Проведено порівняння вмісту 11 елементів (Al, Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Si, Zn) в плодових тілах 9 видів нівальних міксоміцетів та їх субстратах, зібраних в італійських та французьких Альпах. Виявлена здатність нівальних видів акумулювати високотоксичні важкі метали Cd та Pb, а також Zn і Cu

Ключові слова: важкі метали, міксоміцети, нівальні види, біоакумуляція, моніторинг, екологічна безпека, паспортизація

Проведено сравнение содержания 11 элементов (Al, Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Si, Zn) в плодовых телах 9 видов нивальных миксомицетов и их субстратах, собранных в итальянских и французских Альпах. Обнаружена способность нивальных видов аккумулировать высокотоксичные тяжелые металлы Cd и Pb, а также Cu и Zn

Ключевые слова: тяжелые металлы, миксомицеты, нивальные виды, биоаккумуляция, мониторинг, экологическая безопасность, паспортизация

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THE ANALYSIS OF METALS BIOTRANSFORMATION BY ALPINE NIVICOLOUS MYXOMYCETES FROM SUBSTRATES

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1. Introduction

In recent decades metallomics is developing actively, it is an analytical scientific field which studies the content and functions of metals in biological systems [1]. The metallomics overcomes interdisciplinary barriers between chemistry, biology, physics, medicine and ecology, revealing the features of accumulation, transport, metabolism and toxicity of metal ions. The importance of metals for the life of living organisms is no doubt, but their role in the functioning of biological systems is still not fully understood. In the sphere of environmental safety there are studies about accumulation of heavy metals by plants [2], animals [3], fungi [4] and microbiological objects [5], but very little is known about the bioaccumulative properties of myxomycetes [6]. Whereas myxomycetes combine both fungi and protists features, they are common favorable objects for different modeling researches. On the vegetative stage of the life cycle, myxomycetes plasmodia actively absorbs bacteria, single cells and hyphae of fungi, as well as organic and inorganic particles of soil and substrates, accumulating elements in different forms and playing the role of a native bioconcentrator. Generative stage of myxomycetes is represented by the sporocarps, whose analysis can evaluate the environmental situation, because all elements accumulated by myxomycetes, were collected from their immediate environment. In addition to bioindication and bioremediation potential, myxomycetes can help in discovering the mechanisms of resistance to unfavourable environmental conditions, especially it concerns nivicolous myxomycetes. This environmental group is associated with alpine, subalpine and arctic zones, where nivicolous myxomycetes produce sporophores in spring on plant substrates near the melting snow. Nivicolous species are able to survive in conditions of extreme temperatures and intense insolation, so such study will help to reveal the mechanisms of adaptation of living organisms to unstable environmental conditions. The study of the elements contents in the fruiting bodies of nivicolous myxomycetes will contribute to the research of biogeochemical cycle of elements and help to find out the ways of chemical elements transformation in the ecosystems food chains. For the sphere of the environmental safety, it is especially important to find out ways of heavy metals transformation in the environment, and myxomycetes are very convenient experimental organisms for realization of these tasks due to their special biological characteristics and widespread distribution.

2. Literature review and problem statement

Despite the relevance of research of transformation of heavy metals in ecosystems and the potential of myxomycetes to bioaccumulation, information on the concentrations of elements in samples of myxomycetes is represented only in a few publications. The concentration of aluminum, iron, zinc, copper, mercury and cadmium in fruit bodies of seven myxomycetes: *Fuligo septica*, *Lycogala epidendrum*, *Tubifera ferruginosa*, *Symphytocarpus flaccidus*, *Amaurochaete atra*, *Ceratiomyxa fruticulosa*, *Stemonitis* sp., and two uncertain plasmodium species was determined [7]. There was a significant content of metals in general and enormously high concentration of zinc (2200–20000 mg/kg) in herbarium and field specimens of *F. septica*. The average content of zinc was 240 times higher than in leaves of blueberry (9.7–160 mg/kg) collected near myxomycetes to determine the background level of contamination of the investigated areas. In addition, all investigated myxomycetes except *Stemonitis* sp., demonstrated the ability to accumulate Cd, and the content of the element (11 mg/kg) in some *Symphytocarpus flaccidus* samples was more than 300 times higher than the background (0.034 mg/kg).

Next research of the elements in myxomycetes was carried out after 10 years only [8]. In herbarium specimens of *Fuligo septica, Reticularia splendens, R. lycoperdon, Tubifera ferruginosa* and *Lycogala epidendrum*, received from Australia, Canada, USA and Switzerland, the content of 60 elements was analyzed. However, for 50 of them, including rare earth metals, as well as As, Sb and Se, concentrations were so low that these data were not included in the publication. The extraordinary accumulation ability of *F. septica* was confirmed and it was found that this species also accumulates calcium, iron, barium, cadmium, strontium and manganese. In comparison with other studied samples, *Tubifera ferruginosa* wasn't distinguished by special accumulative properties, but contained slightly elevated concentrations of heavy metals iron, zinc and magnesium. In *Reticularia lycoperdon* there was a high amount of rare metal bismuth (20 mg/kg) compared to other myxomycetes (<0.05 mg/kg). It turned out that *Lycogala epidendrum* has a tendency to accumulate Sn, while as in other living organisms this element is present only in trace amounts. Also in *L. epidendrum* the tendency to accumulate copper (52–84 mg/kg) was revealed, while other four species are not accumulating this metal (3–14 mg/kg). Given that the two previous works have been focused on the extraordinary properties of *F. septica*, it is not surprising that the next research was about zinc accumulation by this species. It was the comparison of zinc content in 19 samples of *F. septica* and in its substrates, collected in Russia, Lithuania, Uzbekistan, Kyrgyzstan, Tajikistan and North Korea [9]. The average value of zinc concentration in substrates proved 555.75 mg/kg, and in myxomycetes – 25 times higher (14234.38 mg/kg), and in some cases the ratio reached 72. The phenomenal ability of *F. septica* to hyperaccumulate Zn was explained recently. Polish researchers found that the *F. septica* resistance to toxic influence of zinc and other heavy metals is connected with yellow pigment fuligorubin A with a chemical structure, which allows it to chelate metals and convert them to inactive forms [10]. After that, USA researchers expanded the range of experimental species and used the combined methods [11]. Using electron microscopy and X-ray microanalysis, the distribution of metals among myxomycetes morphological structures was investigated. Besides *F. septica*, the species from genera *Physarum, Hemitrichia, Trichia* and *Stemonitis* were involved. Quantitative content of elements was determined in three species of myxomycetes. For five metals Fe, Mn, Cu, Zn and Al, the method of emission spectroscopy with inductively coupled plasma was used, and Pb was identified by atomic absorption spectrometry. The highest concentration of Pb, Mn and Zn was found in *F. septica*, compared with *Trichia favoginea* and *Stemonitis splendens*.

