiting bodies of shiitake mushrooms were washed with distilled water, air-dried at room temperature, and then ground on a laboratory electric oven SNOL 58/350 at 105°C until a constant weight was obtained.

The dried mushrooms were ground to a powder using a blender. A 0.2 g sample was weighed on an analytical scale, transferred to an autoclave, 3.0 ml of concentrated nitric acid (65% nitric acid Merck, Germany) was added, and incubated for 30 min. Then they were mineralised in a MARS-One microwave oven (CEM, USA). The mineralisation programme included 3 stages: heating, mineralisation, and cooling. The total mineralisation time was 25 minutes. After cooling, the sample was brought to 10.0 ml with deionised water (18Ω). Deionised water was prepared using the Distillacid TM BSB-939-IR system (Berghof, Germany).

The content of bioelements was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) using an Optima 2100 DV device from Perkin-Elmer (USA).

For the calibration of the device, an ICP-multi-element standard solution containing 23 chemical elements No. 111355.0100 (Merck, Germany) was used. The analysis of Mg, Al, Cd, Pb, Mn, Zn, Fe, Co, Cu, Ni Se, Mo, Cr, V, Ti Ge was performed in the axial plasma view mode. The limits of detection (LOD) for vanadium, magnesium, copper, ferrum, selenium, and zinc were 4.0 µg/l, 0.05 µg/l, 0.3 µg/l, 0.3 µg/l, 3 µg/l, and 0.4 µg/l, respectively. To ensure accuracy, the measurement was repeated three times. In this regard, the experimental value was considered accurate if the relative standard deviation did not exceed 2%. External quality control of laboratory tests for determining the content of toxic metals and essential trace elements in reference and test materials of plant origin was carried out in accordance with the programme of the Centre for Metrology and Laboratory Services for the following elements Fe, Cu, Zn, Se, Mn, Cd, Pb.

The mathematical processing of the obtained results was performed using the WinLab32 software of the OES-ISP device in the Windows XP prof operating system, and the statistical processing was performed using the Microsoft Excel software package according to [28].

Results of the research and their discussion

The results of the research allowed us to state that the introduction of different amounts of Cr, Se, Ge, Fe citrates into the substrate did not affect the yield, appearance, taste and smell of shiitake mushrooms. The elemental composition of the enriched mushrooms per 100 g of fresh mushrooms (in comparison with the control) is given in Table 2.

The analysis of the data obtained makes it possible to observe several clear patterns. The enrichment of the substrate with citrates Cr, Se, Ge, Fe leads to their accumulation by shiitake mushrooms and also affects the trace element composition of mushrooms in general, compared to the control. The introduction of a certain trace element into the substrate leads to an increase in its content in the fruiting body of the fungus (except for Ge), but not proportionally (Table 2, Fig. 1).

