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The assessment of heavy metal accumulation by myxomycetes

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Summary. The assessment of heavy metal accumulation by myxomycetes (slime moulds) has been made. The importance of these organisms for environmental safety monitoring was specified. Ecological and physiological mechanisms of ability of myxomycetes to concentrate metals and other elements from their substrates were analyzed. For five common myxomycetes species with relatively big fruiting body were compared chemical elements concentrations. *Fuligo septica* (L.) Wigg. was proposed as perspective bioindicator for the detection of Zn on the grounds of the ability to accumulate this metal and by reason of this species have widespread distribution in terrestrial ecosystems. Prospects of using slime molds as objects for bioremediation of contaminated soils were considered.

Key words: environmental safety, risk assessment, heavy metal, myxomycetes.

INTRODUCTION

An important place in environmental safety has problem of heavy metals accumulation in nature. Heavy metals in soils and forest litter are derived from anthropogenic pollutant and natural soil weathering sources. [14]. There are 4 level of danger for heavy metals: high (As, Cd, Hg, Se, Pb, Zn), tempered (Co, Ni, Mo, Cu, Cr, Sb), low (Ba, V, W, Mn, Sr) and unknown (Ge, Sn, Ce, La, Bi, Y, Rb, Cs, and others) [9]. According to modern concepts, living organism forms and controls in the biosphere flows of matter and energy, ensuring consistency of environmental parameters [6]. The main reactions of a living organism associated with the toxic effects of excess elements are: changes in cell membrane permeability (Au, Ag, Cd, Cu, F, Hg, I, Pb); competition for vital metabolites (As, Sb, Te, W, F); a high affinity with the phosphate groups and the active centers of ATP and ADP (Al, Zr, and probably all of the heavy metals); substitution of vital ions (Cr, Li, Pb, Sr); capture molecules positions held important functional groups [13].

The proportionality between content of element in soils and their removal to living organisms is direct not for all heavy metal. It is the case for Ni and Cr, but for Zn, Mn and Cd was shown limited transition metals in phytocenosis biomass. For example, increases Zn concentration in soils in more than 20 times, will give its removal in 1,5 times only. The restriction of transition metals in the aboveground part was observed for Fe, Cr, Pb. For fungi the highest transition index is for Hg, Cd, Cu, Zn and Se [13].

Living organisms involved in all of the elementary soil and biological processes that directly or indirectly affect the mobility of trace elements. In different trophic levels are actively involved in the stabilization medium, acting both as original geochemical barriers and as storage of chemical elements in trophic chains ecosystems. The deformation of the biogeochemical cycles with chemical environmental pollution can destabilize many processes in ecosystems [1].

Myxomycetes have been documented to occur virtually all types of terrestrial ecosystems, and extend geographically from the Polar Regions to the tropics, wherever detritus is present. Even 1cm^3 of soil can contain to 20,000 individual myxomycetes cells [2]. One of ecological role of these organisms is utilizing organic matter from various microhabitats. This suggests that myxomycetes are important in nutrient cycling as members of the detritus food chain [11]. Plasmodia of slime molds (myxomycetes) obtain nutrients by ingesting bacteria, protists, fungal hyphae and spores, and particles of organic matter from their immediate environment [15]. Because myxomycetes are ubiquitous in terrestrial ecosystems and accumulate material only from their immediate environment, they can be using in biomonitoring heavy metal contamination at different locations in environmental safety [8]. Some myxomycetes appear to be tolerant of high levels of heavy metals and apparently accumulate them vigorously [14].

PURPOSE OF WORK

The objective of this study is to make assessment of heavy metal accumulation by myxomycetes. According with this purpose was planed to 1) analyze data about heavy metal accumulation by myxomycetes; 2) examine ecological and physiological mechanisms of ability of myxomycetes to concentrate metals and other elements from their substrates; 3) determine potential of these organisms for environmental safety monitoring for bioindications and bioremediation of heavy metal.

MATERIAL AND METHODS

Material for this study is result of myxomycetes research for more than 20 years in their native habitats in Ukraine [5], and also in forest, forest-steppe, desert, mounting, tropical vegetation over world. Samples of myxomycetes were collected from protected areas and from territory with anthropogenic impact for study patterns of myxomycetes distribution. A series of monitoring plots were established in an affected area and adjacent unaffected sites for compere myxomycetes developed under natural conditions in the field and on polluted urban areas.