The latest work carried out in the Philippines showed that the contents of Cr and Mn in sporophores of *Arcyria cinerea*, *Physarum album* and *Ph. pusillum* were higher than the concentration of these metals in myxomycetes substrates [12]. The comparison with laboratory experiments in sterile water agar showed that bioabsorbtion by *Physarum album*, *Ph. cinereum* and *Ph. melleum* depends on bacteria absorbed by myxomycetes plasmodia in nature. Philippine researchers believe that "microbial cocktails" of myxomycetes with large plasmodia in combination with bacterial isolates can be used for bioremediation of contaminated sites. In other mentioned publications, the practicability of using myxomycetes to study the impact of toxic metals on the ecosystem was also emphasized [10]. As myxomycetes are ubiquists and accumulate substances directly from their habitats, they can be used for biomonitoring of heavy metals [11]. There is a hope that after the identification of genes corresponding to the toxic elements sequestration mechanism, their cloning and implantation into plants with a larger biomass for bioremediation of contaminated areas will be possible [9].

None of the above studies involved nivicolous myxomycetes, although the characteristics of their physiology may be associated with bioabsorption of elements from the environment. It is no coincidence that nivicolous species are virtually impossible to cultivate in the laboratory, because they require specific conditions, which are available only in their natural environment. Besides such factors as temperature, humidity and insolation, it is possible that metals contained in the soil and substrates also have a significant impact on the development of nivicolous myxomycetes. In this regard, the study of the elements transformation patterns by nivicolous myxomycetes was started in the Ukrainian Carpathians [13]. The data about accumulation of Ca, Mn, Mg, Al, Ni, and toxic elements Pb and Cd in fruit bodies of *Diderma globosum, D. meyerae, D. niveum, Didymium dubium, Lamproderma splendens, L. ovoideoechinulatum, L. spinulosporum, Meriderma echinulatum, Physarum albescens* and *Ph. alpestre* were obtained, but there was no comparison of the content of these elements in the substrates. The new research was carried out in the Italian and French Alps, elements were analyzed as not only in myxomycetes, but also in their substrates.

3. The purpose and objectives of the study

The study aims to identify the patterns of elements accumulation by alpine nivicolous myxomycetes compared to their substrates to determine their bioaccumulation capabilities and ways of heavy metals transformation.

To achieve this goal, the following tasks were formulated:

– to examine the distribution of 11 elements in fruiting bodies of 9 myxomycetes species and their substrates, and calculate transformation ratios to identify species with the highest bioaccumulation properties;

– to determine the ability of the studied nivicolous myxomycetes to accumulate heavy and toxic metals;

– to compare samples of the same species from different countries for establishing correlations between the concentrations of elements in myxomycetes and environmental conditions;

– to investigate the possibility of a connection between the ratio of elements in the substrate and slime molds and the features of the life cycle of plasmodia.

4. Materials and methods of study of the elements content in the myxomycetes fruiting bodies and their substrates

4. 1. Materials for research, sampling and identification of specimens

For the study of heavy metals and other elements, 10 samples of nine myxomycetes species were selected in the spring of 2014–2015 on the edge of melting snow in the Alps. Samples of myxomycetes were found in the following localities:

1) *Trichia decipiens* (Pers.) T. Macbr. – France, the Alps, surroundings of Engins near Grenoble in the fir forest at an altitude of 1350 m, on the rotten trunk of *Picea abies* (L.) H. Karst. covered with moss, 03.11.2014;

2) *Diderma fallax* (Rostaf.) Lado with *Lamproderma echinosporum* Meyl. – the same location, in open alpine meadows at an altitude of 1100 m, on living blackthorn twigs *Prunus spinosa* L., 13.03.2015;

3) *Lamproderma pseudomaculatum* Mar. Mey. & Poulain – the same location on fallen twigs, 17.3.2015;

4) *Lamproderma arcyrioides* (Sommerf.) Rostaf. – the same location, on the edge of a beech forest, on a living branch of *Fagus sylvatica* L., 23.03.2015;

5) *Physarum vernum* Sommerf. – the same location, on the dry leaf of *Fagus sylvatica* L., 23.03.2015;

6) *Diderma alpinum* (Meyl.) Meyl. with *Physarum alpestre* Mitchel, S. W. Chapm. & M. L. Farr – Italy, the Alps, near Bagni di Vinadio, province of Cuneo, at an altitude of 1305 m, on living twigs of an alder bush *Alnus glutinosa* (L.) Gaertn., 29.04.2015;

7) *Diderma globosum* Pers. – the same location, on dry grass, 29.04.2015;

8) *Lamproderma pseudomaculatum* Mar. Mey. & Poulain – the same location, on dry fallen twigs, 29.04.2015.