Table 2 – Content of elements in shiitake mushrooms, n=3, p ≤ 0.02

Trace element	Trace element content in shiitake mushrooms, µg/100g												
	Control	Samples of shiitake fungi cultivated on substrates enriched with											
		Chromium citrate, mg/kg			Selenium citrate, mg/kg			Germanium citrate, mg/kg			Ferric citrate, mg/kg		
		0.3	1.0	3.0	0.3	1.0	3.0	0.3	1.0	3.0	20.0	50.0	100.0
Mg	10435.38	14407.84	13625.34	14501.66	17780.55	16686.21	17913.50	16340.01	14459.06	14501.66	15594.06	12627.53	12500.76
AI	42.34	58.04	58.69	52.12	50.19	53.54	42.86	29.86	34.62	28.83	51.35	42.47	45.95
Cd	0.36	1.67	0.76	0.46	0.37	2.57	2.14	3.32	1.33	1.38	2.41	1.15	1.40
Pb	3.35	2.83	2.83	3.09	1.44	1.07	1.79	1.80	4.38	3.99	5.79	4.38	6.31
Mn	20.85	51.99	49.03	52.64	65.12	85.84	141.83	97.43	86.74	42.34	43.11	29.73	28.83
Zn	168.21	505.28	474.65	425.35	445.95	874.52	910.81	1101.41	334.23	209.27	1270.14	215.19	209.27
Fe	995.37	596.40	411.33	365.64	280.95	308.88	413.77	1387.90	1448.00	1499.10	1938.48	2463.45	2616.99
Co	0.01	0.02	0.05	0.08	0.04	0.04	0.01	0.01	0.08	0.07	0.05	0.06	0.08
Cu	32.30	43.76	29.47	34.75	48.52	121.11	64.86	89.06	65.38	44.79	88.16	47.23	64.99
Ni	3.22	2.19	3.60	5.28	2.45	3.60	3.09	2.45	2.06	1.67	2.83	2.83	2.70
As	0.40	0.30	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.18	0.93	0.14
Se	1.80	1.80	5.28	9.91	26.90	90.73	131.66	2.06	1.03	1.03	1.42	1.80	2.83
Mo	0.35	0.46	0.30	0.45	0.35	0.40	0.36	0.54	0.66	0.75	0.99	0.93	1.25
Cr	1.48	1.34	3.09	9.91	13.51	4.12	4.38	9.65	4.47	3.64	13.26	4.63	4.38
V	0.36	0.88	1.17	1.49	2.96	2.70	2.19	0.88	0.50	0.35	0.75	0.36	0.37
Ti	1.27	0.01	0.03	0.02	1.93	1.93	0.02	0.12	0.23	0.33	5.28	6.05	0.13
Ge	1.79	6.05	2.61	3.72	2.70	2.65	0.22	3.44	1.66	1.65	1.18	1.61	2.65