The analysis of heavy metal concentrations have been made with utilizing of literature data [8, 10, 12, 16, 18]. In these studies fruiting bodies of slime molds were analyzed by atomic absorption (Pb) or inductively coupled plasma emission spectrometry (Fe, Mn, Cu, Zn, Al) to determine heavy metal concentration. Individual mature fruiting bodies were prepared for light microscopy (LM). Additional specimens were prepared for electron microscopy (TEM) and X-ray microanalysis (EDX) to determine location or accumulation of heavy metals in various regions of the fruiting body. [3, 8].

For assessment of heavy metals accumulation in myxomycetes fruiting body and plasmodium the transition index using:

$$K_t = \frac{C_m}{C_s}, \qquad (1)$$

where: K_t - transition index of heavy metals; C_s - heavy metals concentration in soil; C_m - heavy metals concentration in myxomycetes.

It is suggested to use the total risk summary, while the influence of several factors:

$$i_{ir}^{sum} = \sum_{i=1}^{n} i_{ir_i}, \qquad (2)$$

where: n – number of risk factors, i_{ir_i} – risk performance for the i-th factor [17].

The analytical methods used demonstrate bioaccumulation of heavy metals in myxomycetes.

RESULT OF RESEARCH

Preliminary compere of myxomycetes assemblage on protected nature territory and on contaminated areas didn’t reveal reduction of species abundance, but it find of change of species composition, which can be caused by pollution. Both the biodiversity and relative abundance of the myxomycetes determined in field show that distribution of these organisms depends on the availability of suitable substrates and climate. Also there are some correlations between the densities of myxomycetes and various environmental parameters. Thus feasibility of utilizing these organisms to assess the accumulation of heavy metals in the environment has been reported in several researches.

For study of environmental pollution effect to myxomycetes in south-west Finland the following metals were selected: Al and Fe (major soil constituents), Zn and copper (essential plant nutrients), and mercury and cadmium (notoriously toxic metals) [12]. Then fifteen collections from Australia, Canada, New Mexico and Switzerland were analyzed for about 60 chemical elements. Some myxomycetes species demonstrate tolerate to incredibly high levels of heavy metal accumulation. The results of this research were processed and present on graphs (Fig. 1-5) for five myxomycetes species: *Fuligo septica* (L.) Wigg., *Reticularia splendens* Morgan, *R. lycoperdon* Bull., *Tubifera ferruginosa* (Batsch) J.F. Gmel. and *Lycogala epidendrum* (L.) Fries. This species have relatively big fruiting body and three of them (*F. septica, L. epidendrum, T. ferruginosa*) are in many habitats around the world.

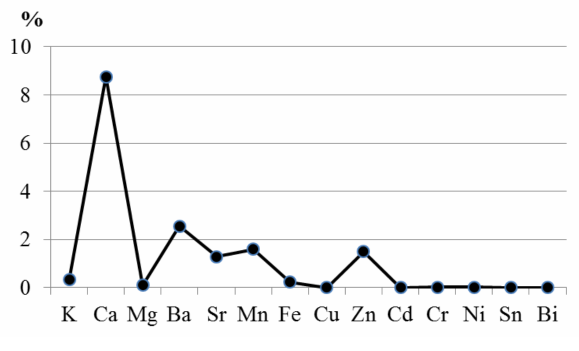

Fig. 1. Concentrations of metals in *Fuligo septica*: total contain on the average 16,39% on dry matter of fruiting body

F. septica (Physarales) produces the largest plasmodium and aethalium of any slime mold (from 2 to 20 cm in diameter and sometimes even more). High level Ca (4800…1120 mg/kg based on dry matter) is typical for species from order Physarales (Fig. 1). Calcium is essential element for building thick lime cortex and other structures of fruiting body. This is why mobile vegetative stage plasmodium contained much less Ca, than generative stage of its life cycle. The presence of high concentrations of the mildly toxic metals strontium (237–2190) and Ba (294–15190 mg/kg based on dry matter) is surprising. These metals are belonging to the same chemical group and could, therefore, be simultaneously absorbed with the calcium from the soil. The element radium also chemically rather close and with gamma spectrometry found indeed evidence of a low, but significant concentration of Ra 226 (670 Bq/kg).