Samples with fragments of substrates were kept in separate cardboard boxes. Determinations of specimens were made with the use of "Les Myxomycètes" [14]. Identification was based on the characteristics of peridium, capillitium, spores and the overall structure of the myxomycetes fruiting body. Microscopic characteristics were investigated using a light microscope Carl Zeiss Axiostar with lenses Zeiss Acroplan 40x, 100x, 400x and 630x.

4. 2. Determination of elements in myxomycetes samples

All myxomycetes specimens and their substrates were analyzed for contents of 11 elements: Al, Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Si and Zn. The methods of emission spectroscopy with inductively coupled plasma, atomic absorption spectrometry, electron microscopy and X-ray microanalysis were used for determination of the elements in the myxomycetes fruiting bodies [8, 11]. The first method is often used to determine the exact concentration of most elements. Therefore, measurement of elements in the myxomycetes fruiting bodies and substrates was carried out by atomic emission spectrometry with inductively coupled plasma (AES-ICP) in the laboratory of analytical chemistry and monitoring of toxic substances of the SI "Institute for Occupational Health of NAMS of Ukraine". The samples were prepared according to the methods of analytical chemistry, and measurements were made by the device Perkin Elmer Optima 210 DV (USA). 0.1 g of the sample was taken, 2.0 mL of concentrated HNO_3 (Merck) was added, and after some time it they were by closed method in the microwave Berghof MWS-2. The resulting transparent miniralizate was dissolved in deionized water (18Ω) to a volume of 10 ml for analysis by AES-ICP. Optima 2100 DV is an optical spectrometer with semiconductor solid-state detector with inductively coupled plasma as the excitation source. In the spectrometer, spectral background correction is implemented using a multispectral filtering algorithm (MSF and IEC). Management and control of the spectrometer were made by WinLab32 software in the operating system Windows XP prof. The data were mathematically processed by the device and displayed on a monitor in the proper format [15].

4. 3. The calculation of ratios and graphical representation of results

The ratios of the elements in the fruiting bodies of the studied myxomycetes and substrates on which they produced sporophores, were calculated using the formula:

$$K_t=C_m/C_s,$$

where K_t – conversion factor of the elements in the "substrate-myxomycetes" system, C_m – element content in the fruit bodies of myxomycetes, C_s – element content in the substrate. This ratio is called the coefficient of biological absorption [2] or bioconcentration factor (BCF) [12]. Since in this study there is no direct evidence that elements absorption proceeded from substrates, this ratio is called the coefficient of transition of the elements in the "substrate-myxomycetes" system (K_t).

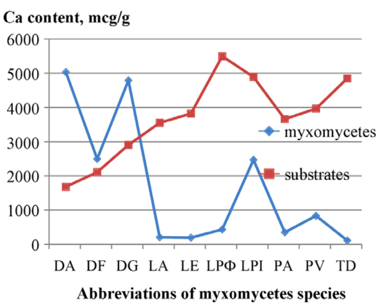
The results were processed using Excel and presented in graphs and tables. The research was held at the laboratory of the department of labour and environmental protection at the faculty of engineering systems and ecology of the Kyiv National University of Construction and Architecture (Ukraine).

5. The analysis of elements in nivicolous myxomycetes and their substrates

Nine species of myxomycetes were analyzed, they belong to 4 genera of Physaraceae, Stemonitaceae and Trichiaceae families in Physarales, Stemonitales and Trichiales orders of the class Myxomycetes of Amoebozoa subkingdom. Eight species belong to the ecological group of nivicolous myxomycetes, which form sporocarps on the edge of melting snow in spring on open alpine areas. In addition, for comparison *Trichia decipiens* was studied, which, although it does not belong to nivicolous species, but also was collected in the alpine region of the Alps. Four samples (*Diderma alpinum, D. globosum, Lamproderma pseudomaculatum* and *Physarum alpestre*) were selected in the Italian Alps, and six (*Diderma fallax, Lamproderma arcyrioides, L. echinosporum, L. pseudomaculatum, Physarum vernum* and *Trichia decipiens*) – in the French Alps, but only *Lamproderma pseudomaculatum* was common for both areas.

5. 1. The content of elements in the fruiting bodies of myxomycetes and their substrates

The highest concentration of Ca (5494.71 mcg/g) was found in dry twigs of the substrate of *Lamproderma pseudomaculatum* from France. And the same substrate of this myxomycetes species from Italy demonstrated minimal concentrations of Cd – 0.004 mcg/g. In general, according to the average concentration, the elements in the substrates formed the next sequence: Ca (3693.13)>Mg (471.08)>Al (55.49)>>Fe (43.15)>Mn (35.8)>Si (29.08)>Zn (14.91)>Pb (2.93)>>Cu (0.42)>Ni (0.28)>Cd (0.05 mcg/g). It is interesting that the average concentration of the elements for fruiting bodies of the studied myxomycetes has a different sequence: Ca (1692.38)>Mg (1338.87)>Fe (996.1)>Al (199.59)>>Si (125.29)>Mn (50.47)>Cu (17.32)>Pb (13.22) Ni (2.63)>>Cd (1.7)>Zn (0.04 mcg/g). According to the obtained average concentrations of the elements, the coefficients of transition from substrates to myxomycetes are reduced in the following direction: Cu (41.24)>Cd (34)>Fe (23.08)>>Ni (9.39)>Pb (4.51)>Si (4.31)>Al (3.59)>Mg (2.84)>>Mn (1.41)>Ca (0.46)>Zn (0.003). However, it should be considered that the concentration of each element may significantly differ from the average, depending on the samples of myxomycetes and their substrates. The highest concentration compared with other elements was for Ca: 5033.33 mcg/g in *Diderma alpinum* and 4790 mcg/g in *D. globosum*. In most of the studied myxomycetes, calcium content in the substrate was higher than its concentration in the fruiting bodies (Fig. 1).