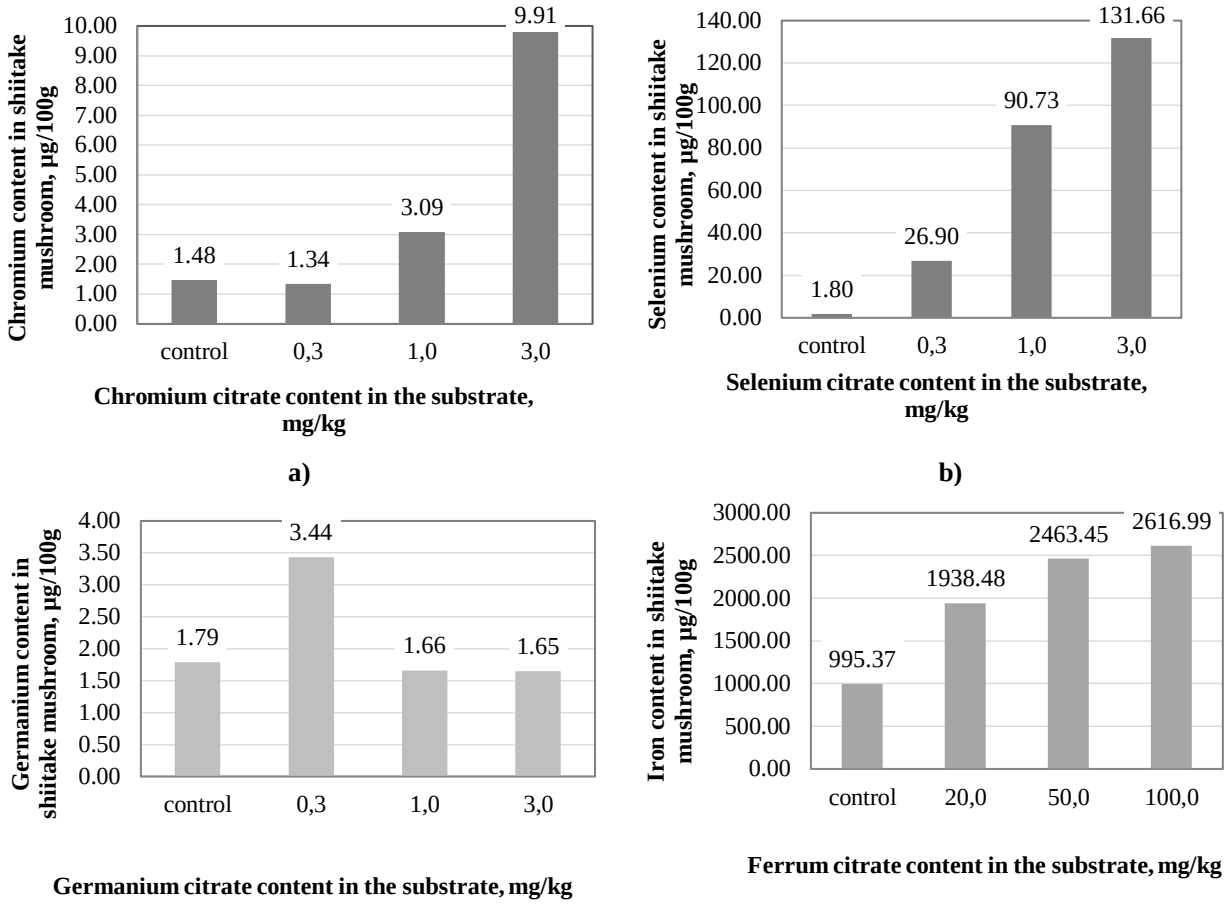


Fig. 1. Accumulation of bioelements by the fruiting body of Shiitake mushrooms depending on their content in the substrate: a) Chromium accumulation; b) Selenium accumulation; c) Germanium accumulation, d) Ferrum accumulation

Based on the data obtained, it can be assumed that further increase in the trace element citrate concentration in the substrate will not contribute to its significant accumulation in the fungus. That is, a certain "protection mechanism" operates in the fungus-substrate biosystem, which does not allow to reach the maximum toxic concentration of the trace element that would inhibit the growth of the fungus.

It is known that the level of accumulation of micro- and macroelements by mushrooms, including shiitake, depends on a number of factors, such as climatic conditions, type and nutritional value of the substrate, etc. Analysing the literature data on the content of bioelements in Lentinula edodes mushrooms, the results of different studies are quite variable.

In [29], the content of selected macro- (K, Ca) and microelements (Mn, Fe, Cu, Zn, Se) was studied. Major element ranged in fruiting bodies of mushroom collection suggest that K (13.2–62.2 g kg^{-1}), P (7.8–54.5 g kg^{-1}) and Mg (3.4–6.5 g kg^{-1}) as most abundant elements followed by Ca (179.8–1698 mg kg^{-1}), Na (191.3–3448 mg kg^{-1}) and Al (15.3–79.5 mg kg^{-1}). The Al levels resemble the amounts reported for Al accumulating mushroom species. Most abundant trace/heavy metals in the mushroom collection were Zn (59.3–283.9 mg kg^{-1}) followed by Fe (44.4–125.1 mg kg^{-1}), Mn (18.2–73.2 mg kg^{-1}) and Cu (13.7–182.4 mg kg^{-1}). Only trace amounts of toxic heavy metals, Cd (0.27–3.7 mg kg^{-1}), Co (0–0.87 mg kg^{-1}), Ni (0–0.54 mg kg^{-1}) and Pb (0–2.2 mg kg^{-1}) were found in mushroom collection.

The authors of [30] have been investigated the content of 62 elements in the fruiting bodies of Lentinula edodes (Shiitake mushroom) cultivated commercially in Poland on various substrates from 2007–2015. The general mean content (mg kg^{-1} dry weight (DW)) of the studied elements ranked in the following order: K (26,335) > P (11,015) > Mg (2,284) > Ca (607) > Na (131) > Zn (112) > Fe (69) > Mn (33) > B (32) > Rb (17) > Cu (14.5) > Al (11.2) > Te (2.9) > As (1.80) > Cd (1.76) > Ag (1.73) > Nd (1.70) > Sr (1.46) > Se (1.41) > U (1.11) > Pt (0.90) > Ce (0.80) > Ba (0.61) > Co (0.59) > Tl (0.58) > Er (0.50) > Pb (0.42) > Li (0.40) > Pr (0.39) > Ir (0.37) > In (0.35) > Mo (0.31) > Cr (0.29) > Ni (0.28) > Sb (0.26) > Re (0.24) > Ti (0.19) > Bi (0.18) > Th (0.12) > La (0.10) = Pd (0.10) > Os (0.09) = Zr (0.09) > Rh (0.08) > Ho

(0.07) > Ru (0.06) > Sm (0.04) = Eu (0.04) = Tm (0.04) > Gd (0.03) > Sc (0.02) = Y (0.02) > Lu (0.01) = Yb (0.01) = V (0.01).