Even more amazing is the presence of a staggering amount of Mn (116–4570 mg/kg based on dry matter) compared with a relatively low Fe (232–478 mg/kg based on dry matter) content. These metals are chemically close, but in most organisms, e.g. fungi, iron predominates over.

As it was shown in several study, *F. septica* has an enormous affinity for zinc Zn (395–3600 mg/kg based on dry matter) [16**]**. It contains on the average 240 times more than the *Vaccinium* (10-160 mg/kg in blueberry leaves). The high amount of zinc in *F. septica* is rather intriguing, since it is much more than ever encountered in macromycetes which contain on the average 100 mg/kg on dry matter [12]. In other study the biomass collected ranged from 305 to 968 mg, whereas Zn concentrations in plasmodia of *F. septica* ranged from 8400 to 23000 mg/kg (-1) dry wt. It is remarkably, that forest litter on which this species was found had Zn concentrations of only 25 to 130 mg kg (-1) dry wt. [18]. A higher concentration of Zn is in hypothallus regions of *F. septica* near the base of the fruiting structure than in other areas of the fruiting body. These metals appeared to be "complexed" and thus may not affect myxomycete growth or reproduction. Tolerance to high levels of heavy metals may be related to the ability to sequester them in regions where reproduction is unaffected [8]. They suggested that the metal probably affords protection from some more dangerous factor by acting as an enzyme activator in detoxification systems. This hyperaccumulation ability of Zn in *F. septica* seems to be unique to this species. The mechanism of this metal resistance is now understood: *F.septica* produces a yellow pigment called fuligorubin A, which has been shown to chelate metals and convert them to inactive forms [7].

In addition, in lesser but still significant amounts, Ba, Cd, Fe, Mg, and Sr were found in *F. septica* in amounts much greater than in macromycetes and micromycetes. The iron and cadmium content are also higher than the amounts measured in the substrate, but the concentration rates are much smaller [16**].**

Fruiting bodies of *T. ferruginosa* crowded together and compressed, forming raspberry-like forms. Individual fruit bodies are less than 0,5 mm wide and are up to 3-5 mm high, but compressed clusters can be up to 15 cm or more in length.

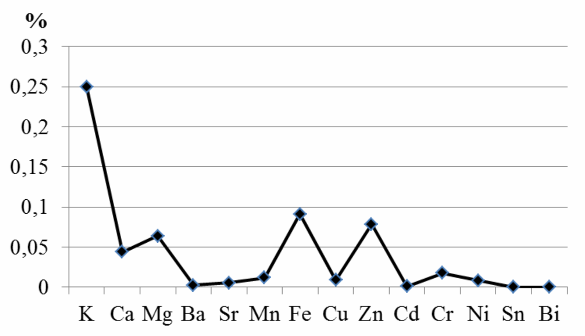

Fig. 2. Concentrations of metals in *Tubifera ferruginosa*: total contain on the average 0,58% on dry matter of fruiting body

Tubifera ferruginosa (Liceales) seems to be poor in metals (Fig. 2). In fruiting body of this species K contains in the highest concentration (210–290 mg/kg based on dry matter) in compare with other metal, but it is less, then in *F. septica* (220–390 mg/kg based on dry matter) and *R. lycoperdon* (380–480 mg/kg based on dry matter). This species apparently only concentrates heavy metals iron (67–115), zinc (74–83) and magnesium (61-68 mg/kg based on dry matter), but the levels are far less spectacular. The calcium concentrations of *Tubifera* (28–61 mg/kg based on dry matter), are less than in *F. septica*, but significantly higher than those measured in macromycetes. In fungi the non-metal phosphorus plays a key role in the intracellular transport of many metals (as soluble complex phosphates), and its level is indeed positively correlated with the heavy metal concentrations present. These observations do not seem to apply to slime moulds, since the phosphorus content of *Tubifera ferruginosa* [16].