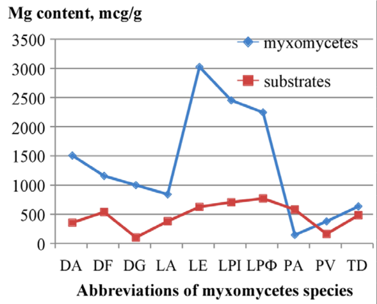


№	Species	Ca content, mcg/g		K_t
		myxo-mycetes	sub-strates	
1	DA	5033,33	1684,15	2,99
2	DF	2496	2108,21	1,18
3	DG	4790	2905,5	1,65
4	LA	207	3550,82	0,06
5	LE	195	3822	0,05
6	$LP_Ф$	435	5494,71	0,08
7	LP_I	2470	4891,25	0,51
8	PA	350	3659,76	0,1
9	PV	835	3965	0,21
10	TD	112,5	4849,91	0,02
	AV	**1692,38**	**3693,13**	**0,69**

Fig. 1. The content of calcium in the fruiting bodies and substrates of myxomycetes: DA – *Diderma alpinum*, DF – *D. fallax*, DG – *D. globosum*, LA – *Lamproderma arcyrioides*, LE – *L. echinosporum*, LP – *L. pseudomaculatum* (LPФ – sample from France, LP_I – sample from Italy), PA – *Physarum alpestre*, PV – *Ph. vernum*, TD – *Trichia decipiens*, AV – average, K_t – coefficient of transition

While for genus *Lamproderma* and for *Trichia decipiens* it is an expected effect, but for the genus *Physarum* the tendency of reducing the calcium concentration compared with their substrates is unexpected. Because the species from Physarales are characterized by the accumulation of lime in their morphological structures. But the species of the genus *Diderma*, which also belong to the order Physarales, confirmed the fact of lime concentration, although the coefficients of transition for Ca didn't demonstrate impressive differences.

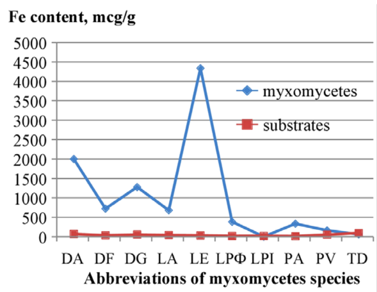
The highest magnesium content was in *Lamproderma echinosporum* – 3024.5 mcg/g and slightly lower in *L. pseudomaculatum*: 2452.51 mcg/g of Mg was found in sporangia of this species from Italy, while in samples from France – 2245.2 mcg/g. However, fruiting bodies of *Physarum alpestre* had lower concentration of magnesium (146.07 mcg/g) than in their substrate (580.33 mcg/g). Thus, despite the overall high Mg content in sporophores of myxomycetes, small K_t values (from 0.25 to 9.72) call into question-the studied species ability to accumulate this element (Fig. 2).



№	Species	Mg content, mcg/g		K_t
		myxo-mycetes	sub-strates	
1	DA	1507,88	356,57	4,23
2	DF	1157,2	538,67	2,15
3	DG	999,4	102,79	9,72
4	LA	841,6	380,76	2,21
5	LE	3024,5	627,56	4,82
6	LP_I	2452,51	706,71	3,47
7	$LP_Ф$	2245,2	771,85	2,91
8	PA	146,07	580,33	0,25
9	PV	378,7	162,3	2,33
10	TD	635,68	483,27	1,31
	AV	**1338,87**	**471,08**	**3,34**

Fig. 2. The content of magnesium in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)

Among all the samples of myxomycetes, the highest iron content was found in *Lamproderma echinosporum* – 4337.53 mcg/g (Fig. 3).



№	Species	Fe content, mcg/g		K_t
		myxo-mycetes	sub-strates	
1	DA	2000,16	70,92	28,2
2	DF	722,28	34,74	20,79
3	DG	1277,88	55,2	23,15
4	LA	680,61	40,56	16,78
5	LE	4337,53	31,2	139
6	$LP_Ф$	384	18,36	20,92
7	LP_I	0,6	19,5	0,03
8	PA	336	13,44	25
9	PV	162	51,6	3,14
10	TD	60	96	0,63
	AV	**996,11**	**43,15**	**27,76**

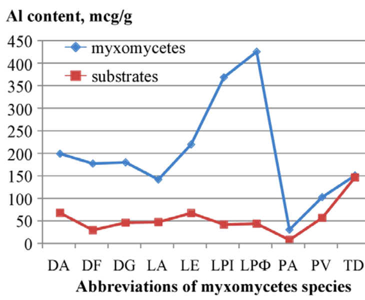
Fig. 3. The content of iron in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)

It is interesting that the sample of *L. pseudomaculatum* from Italy is characterized by the lowest content of Fe (0.6 mcg/g), even compared with a sample from France (384 mcg/g) of the same species on a similar substrate. Also the lower content of Fe (60 mcg/g) than in the substrate (96 mcg/g) was found for *Trichia decipiens*. It should be noted that for a number myxomycetes, relatively high rates of iron K_t from substrates in myxomycetes were observed: *Diderma alpinum* – 28.2, *D. globosum* – 23.15, *Lamproderma pseudomaculatum* from France – 20.92, *D. fallax* – 20.79, *L. arcyrioides* – 16.78.