Mirończuk-Chodakowska et al. [31] investigated bioelements content in 21 species of edible mushrooms: eighteen species of wild mushrooms and three species of popular cultivated mushrooms. The mean Cu, Mn, Se, and Zn content (in μg/g, dry mass DM) ranged from 10.6–123.1, 12.2–41, 0.13–13.3, and 68.3–184, respectively.

Regarding the accumulation of toxic elements by shiitake fruits depending on the content of Cr, Se, Ge, Fe citrates in substrates, it can be noted that modification of the trace element composition of shiitake mushrooms does not lead to the accumulation of toxicants above the level of the maximum permissible concentration [32,33] (Fig. 2), which correlates with our previous studies [34].

According to the results of the study, there is a tendency to change the accumulation of various bioelements by the fruits of shiitake mushrooms, depending on the kind and concentration of Cr, Se, Ge, Fe citrates in the substrates, compared to control samples (Table 2, Fig. 3-5). The effect of synergism and antagonism of trace elements for biological organisms is widely known, and we have partially observed it for shiitake mushrooms in previous studies [35]. This effect, after a detailed study, can be used for the biosynthesis of raw materials comprehensively enriched with essential bioelements.

An increase of chromium citrate concentration in the substrate leads to an increase in the chromium content of the shiitake mushroom and a decrease in the iron content. Along with chromium, the amount of selenium and cobalt increases. The trace element pairs zinc-manganese and copper-molybdenum behave in a similar way. With an increase in the concentration of chromium citrate in the substrate to 0.3 mg/kg, its content in shiitake mushrooms increases, and with the introduction of more chromium citrate, the chromium content in the fruit remains at a stable level. Simultaneously with an increase in the concentration of chromium and cobalt in the shiitake mushroom and a decrease in iron content, the nickel content decreases (at 0.3 mg/kg of chromium citrate), and at higher concentrations of chromium citrate, its content increases similarly to the concentration of chromium and cobalt against the decrease in iron concentration (Fig. 3).

