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BIOACCUMULATIVE AND BIOINDICATIVE ROLE OF FUNGI IN THE ENVIRONMENT

BIOAKUMULACYJNA I BIOINDYKACYJNA ROLA GRZYBÓW W ŚRODOWISKU

Abstract: Mycelial construction of mushrooms is considered as a good biomass for heavy metals binding as well as lots of elements, including toxic and radioactive ones. Between various fungi known to bind heavy metals, mycelium of *Pleurotus* spp. can be used in Cd indication and accumulation, by means of fungal biomass. In this work possible role of *Pleurotus ostreatus* in accumulation of radionuclides is presented. This is important because this fungus is one of broadly cultivated mushrooms, and it can grow as saprobic organism on various substrates. Naturally occurring fruiting bodies of *P. ostreatus* were used in studies as a potential marker of atmospheric radioactive pollution. The samples of fruiting bodies in different age (young, mature and old) were collected in the center of Opole. Measurements of the gamma radionuclides activity concentration in dried samples were carried out by means of a gamma-spectrometer with a germanium detector HPGe (Canberra) of high resolution. Potassium concentration in fruiting bodies was similar in each sample, though it was the lowest in the young specimen. Activity concentration of Cs-137 was also related with the age of the mushroom. The biggest concentration was determined in old specimen, while in the young and mature ones it was similar. In the mature and old mushrooms activity concentration of Pb-210 was lower than minimum detectable activity. Pb-212 was determined only in fruiting bodies of old specimen.

Keywords: mushrooms, radionuclides, environmental pollution indication

Introduction

Mushrooms are investigated as a source of numerous elements and, among them, also these classified as toxic and radioactive [1, 2].

Pleurotus ostreatus is one of broadly cultivated mushrooms as well as it can grow as saprotrophic organism on various substrates, f. ex. trunks. One of most frequently cultivated is an oyster mushroom (*Pleurotus osteratus*). An oyster mushroom is cultivated for not long time, the first information about its cultivation becomes from 1917, but before it was collected in natural environment, also naturally infected trunks were collected and stored in wet conditions waiting for fructification. The first scientific name of oyster mushroom was *Agaricus ostreatus*, nowadays the number of 36 species were described in the genera of *Pleurotus*. The genera *Pleurotus* is classified in the phylum *Basidiomycota.* Mycelium of basidiomycetous fungi is characterized by clump connections occurring on vegetative hypha (Fig. 1), which is a typical hallmark for this kind of mushrooms, as well as for genus *Pleurotus*. Only two species of *Pleurotus* are not edible, and 25 of them are cultivated broadly in the world. Between cultivated mushrooms the genera of *Pleurotus* has the biggest number of cultivated species and cultivars. In central Europe an oyster mushroom can grow saprotrophically on the dead or living trunks of deciduous trees and

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naturally on the ground. Fruiting bodies occur in November and December. Naturally occurred mycelium grows saprotrophically and do not need many nutritional requirements [3]. This makes it easy in cultivation on various lignin and cellulose substrates and wastes. Fruiting bodies of oyster mushroom grow in big groups consisted of bigger or smaller ones, on the same base [4]. They are a good source of mineral substances. There were found 3 g of potassium, 150 mg of calcium and 125-757 mg of sodium in 100 g of dry mass of fruiting bodies of oyster mushroom [5]. They contain also trace amounts of iodine, fluorine, copper, zinc, mercury and manganese [6, 7]. Mushrooms can collect also lots of elements including the toxic and the radioactive ones. Mushrooms or single mycelia are considered as good biomass for binding heavy metals. Between various fungi known to bind heavy metals, mycelium of *Pleurotus sajor-caju* can be used to remove Cd by means of fungal biomass [8].

In our work the naturally occurring *P. ostreatus* fruiting bodies were used as a potential marker of atmospheric radioactive pollution.