The highest content of aluminum was found in the samples of *Lamproderma pseudomaculatum* from France 368.68 mcg/g, and the lowest – in the fruiting bodies of *Physarum alpestre* – 30.49 mcg/g (Fig. 4).

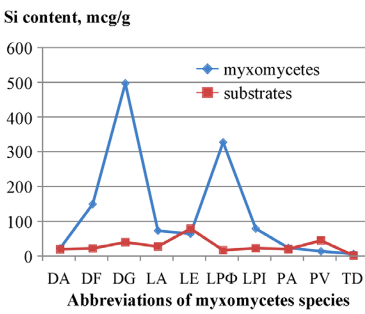
The smallest values of K_t for Al are characteristic of the species *Physarum vernum* (1.81) and *Trichia decipiens* (1.03), and the largest – *Lamproderma pseudomaculatum* from Italy (9.96) and France (8.78). Currently, a special capacity for accumulation of aluminum by the studied myxomycetes was not observed, as indicated by small values of the coefficient of transition for this element.

The highest silicon content was found in the fruiting bodies of *Diderma globosum* (496.92 mcg/g), and the lowest – in *Trichia decipiens* (5.45 mcg/g). In the samples of *Physarum vernum* (13.64 mcg/g) and *Lamproderma echinosporum* (63.63 mcg/g), the concentration of Si was smaller than in their substrates – 44.42 and 78.78 mcg/g, respectively. However, some species are characterized by relatively high K_t, specifically *Lamproderma pseudomaculatum* from France (19.78) and *Diderma globosum* (12.62) (Fig. 5).



№	Species	Al content, mcg/g		K_t
		myxomycetes	substrates	
1	DA	199,1	67,71	2,94
2	DF	177,25	29,57	5,99
3	DG	180	46,09	3,91
4	LA	141,8	47,29	3
5	LE	219,79	67,36	3,26
6	$LP_Ф$	368,68	42,01	8,78
7	LP_I	425,4	43,89	9,69
8	PA	30,49	7,73	3,94
9	PV	102,81	56,72	1,81
10	TD	150,66	146,55	1,03
	AV	**199,59**	**55,49**	**4,44**

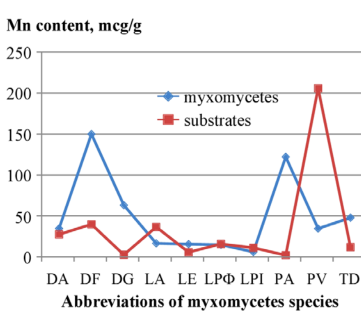
Fig. 4. The content of aluminum in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)



№	Species	Si content, mcg/g		K_t
		myxomycetes	substrates	
1	DA	22	19,27	1,14
2	DF	149,28	21,87	6,83
3	DG	496,92	39,39	12,62
4	LA	72,72	27,15	2,68
5	LE	63,63	78,78	0,81
6	$LP_Ф$	327,24	16,54	19,78
7	LP_I	78,78	22,36	3,52
8	PA	23,33	19,7	1,18
9	PV	13,64	44,42	0,31
10	TD	5,45	1,33	4,1
	AV	**125,3**	**29,08**	**5,3**

Fig. 5. The content of silicon in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)

The concentration of manganese has considerable variations in values in both myxomycetes, and their fruiting bodies. In four cases, the manganese content in the substrates was higher than the concentration of this element in myxomycetes sporocarps: *Lamproderma arcyrioides*, both samples of *L. pseudomaculatum* and *Physarum vernum*. In the latter case, the difference of concentrations is significant (Fig. 6).

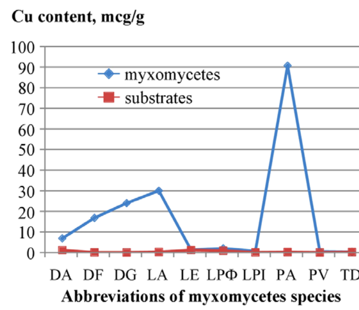


№	Species	Mn content, mcg/g		K_t
		myxomycetes	substrates	
1	DA	34,67	27,36	1,27
2	DF	150	39,72	3,78
3	DG	63,12	2,5	25,24
4	LA	16,43	36,48	0,45
5	LE	15,48	5,59	2,77
6	$LP_Ф$	14,71	15,74	0,93
7	LP_I	5,88	11,03	0,53
8	PA	122,09	1,91	63,92
9	PV	34,5	205,94	0,17
10	TD	47,81	11,77	4,06
	AV	**50,47**	**35,8**	**10,31**

Fig. 6. The content of manganese in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)

The maximum concentration of Mn was in *Diderma fallax* – 150 mcg/g, and the minimum – in *Lamproderma pseudomaculatum* from Italy (5.88 mcg/g). The low Mn content in the substrate (1.91 mcg/g) with a high concentration of this element in *Physarum alpestre* caused a high value of the coefficient of transition (K_t=63.9).