Two species genus *Reticularia* (Liceales) are not so common as *F. septica*, *T. ferruginosa* and *L. epidendrum*, but they especially analyzed for showing distinguish in metal composition for related slime molds (Fig. 3, 4). Both have rather large pulvinate fruiting bodies (from 1 to 8 cm in diameter), but *R. splendens* usually forming a white hypothallus ring about the base of the aethalium.

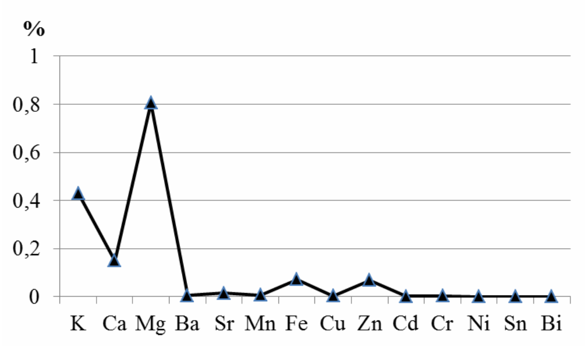

Fig. 3. Concentrations of metals in *Reticularia splendens* total contain on the average 1,56% on dry matter of fruiting body.

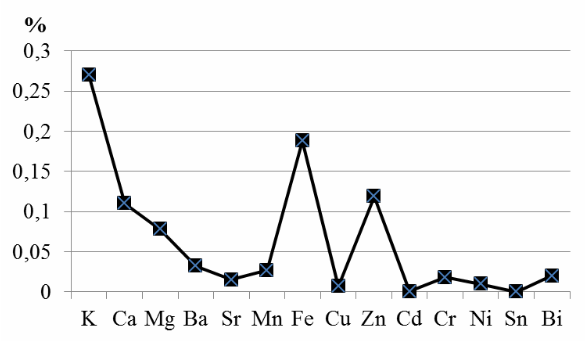

Fig. 4. Concentrations of metals in *Reticularia lycoperdon*: total contain on the average 0,89% on dry matter of fruiting body

Most significant deferens between species of same genus is high concentration Mg (805 mg/kg based on dry matter) in fruiting body of *R. splendens,* while *R. lycoperdon* contains only 78 mg/kg (based on dry matter) of this element. In contrast to other species of this genus, *R. splendens* demonstrate a bit lower level of K (380-480) and Ca (150 mg/kg based on dry matter); in case of *R. lycoperdon*, these data take the meaning 270 and 110 mg/kg (based on dry matter) respectively. In turn *R. lycoperdon* has high content of Fe (188), Zn (119), Ba (32) and Mn (27 mg/kg based on dry matter), while as in *R. splendens* these elements have a concentration 65-78, 69, 5-6 and 5-7 mg/kg (based on dry matter) in accordance with the order of citation elements [16].

L. epidendrum is most well known and most common species of slime mold. It has carmine-pink plasmodium, which developing into a grey-brown crowded aethalium 3-10 mm in diameter.

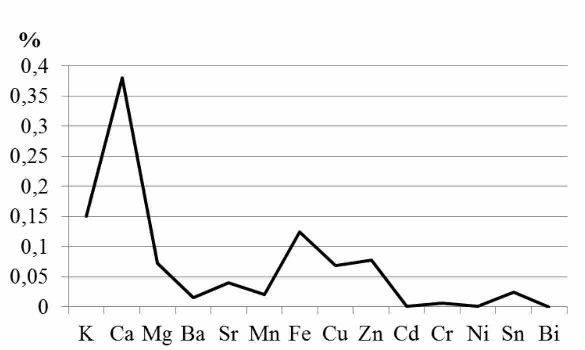

Fig. 5. Concentrations of metals in *Lycogala epidendrum*: total contain on the average 0,98% on dry matter of fruiting body

L. epidendrum (Liceales) seems to bioaccumulate copper (52–84 mg/kg based on dry matter), whereas the four other slime moulds appear to exclude this metal (Fig. 5). In most *L. epidendrum* specimens was observed not less than 20 mg/kg tin (19–30 mg/kg based on dry matter), a metal that usually occurs only in traces (<1 mg/kg) in plants, animals and fungi. It is noteworthy that no accumulation of elements from the same group such as arsenic and antimony could be found [16].