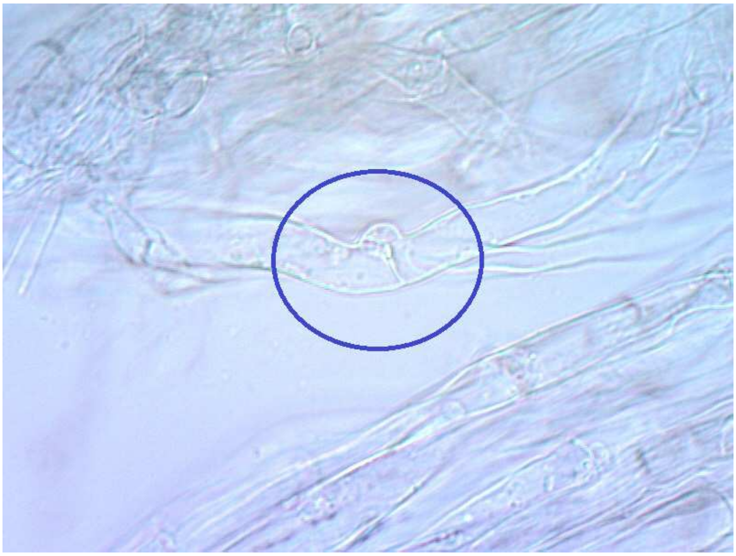

Fig. 1. Hyphae of *P. ostreatus* with a clump connection (marked in the ring) (photo by E. Moliszewska)

Materials and methods

The samples of *P. ostreatus* fruiting bodies were collected from the trunk of living lime-tree (*Tilia cordata*) growing on the side of the average traffic road in the center of Opole. They were collected during two winter seasons: the end of November (2012), the end of December (2013) and the end of February (2014). Additionally the age of the fruiting bodies was qualified as young specimens (Fig. 2), mature (Fig. 3) and old specimens. For determination of the gamma radionuclides activity dried samples of fungi fruiting body were prepared. Samples were oven dried in 105°C since the mass of the sample was stable and then dried by mortar.

Fig. 2. Young fruiting bodies of *P. ostreatus* (photo by E. Moliszewska)

The measurement of radionuclide activity in fungal samples was carried out by means of a gamma-spectrometer with a germanium detector HPGe (Canberra) of high resolution: 1.29 keV (FWHM) at 662 and 1.70 keV (FWHM) at 1332 keV. Relative efficiency: 21.7%. Energy and efficiency calibration of the gamma spectrometer was performed with the standard solutions type MBSS 2 (Czech Metrological Institute, Praha), which covers an energy range from 59.54 to 1836.06 keV. The geometry of the calibration source was a Marinelli container (447.7 ±4.5 cm^3), with density 0.99 ±0.01 g/cm^3, containing Am-241, Cd-109, Ce-139, Co-57, Co-60, Cs-137, Sn-113, Sr-85, Y-88 and Hg-203. The geometry of sample container was a similar Marinelli of 450 cm^3. Time of measurement was 24 h for all samples. Measuring process and analysis of spectra were computer controlled with the use of software GENIE 2000.

Some radionuclides were determined in each sample, and some of them appeared in determinable concentrations only in single samples. Results were shown as the activity concentrations of radionuclides (a), and the measurements uncertainties (Δa). The values lower than minimum detectable activity (MDA) are marked with < MDA.

Fig. 3. Mature fruiting bodies of *P. ostreatus* (photo by E. Moliszewska)

Results

The following radioisotopes were determined in fruiting bodies of *P. ostreatus* samples: Pb-212 (thorium decay chain), Pb-210 (radium decay chain), K-40 and artificial Cs-137. In Table 1 the half-life times of the determined radioisotopes are shown. Among the radioisotopes determined in fungal samples, K-40 is the most stable.