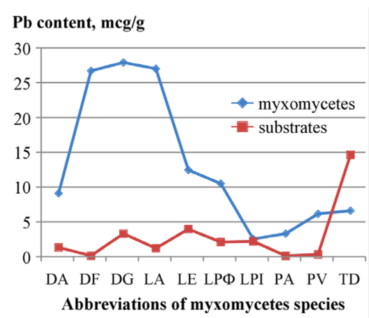
Some species of slime molds show a pattern of copper accumulation, because in absolutely all investigated samples the concentration of Cu exceeded the content of this element in the substrates. This is especially true for *Diderma globosum* (K_t=960), *Physarum alpestre* (K_t=412.4), *D. fallax* (K_t=280) and *Lamproderma arcyrioides* (K_t=103.4). All substrates were characterized by a very low content of Cu, which was in the range of 0.025 to 1.21 mcg/g. Most copper appeared in *Physarum alpestre* (90.72 mcg/g), and the least was in *Trichia decipiens* (0.33 mcg/g) (Fig. 7).



№	Species	Cu content, mcg/g		K_t
		myxomycetes	substrates	
1	DA	6,9	1,18	5,85
2	DF	16,8	0,06	280
3	DG	24	0,025	960
4	LA	30	0,29	103,4
5	LE	1,26	1,21	1,04
6	$LP_Ф$	2,02	0,91	2,22
7	LP_I	0,67	0,064	10,47
8	PA	90,72	0,22	412,4
9	PV	0,5	0,028	17,86
10	TD	0,33	0,23	1,43
	AV	**17,32**	**0,42**	**179,5**

Fig. 7. The content of copper in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)

Lead ranks fifth among the average values of the coefficient of transition (K_t=4.51). The lead content in the substrates was consistently lower than in the fruiting bodies of myxomycetes, ranging from 0.12 to 3.96 mcg/g, except *Trichia decipiens*, where the concentration of Pb in the wood of *Picea abies* (14.63 mcg/g) was twice as much as in the sporangia of myxomycetes (6.6 mcg/g). The ability to accumulate Pb was shown by *Diderma fallax*, judging by the high value of K_t=190.7. The maximum amount of lead was found in three species of myxomycetes: *Diderma globosum* – 27.9, *Lamproderma arcyrioides* – 27 and *D. fallax* – 26.7 mcg/g. The least Pb was found in *Lamproderma pseudomaculatum* from Italy – 2.53 and *Physarum alpestre* – 3.3 mcg/g (Fig. 8).

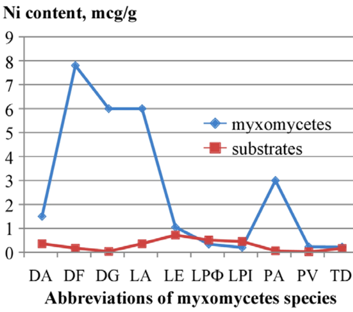


№	Species	Pb content, mcg/g		K_t
		myxomycetes	substrates	
1	DA	9,1	1,32	6,89
2	DF	26,7	0,14	190,7
3	DG	27,9	3,3	8,46
4	LA	27	1,2	22,5
5	LE	12,45	3,96	3,14
6	$LP_Ф$	10,5	2,09	5,02
7	LP_I	2,53	2,2	1,15
8	PA	3,3	0,12	27,5
9	PV	6,15	0,33	18,64
10	TD	6,6	14,63	0,45
	AV	**13,22**	**2,93**	**28,45**

Fig. 8. The content of lead in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)

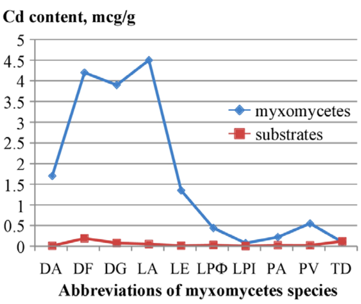
The content of nickel is stable low in the substrates and it is within 0.02–0.72 mcg/g. However, the coefficient of transition for this element from a substrate to the fruiting body of *Diderma globosum* reaches 153.8. Also this species showed relatively high concentrations of nickel – 6 mcg/g, the same as in *Lamproderma arcyrioides*, but the highest content of Ni was noted in *Diderma fallax* – 7.8 mcg/g (Fig. 9).

The substrates demonstrated consistently high levels of Cd, ranging from 0.004 to 0.19 mcg/g. Myxomycetes showed the ability to accumulate this element, based on the coefficient of transition: 170 for *Diderma alpinum*, 103 for *Lamproderma echinosporum*, 86.54 for *L. arcyrioides* and 50.65 for *D. globosum*. The maximum content of Cd was found in three species of myxomycetes: *Lamproderma arcyrioides* – 4.5, *Diderma fallax* – 4.2 and *D. globosum* – 3.9 mcg/g. The minimum content of Cd from all samples was in *Lamproderma pseudomaculatum* from Italy – 0.047 mcg/g. For Cd, the second largest average value of the coefficient of transition from the substrate to myxomycetes, equal to 34 was obtained (Fig. 10).



№	Species	Ni content, mcg/g myxo-mycetes	sub-strates	K_t
1	DA	1,5	0,36	4,17
2	DF	7,8	0,17	45,88
3	DG	6	0,039	153,8
4	LA	6	0,36	16,67
5	LE	1,05	0,72	1,46
6	LP_{Φ}	0,34	0,51	0,67
7	LP_I	0,2	0,45	0,44
8	PA	3	0,06	50
9	PV	0,23	0,02	11,5
10	TD	0,22	0,16	1,38
	AV	**2,63**	**0,28**	**28,6**

Fig. 9. The content of nickel in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)