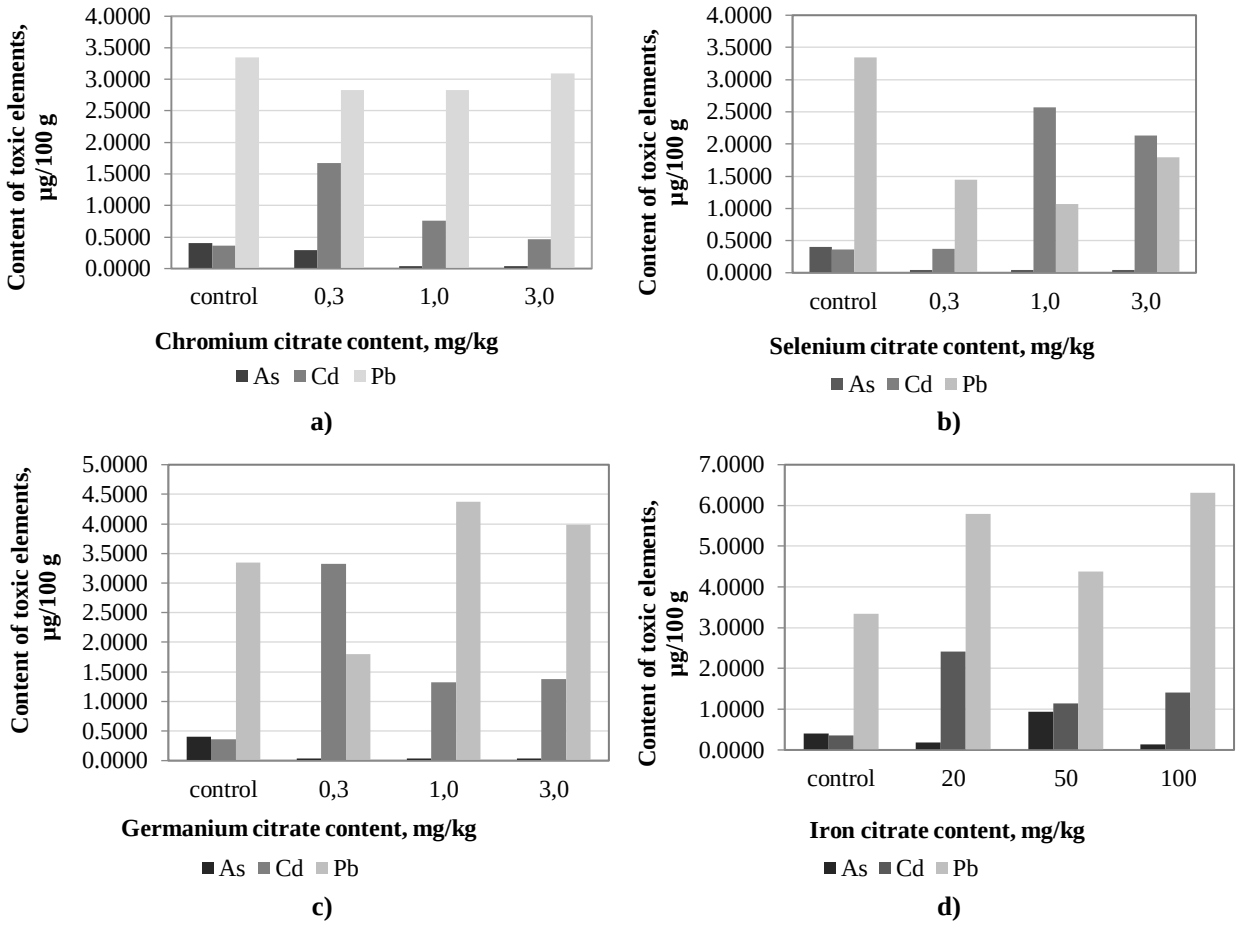


Fig. 2. Accumulation of toxic elements by the fruiting body of shiitake mushrooms depending on the content of citrates in the substrate: a) Cr citrate; b) Se citrate; c) Ge citrate; d) Fe citrate