Table 1

Half-life times of the radioisotopes determined in soil samples

Pb-212	**Pb-210**	**K-40**	**Cs-137**
10.64 h	22.2 a	$1.25 \cdot 10^9$ a	30.1 a

It was observed that K-40 concentrations in fruiting bodies of *P. ostreatus* were similar to each other, though it was the lowest in the young specimen (Table 2). The Cs-137 is present in environment and it is also determined in the investigated mushroom samples. Both, direct atmospheric deposition on the surface and transport from background via mycelium, could deliver Cs-137 to the fruiting bodies. Activity concentration of this radioisotope was also related with the age of the mushroom. The biggest concentration was determined in old specimen, while in the young and mature ones it was similar (Table 2).

The Pb-210 radioisotope was found in young mushrooms (Table 2), though the measurement uncertainty was significant. In the mature and old mushrooms activity concentration of Pb-210 was lower than MDA.

The Pb-212 isotope was determined only in old mushrooms at the level 1.27 Bq/kg (Table 2).

Table 2

The gamma radionuclides activity in dried samples of fruiting bodies

Time (age)	$a_{K\text{-}40}$ [kBq/kg]	$\Delta a_{K\text{-}40}$ [kBq/kg]	$a_{Cs\text{-}137}$ [Bq/kg]	$\Delta a_{Cs\text{-}137}$ [Bq/kg]	$a_{Pb\text{-}210}$ [Bq/kg]	$\Delta a_{Pb\text{-}210}$ [Bq/kg]	$a_{Pb\text{-}212}$ [Bq/kg]	$\Delta a_{Pb\text{-}212}$ [Bq/kg]
November 2012 (mature)	1.059	0.040	4.76	0.64	< MDA	–	< MDA	–
December 2013 (young)	0.941	0.039	5.30	0.75	45.2	44.9	< MDA	–
February 2014 (old)	1.050	0.033	10.08	0.46	< MDA	–	1.27	0.28

Discussion

Biosorption is an area of increasing biotechnological interests. Especially since the removal processes of potentially toxic and/or valuable metals and radionuclides from contaminated sources can be used to detoxify them prior to environmental elimination [9]. Mushrooms may accumulate great concentrations of metals. Some of them are involved in fungal growth, metabolism and differentiation (*eg* K, Na, Mg, Ca, Mn, Fe, Cu, Zn, Co and Ni), while others, *eg* Rb, Cs, Al, Cd, Ag, Au, Hg and Pb have probably no essential functions. Some of them, such as mercury, cadmium, lead and copper can cause morphological abnormalities, reduce growth and increase mortality and mutagenic effects in humans [10, 11]. Yilmaz et al [12] found that fungal species growing on wood contain, in general, lower concentrations of heavy metals than fungi growing on soil. This observation is proper for cultivated mushrooms, but naturally occurring ones can grow on different, contaminated substrates, as *eg* fruiting bodies of tested in this research mushrooms. The source of contamination was mostly city traffic and precipitation, so consumption of such mushrooms as food may lead to intoxication. In this way selected food products may deliver to an average person certain doses of both natural and artificial radioactive isotopes [13].

The abundance of K-40 in potassium is constant and is 0.0119%. Because of the constant content, activity concentration of K-40 is the measure of the total potassium concentration. It seems that tested specimens do not collect continuously this element. Desorption of immobilized cesium cations is supported by the ones of similar size, like K^+ or NH_4^+. The Cs-137 and K-40 isotopes have similar chemical properties, but their origin in nature is different. In opposite to Cs-137, the K-40 radioisotope occurs naturally in environment.

The Cs-137 isotope is the artificial radionuclide, with half-life time of 30.1 years. Its circulation in environment started about 70 years ago. It was a product of a number of nuclear tests performed in 50. and 60. of 20th century [14]. Environment was contaminated also as a result of a number of incidental, uncontrolled releases, like, for example, accidents in Chernobyl (1986) and Fukushima Dai-Ichi (2011) nuclear power plants [15-17]. The Cs-137 is present in environment up to now, and it was also determined in the investigated mushroom samples. Probably growth of the fruiting bodies of *P. ostreatus* was associated with continuous intake of Cs-137 from surrounding area.