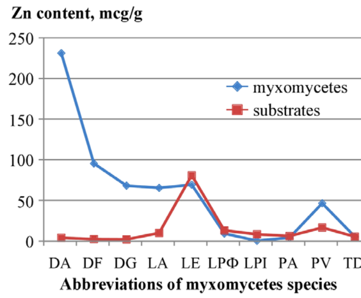


№	Species	Cd content, mcg/g myxo-mycetes	sub-strates	K_t
1	DA	1,7	0,01	170
2	DF	4,2	0,19	22,11
3	DG	3,9	0,077	50,65
4	LA	4,5	0,052	86,54
5	LE	1,35	0,013	103,8
6	LP_{Φ}	0,44	0,029	15,17
7	LP_I	0,074	0,004	18,5
8	PA	0,22	0,024	9,17
9	PV	0,55	0,021	26,19
10	TD	0,1	0,12	0,83
	AV	**1,7**	**0,05**	**50,3**

Fig. 10. The content of cadmium in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)

Among the other studied samples, much higher zinc content was in *Diderma alpinum* – 231.06 mcg/g, and the minimum amount was recorded in the samples of *Lamproderma pseudomaculatum* from Italy – 0.36 mcg/g. In the latter species, Zn content in the sporangia was lower than in the substrates that affected the value of the coefficient of transition: K_t=0.04 for the samples from Italy and 0.71 – for the samples from France. The same trend is typical for two species: K_t=0.85 for *Lamproderma echinosporum*, and 0.69 for *Physarum alpestre*. On the other hand, the highest coefficients of transition for Zn from substrates to myxomycetes were recorded for all three studied species of the genus *Diderma*: *D. alpinum* K_t=57.05, *D. fallax* – 40.09, *D. globosum* – 33.69 (Fig. 11). This could indicate the zinc bioaccumulation ability of some species of *Diderma*. It should be noted that zinc is the last in the gradation of the average values of the coefficient of transition – K_t=0.003.

In general, the highest content was found for the three elements in the fruiting bodies of five species of myxomycetes: Ca – 5033.33 mcg/g in *Diderma alpinum*, in *D. globosum* – 4790, in *D. fallax* – 2496 and in *Lamproderma pseudomaculatum* from Italy – 2470; Fe – 4337.53 in *L. echinosporum*, 2000.16 – in *D. alpinum* and 1277.88 – in *D. globosum*; Mg – 3024.5 in *L. echinosporum*, 2452.51 – in *L. pseudomaculatum* from Italy, 2245.2 – in *L. pseudomaculatum* from France, 1507.88 – in *D. alpinum* and 1157.2 – in *D. fallax*. Herewith, the transition coefficient of the elements from substrates to myxomycetes exceeded 100 units only in 8 % of samples: for Cu – 960 in *D. globosum*, 412.4 – in *Physarum alpestre*, 280 – in *D. fallax* and 103.4 – in *Lamprederma arcyrioides*; for Pb – 190.7 in *D. fallax*; for Cd – 170 in *D. alpinum* and 103.8 in *L. echinosporum*; for Ni – 153.8 in *D. globosum* and for Fe 139 – in *L. echinosporum*. In 25 % of the samples, K_t had a value from 10 to 100, in 45 % – from 1 to 10 and in 22 % K_t was less than one.



№	Species	Zn content, mcg/g myxo-mycetes	sub-strates	K_t
1	DA	231,06	4,05	57,05
2	DF	95,42	2,38	40,09
3	DG	68,16	2,023	33,69
4	LA	65,43	9,99	6,55
5	LE	69,3	80,92	0,85
6	LP_{Φ}	9,29	13,09	0,71
7	LP_I	0,36	8,33	0,04
8	PA	4,29	6,19	0,69
9	PV	46,43	16,66	2,79
10	TD	5,48	5,47	1
	AV	**59,52**	**14,91**	**14,35**

Fig. 11. The content of zinc in the fruiting bodies and substrates of myxomycetes (abbreviations as in Fig. 1)

5. 2. The dependence of concentrations of elements on the types of substrates and location of samples

The comparison of two specimens of *Lamproderma pseudomaculatum* from different countries from the same substrate could provide the insight about the influence of terrain on the elemental composition in species. The content of elements in the substrates is almost the same, only the concentration of Cu in fallen branches from France is 14 times higher than it in same substrates from Italy, and the Fe content in the fruiting bodies of *L. pseudomaculatum* from France is 640 higher than that from Italy. This is the reason why the coefficient of transition has 700 times of the difference for iron: $K_t(Fe)_I$=0.03 and $K_t(Fe)_F$=20.92. Among the coefficients for other elements, the following should be mentioned: $K_t(Si)_F$=19.78, $K_t(Cd)_I$=18.5, $K_t(Cd)_F$=15.17 and $K_t(Cu)_I$=10.5. In general, for Si, Fe, Cu and Pb there are significant differences in the concentrations in *L. pseudomaculatum* from France and Italy, which shows the influence of environmental factors on the content of elements.