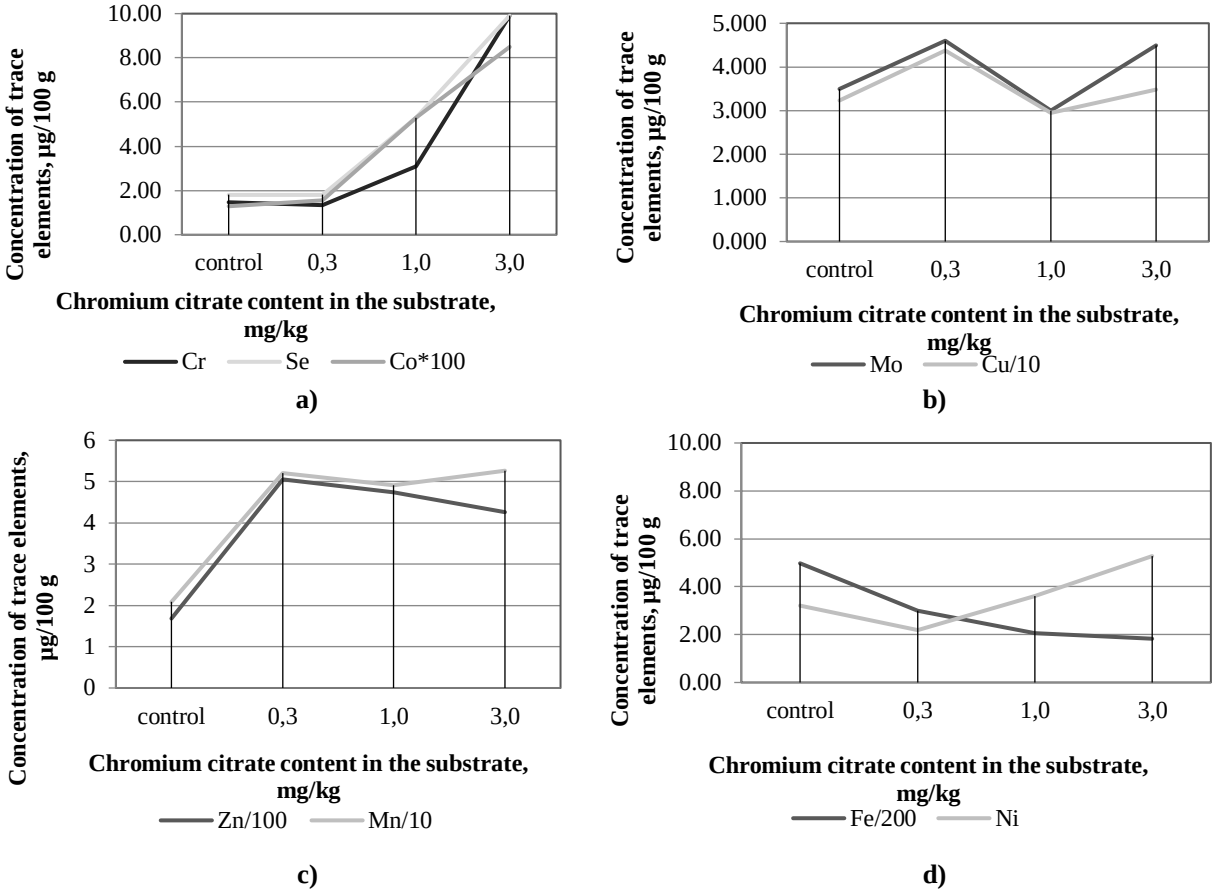


Fig. 3. Accumulation of some bioelements by the fruiting body of Shiitake mushrooms depending on the content of Chromium citrate in the substrate: a) accumulation of Cr, Se, Co*100; b) accumulation of Mo, Cu; c) accumulation of Zn/100, Mn/10; d) accumulation of Fe/200, Ni

Taking into account the high content of Fe in the primary wood substrate, to modify the trace element composition of mushrooms, Fe citrate was added in much larger quantities. In accordance with Le Chatelier's principle, this did not lead to a significantly proportional increase in its content in the shiitake mushroom, but instead, a tendency to accumulate Chromium was observed. A particularly large amount of Chromium was absorbed by the fungus at the initial enrichment of the substrate with Ferric citrate (20 mg/kg). In the pairs of Zinc - Manganese and Copper - Molybdenum, an increase in their concentration in the shiitake fungus was also observed when 20 mg/kg of Ferric citrate was added to the substrate and a further decrease with an increase in the citrate concentration (Fig. 4). The Nickel content first decreases and then remains stable, and Cobalt tends to accumulate (Table 2).

The addition of germanium citrate to the substrate has practically no noticeable effects, including an increase in its content in shiitake mushroom, but there is a slight increase in Magnesium and Zinc compared to the control (Table 2).

However, the addition of selenium citrate has a significant effect on the trace element balance of the mushroom (Fig. 5). The addition of selenium citrate to the substrate leads to an increase in its content in the shiitake mushroom. At the rate of 0.3 mg/kg, the content of Cromium increases and the content of Ferrum decreases; further increase in the concentration of selenium citrate in the substrate has virtually no effect on the accumulation of these elements by the fungus.

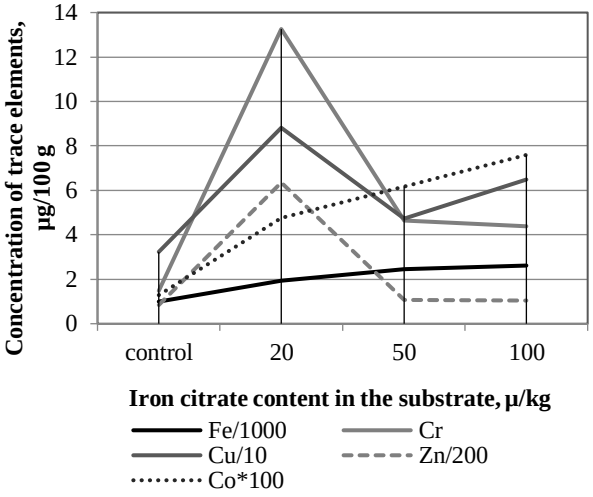


Fig. 4. Accumulation of some bioelements by the fruiting body of shiitake mushrooms depending on the content of Fe citrate in the substrate