It is worth noting that in the five slime molds the essential element Mn is present at the same concentrations as those generally observed in macromycetes. The magnesium content of mushrooms is subject to little variation. The metal K, the principal cation in green plants and mushrooms is very low in slime moulds. The concentrations even lower than those of the Polyporaceae which contain generally about 1% of potassium on dry matter [16].

Other slime mold species (*Symphytocarpus flaccidus* (Lister) Ing & Nann.-Bremek., *Amaurochaete atra* (Alb. & Schwein.) Rostaf., *Ceratiomyxa fruticulosa* (O.F. Müll.) T. Macbr., *Stemonitis* sp.) apparently only concentrates zinc, cadmium and, perhaps copper, but the levels are far less spectacular, then present data [12].

In some stalked myxomycetes species of genus *Hemitrichia*, *Physarum*, *Trichia* heavy metals were detected only in the stalk region. The analytical methods suggest that metal precipitation in the stalk region may provide tolerance to high metal levels. By EDX, metals (Fe, Cr, Mn, Si, Al) were detected as crystalline precipitates in the stalks of fruiting bodies. Viewed by TEM, clusters of bacteria were observed in stalk and hypothallus regions. The bacteria and individual spores in sporangia contained polyphosphate bodies (including P, Ca, K) in their cell cytoplasm. EDX analysis showed the electron-dense precipitate in *Physarum* comprises Fe, Si, Al, Mg, Cl, Cr, and Mn. By contrast, regions of the *Hemitrichia* stalk with deteriorated plasmodial material contained only P and Cl. The most common metals detected in stalk sediment were Fe, Al, and Cr. Precipitates of Al, Si, K, and Cl on outer stalk or peridium walls were seen frequently in all species examined and possibly represent minute soil particles. In all analyzed stalked species, the polyP bodies seen in spores within sporangia and in bacteria from stalk regions contained P, Ca, and occasionally K [8].

For determine the accumulation of heavy metal, myxomycetes were assessed from forest patches on volcanic and ultramafic soils of Philippines. Collected substrates, fruiting bodies, and plasmodia of selected myxomycetes tested for heavy metal were all positive for Cr and Mn. Interestingly, Cr and Mn contens of tested myxomycetes were equal or higher than that of its leaf substrate. The bioabsorption of Cr and Mn by myxomycetes has been assessed, and the heavy metal content of substrates and fruiting bodies determined for selected species [10].

DISCUSSION

For environmental safety would generate a body of novel information relating to the ecological effects of targeted analytes on terrestrial community of living organism dynamics. The concentration of metals depends not only on the biological characteristics of the species and the phases of their development, but also on the environmental situation in the area of their occurring. The resistance of myxomycetes to overage heavy metals may have different mechanisms. It is possible that myxomycetes have tolerance to these chemical elements or make some effective barrier for protection physiological functions. At present, little is known about the chemical forms in which the metals occur in the myxomycetes. Transport of heavy metals depends from pH of environmental. It is considered that true tolerance based on such complex mechanisms of metabolic protection as: change of metabolism; differences in membrane structure and function; selective ions absorption; removal of ions from metabolic processes by deposition or a fixed insoluble forms in different organs and organelles, etc. [13]

The concentration of heavy metals in the forest litter is higher than in living plants [13]. On the other hand, the concentration of some heavy metals in fruiting bodies studied myxomycetes is higher than in their substrates and soil (Fig. 6).

The myxomycetes merit recognition in the scientific community as organisms of special significance that can answer basic biological questions. These organisms apparently play a major role in maintaining the ecological balance that exists between bacteria and other soil organisms. The myxomycetes seem especially well suited to serve as biological indicators in environmental safety for assessing the fundamental differences that exist for the soil microbial system among selected study sites [6].