The coefficients of elements transition from substrates to the fruiting bodies not only show the bioaccumulation ability of myxomycetes, but also demonstrate the "myxomycetes-substrates" relationship. Typically, dry grass is one of the most common substrates for nivicolous myxomycetes, but in these studies, only one specimen was found on a grass litter – *Diderma globosum*. And this species showed high values of the coefficients of transition for many elements: Cu – 960, Ni – 154, Cd – 51, Zn – 34, Mn – 25, Fe – 23, Si – 13. This study showed the strongest relations between *Trichia decipiens* and *Picea abies* wood, as evidenced by the lowest levels of K_t. That is, the content of Al, Cd, Cu, Mg, Ni and Zn are nearly the same or the concentration of elements in the substrate is smaller than in the sporangia of myxomycetes, as in the case of Ca, Fe, Pb and Si. Herewith, *T. decipiens* is the only species belonging to the environmental group of xylophilous myxomycetes in this study. This may explain the phenomenon of the elements concentration similarity in the substrates and sporophores. These studies prove the assumption that *T. decipiens* at the plasmodium stage lives inside the fallen tree trunk, where actively feeds by microorganisms and organic particles, therefore its elemental composition is close to that of the substrates. The observation of the plasmodium in nature is very difficult, while comparing the elemental composition in substrates and myxomycetes was an actual proof that not only sporangium stage is associated with wood, but also it is true for plasmodium of xylophilous myxomycetes. However, fundamental differences of elements concentrations in the "substrates-myxomycetes" system were found for the representatives of nivicolous myxomycetes of the ecological group, which include all other studied samples. This can be explained by the fact that most of their life cycle nivicolous myxomycetes spend as plasmodium, which moves in the soil and plant litter under the snow, selectively accumulating different elements. The substrate, on which nivicolous species were found is primarily serves as a dry place with sufficient insolation for sporulation. That's why, regular correlations of K_t with the types of substrates for the studied nivicolous samples were not found. Accordingly, the analysis of the elemental composition of myxomycetes offers opportunities to study hidden processes of plasmodial life in the field.

6. Discussion of data about bioaccumulation capability of myxomycetes

From the standpoint of environmental safety, among 11 analyzed elements the toxic heavy metals Cd, Pb, Zn are particularly important. The results suggest that nivicolous myxomycetes have the ability to accumulate cadmium and moderate propensity to accumulation of lead, while zinc is the last in the gradation of average values of the transition coefficient Cu and Ni are considered to be moderately toxic heavy metals, what is more Cu is in a top of elements accumulation in this study, and Ni is in the third place. The ability to accumulate copper by myxomycetes is clearly demonstrated by extremely high values of K_t for four species, and total excess of this element in all myxomycetes compared to the substrates. Manganese belongs to low toxic heavy metals and the study revealed no clear trends of bioaccumulation by myxomycetes. In general, the accumulative capability of myxomycetes opens perspectives for their remediation using.

Like other myxomycetes, nivicolous species and their substrates contain the highest level of calcium. This is logical considering the important role of this element in a wide range of vital processes in living organisms. However, in most cases Ca was the only element whose concentration in the substrate was higher than its content in myxomycetes. Fe ranked second in terms of concentration, consistently demonstrating higher content in myxomycetes than in all the analyzed substrates, indicating a moderate capacity for iron bioaccumulation by myxomycetes. In addition, the high content of Mg was found in sporophores, but small values of K_t refute the assumption of the ability of the studied myxomycetes species to exclusive bioaccumulation of these elements. Distribution of Al and a single non-metallic element Si had species-specific character, but does not show apparent trends.

Proceeding from that the concentration of each element quite significantly varied depending on the samples of slime molds and their substrates, it was particularly interesting to analyze the samples of the same species *Lamproderma pseudomaculatum* from different countries, found on the same substrates. Similar elements contents in the fruiting bodies confirm the dependence of the bioaccumulation ability on specific morphological and physiological characteristics intrinsic for each species of myxomycetes. However, Si, Fe, Cu and Pb concentrations present significant differences, indicating the dependence of the elements content in myxomycetes on environmental factors. This fact creates preconditions for using myxomycetes as bioindicators in environmental safety.

The comparison of the elemental composition of myxomycetes and their substrates discovers new aspects of the cycle of matter in nature. But complex analysis of the transformation of heavy metals and other elements in ecosystems requires determining the content of elements not only in myxomycetes and their substrates, but also in the soil, which is planned for further research. Discovering of the ability to accumulate toxic elements reveals the potential of myxomycetes as new objects of bioindication and bioremediation for monitoring and cleaning the environment.

7. Conclusions

1. In ecosystems, myxomycetes play a role of bioconcentrators, as evidenced by the comparative analysis of 11 elements content in fruiting bodies in 9 species of myxomycetes and their substrates. The highest content was found for elements Ca, Fe, and Mg in fruiting bodies of five species of myxomycetes: *Diderma alpinum, D. globosum, D. fallax, Lamproderma echinosporum* and *L. pseudomaculatum*, and transition coefficients were the highest for Cu, Cd, Fe, Ni and Pb. The coefficient of transition of elements from substrates to myxomycetes in 8 % of specimens exceeded 100 units, in 25 % of samples had a K_t value from 10 to 100, 45 % – from 1 to 10, and in 22 % of samples K_t was less than one.

2. In most of the analyzed nivicolous myxomycetes species, the capacity for accumulation of highly toxic heavy metals Cd and Pb, in particular in *Diderma fallax* was found. In addition, *D. alpinum, D. fallax* and *D. globosum* were characterized by bioaccumulation of zinc. The tendency to accumulate copper was noted, as evidenced by the total excess of this moderately toxic heavy metal in all myxomycetes compared to their substrates. Particularly, Cu bioaccumulation ability was found in *Diderma globosum, D. fallax, Physarum alpestre* and *Lamproderma arcyrioides.*

3. Analysis of two samples of *Lamproderma pseudomaculatum* from different countries on the same substrates showed a similar content of elements in their fruiting bodies, indicating a correlation between the ability to bioaccumulation with the specific characteristics of each myxomycetes species. A significant difference in the concentrations of Si, Fe, Cu and Pb shows the dependence of the elements content in myxomycetes on the environmental features, that creates preconditions for their use as bioindicators.

4. The comparison of the elementary content in myxomycetes and their substrates indicates that the elements accumulation takes place at the stage of plasmodium: in xylophilous myxomycetes the content of elements in the fruit bodies corresponds to their concentration in the substrates, but in the case of nivicolous species such correlation is not evident, because their plasmodium lives and feeds mostly in the soil.

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