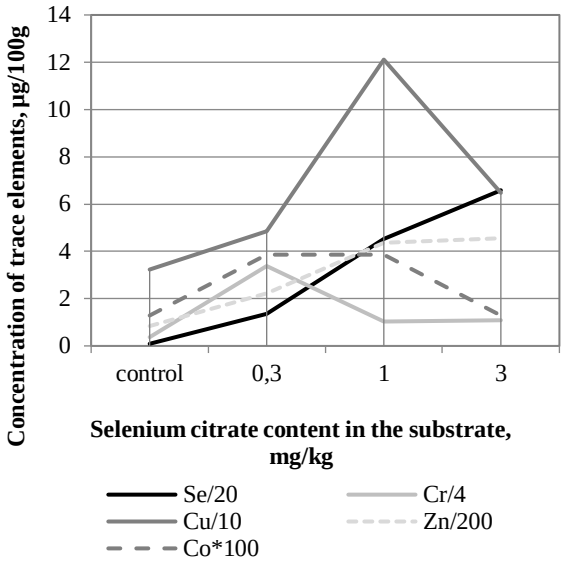


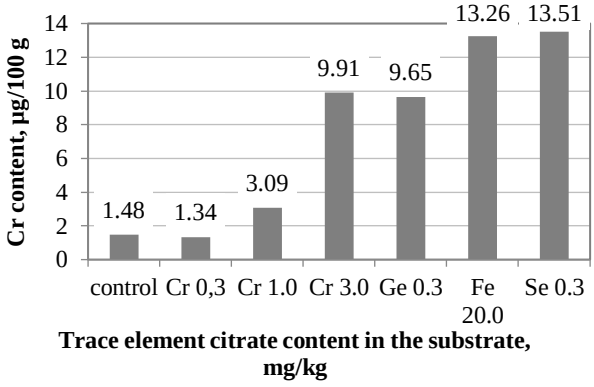
Fig. 5 Accumulation of some biological elements by the fruiting body of shiitake mushrooms depending on the content of selenium citrate in the substrate

In the Zinc-Manganese pair, an increase in their concentration in the shiitake mushroom is observed with an increase in the content of selenium citrate in the substrate. The Molybdenum content remains practically unchanged, and the copper content increases rapidly (at 1 mg/kg of selenium citrate in the substrate), and then begins to decrease. With the addition of 0.3 mg/kg of selenium citrate, the Cobalt concentration increases against the background of a decrease in the Ferrum content, and then returns to the control value through a stability plateau. The Nickel content, on the contrary, decreases and then returns to its original value (Table 2).

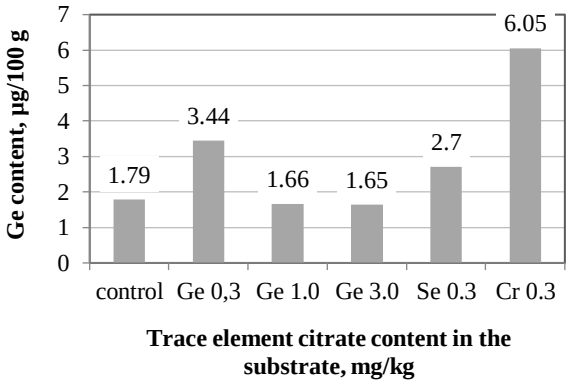
Thus, there is a definite correlation between the content of Chromium, Selenium, Ferrum and Zinc in Shiitake mushrooms. Enrichment of the substrate with Ferrum leads to an increase in Chromium and Selenium in the mushrooms. And the addition of selenium citrate to the substrate enriches the mushrooms with both Selenium and Chromium.

The effect of increasing the concentration of a trace element when small amounts of citrates of synergistic trace elements are added to the substrate is especially significant (Fig. 6).