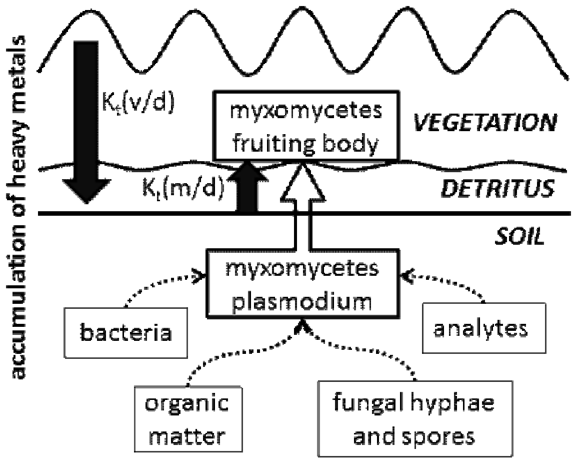

Fig. 6. The scheme of heavy metals accumulation by myxomycetes: heavy metals concentration (K_t - transition index of heavy metals) increases in the direction from substrates to myxomycetes, and it decline from the forest floor to the trees

In perspective environmental ground pollution may be remediated by myxomycete fruiting bodies and plasmodia. The results for *F. septica* proved to be most remarkable. This species hyper-accumulate and concentrate highly toxic levels of Zn several thousand fold greater than site vegetation and lesser significant amounts of Ba, Cd, Fe, Mg, and Sr. The massive, cushion-shaped aethalium of this species has a large yellow plasmodium that may serve as an experimental model to study the uptake and concentration of heavy metals. The biochemical detoxification mechanism of highly toxic levels of zinc in *F. septica* and the cloning of the genes involved could be used in plants with greater biomass for bioremediation of polluted soils [4]. Also *F. septica* may be useful as an indicator of pollution, and further examination of this species (to determine the location of Pb in the fruiting body) should be pursued.

Moreover, the considerable variation in metal concentrations between the two *Fuligo* collections from low elevation indicates that additional collections of all myxomycetes species are needed to determine whether a relationship exists between metal concentration and site elevation [8]. Tree canopy research has shown that aerial pollution results in the decrease of myxomycete species richness at higher elevations. When more environmental parameters are better known myxomycetes may one day serve as the basis for evaluating the impact of pollutants on living trees [4].

Ultimately, a better understanding of this entire system could lead to the development of methodologies utilizing myxomycetes to assess remediation efforts at spill sites. In addition, these methods could be used to monitor the conditions associated with various storage operations such as the leeching of selected analytes [11].

CONCLUSIONS

Living organisms involved in all of the elementary soil and biological processes that directly or indirectly affect the mobility of trace elements. The myxomycetes are organisms of special significance that can answer basic biological questions. Myxomycetes seem especially well suited to serve as biological indicators in environmental safety for assessing the fundamental differences that exist for the soil ecosystem.

1. The assessment of heavy metal accumulation by myxomycetes shows the importance of these organisms for environmental safety monitoring.

2. Myxomycetes have undiscovered potential for bioindications and bioremediation of heavy metal and environmental management.

3. The scheme of heavy metals accumulation by myxomycetes was make to demonstrate that heavy metals concentration increases in the direction from substrates to myxomycetes, and it decline from the forest floor to the trees.

4. The concentration of metals depends not only on the biological characteristics of the species and the phases of their development, but also on the environmental situation in the area of their occurring, in this connection the resistance of myxomycetes to overage heavy metals may have different mechanisms.

5. *Fuligo septica* (L.) Wigg. was proposed as perspective bioindicator for the detection of Zn on the grounds of the ability to accumulate this metal and by reason of this species have widespread distribution in terrestrial ecosystems.

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ОЦЕНКА НАКОПЛЕНИЯ МИКСОМИЦЕТАМИ ТЯЖЕЛЫХ МЕТАЛЛОВ

Аннотация. Осуществлена оценка накопления тяжелых металлов миксомицетами (слизевиками). Определена важность этих организмов для мониторинга в сфере экологической безопасности. Проанализированы экологические и физиологические механизмы поглощения миксомицетами металлов и других элементов из субстратов. Для пяти распространенных видов миксомицетов с относительно крупными плодовыми телами проведено сравнение концентрации химических элементов. *Fuligo septica* (L.) Wigg. предложен в качестве биоиндикатора Zn на основании способности накапливать этот металл и по причине широкого распространения этого вида в наземных экосистемах. Предполагается, что миксомицеты могут стать перспективными объектами для ремедиации загрязненных почв.

Ключевые слова: экологическая безопасность, оценка рисков, тяжелые металлы, миксомицеты.