Thus, the addition of ferric citrate 20 mg/kg, selenium citrate 0.3 mg/kg and germanium citrate 0.3 mg/kg to the substrates leads to an increase of chromium content in mushrooms in a much higher amount than when the substrate is enriched with chromium citrate (Fig. 6a). At the same time, the introduction of 0.3 mg/kg of chromium citrate causes a significant increase in the content of Germanium in mushrooms, and the enrichment of the substrate with 0.3 mg/kg of selenium citrate ensures the accumulation of Germanium in mushrooms at the same level as when applying germanium citrate (Fig. 6b). This fact once again emphasises the crucial importance of the composition of the substrate, which naturally contains large amounts of Ferrum, Manganese and Zinc, for the formation of the trace element balance of the shiitake fungus and the possibility of modifying it by adding additional amounts of trace elements to the substrate.



a)



b)

Fig. 6. Accumulation of Chromium (a) and Germanium (b) by the fruiting body of shiitake mushrooms depending on the citrate content of some biological elements in the substrate (synergistic effect)

Conclusion

Thus, the shiitake substrate-mushroom system acts as a complex system where the Le Chatelier principle, synergism and atognism of myconutrients can be traced in the formation of the trace element composition of the fruiting body of the fungus, depending on the substrate composition.

The study of these processes makes it possible to predictably modify the trace element composition of the fungus, enriching it with one or more biologically active trace elements. The introduction of predictable amounts of trace elements into the L. Edodes substrate allows the synthesis of a balanced raw material base enriched with bioavailable trace elements for the creation of functional foods.

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ОСОБЛИВОСТІ НАКОПИЧЕННЯ БІОЕЛЕМЕНТІВ ГРИБАМИ L. EDODES ПРИ КУЛЬТИВУВАННІ В СУБСТРАТАХ, ЗБАГАЧЕНИХ ЦИТРАТАМИ Cr, Se, Ge, Fe

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Анотація. У роботі досліджено особливості біотрансформації цитратів Cr, Se, Ge, Fe при культивуванні Lentinula Edodes та їхнього впливу на накопичення деяких біоелементів з метою прогнозування біологічної цінності, лікувально-профілактичних властивостей грибів шиїтаке. Для досліджень використовували штам Lentinula Edodes 3790, Mycelia, Belgium. Субстрат для L. Edodes готували з тирси дуба, висівок злакових, лушпиння соняшнику, вторинних продуктів перероблення сої, льону, або коноплі, гіпсу та крейди. Внесення цитратів Cr, Se, Ge, Fe в субстрат здійснювали шляхом його зволоження відповідними розчинами до досягнення в субстратах вмісту цитратів Cr, Se, Ge 0,3, 1,0 та 3,0 мг/кг, цитрату Fe – 20,0, 50,0, 100,0 мг/кг. Вміст біоелементів визначали методом оптико-емісійної спектроскопії з індуктивно зв'язаною плазмою. Згідно результатів досліджень, збагачення субстрату цитратами Cr, Se, Ge, Fe приводить до їхнього акумулювання грибами шиїтаке, а також впливає на мікроелементний склад грибів у цілому, але модифікування мікроелементного складу субстрату не приводить до накопичення біоелементів і токсикантів вище рівня гранично-допустимої концентрації. Внесення в субстрати цитратів заліза 20 мг/кг, селену 0,3 мг/кг і германію 0,3 мг/кг обумовлює збільшення вмісту Хрому в грибах у значно більшій кількості, ніж при збагаченні субстрату цитратом хрому. Внесення 0,3 мг/кг цитрату хрому забезпечує значне збільшення вмісту Германію в грибах, а збагачення субстрату на 0,3 мг/кг цитрату селену забезпечує накопичення Германію на тому ж рівні, як і при внесенні цитрату германію. Таким чином, система субстрат-гриб шиїтаке виступає як складна система де прослідковується дія принципу Ле Шательє, спостерігається синергізм і атогонізм мікронутрієнтів при формуванні мікроелементного складу плодового тіла гриба в залежності від складу субстрату. Дослідження цих процесів дає можливість прогнозовано модифікувати мікроелементний склад гриба, збагачуючи його на один або одночасно декілька біологічно активних мікроелементів. Внесення в субстрат прогнозованих кількостей біоелементів дозволяє L. Edodes синтезувати збалансовану збагачену біодоступними мікроелементами сировинну базу для створення функціональних продуктів харчування.

Ключові слова: L. Edodes, фортифікація, Хром, Селен, мікроелементний склад, функціональні продукти харчування.