The Pb-210 radioisotope found in young mushrooms is the member of the natural uranium decay series. Is half-time time is 22.2 years, which is significantly longer than that for Pb-210 ancestors in decay series. It could be supposed that Pb-210 appeared primarily in mushroom and its presence is not a result of radioactive decay of its closest ancestors in decay series. It could be concluded that this isotope appeared in mushroom surrounding only in a period of time, at the beginning of fruiting bodies growth. Subsequent increase in mass of mushroom caused decrease in Pb-210 concentration and fall of activity concentration below MDA. In old mushrooms the Pb-212 isotope was determined (Table 2). It is a member of the natural thorium decay series. Its half-life time is not long (only 10.6 h). It possible source is Th-228 with half-life time 1.9 years. Occurrence of Pb-212 in fruiting bodies of old specimen supposed deposition of the radioisotope from atmosphere or migration from background. This process was slower than increases in mass of the mushroom. Bishnoi and Garima [18] observed that biosorption of metal ions increase during the lag period or early stages of growth and declines as cultures reached stationary phase (for *Aspergillus niger, Penicillium spinulosum, Trichoderma viride*). The age of fruiting body or its size, are of less importance. Some authors reported higher metal concentrations in younger fruiting bodies. This is explained by the transport of a metal from mycelium to the fruiting body during the start of fructification. During the following increase of the fruiting body mass, the metal concentration decreases. In general the proportion of metal concentrations from atmospheric depositions seems to be of less importance due to the short lifetime of a fruiting body of typical edible mushrooms, which is usually 10 ±14 days [19], but fruiting bodies of naturally occurring *P. ostreatus* can persist longer in their natural position.

Conclusions

Potassium concentration in fruiting bodies was similar in each sample, though it was the lowest in the young specimen. Activity concentration of Cs-137 was also related with the age of the mushroom. The biggest concentration was determined in old specimen, while in the young and mature ones it was similar. In the mature and old mushrooms activity concentration of Pb-210 was lower than minimum detectable activity. Pb-212 was determined only in fruiting bodies of old specimen.

References

[1] Falandysz J, Brzostowski A, Nosewicz M, Danisiewicz D, Frankowska A, Apanasewicz D, et al. Rtęć w grzybach jadalnych z terenu Trójmiejskiego Parku Krajobrazowego. Bromat Chem Toksykol. 2000;2:177-182.

[2] Falandysz J, Lipka K, Gucia M, Kawano M, Strumnik K, Kannan K. Accumulation factors of mercury in mushrooms from Zaborski Landscape Park, Poland. Environ Inter. 2002;28:421-427. DOI: 10.1016/S0160-4120(02)00067-3.

[3] Upadhyay R, Singh ChM. Production of Edible Mushrooms. In: Hofrichter M, editor. Industrial Applications, 2nd Edition. The Mycota X. Berlin Heidelberg: Springer-Verlag; 2010.

[4] Yildiz A, Karakaplan M, Aydin F. Studies on Pleurotus ostreatus (Jacq. ex Fr.) Kum. var. salignus (Pers. ex Fr.) Konr. et Maubl.: cultivation, proximate composition, organic and mineral composition of carpophores. Food Chem. 1998;61(1/2):127-130. DOI: 10.1016/S0308-8146(97)00066-6.
[5] Shah H, Khalil AI, Jabeen S. Nutritional composition and protein quality of Pleurotus mushroom. Sarhad J Agric. 1997;13(6):621-626.
[6] Vetter J. Mineral elements in the important cultivated mushrooms Agaricus bisporus and Pleurotus osteratus. Food Chem. 1994;50(3):277-279. DOI: 10.1016/0308-8146(94)90132-5.
[7] Watanabe T, Tsuchihasi N, Takai Y, Tanaka K, Suzuki A. Effects of ozone exposure during cultivation of oyster mushroom (Pleurotus ostreatus) on chemical components of the fruit bodies. J Japan Soc Food Sci Technol. 1994;41(10):705-708.
[8] Zimmermann M, Wolf K. Biosorption of Metals. In: M. Hofrichter, editor. Industrial Applications, 2nd Edition. The Mycota X. Berlin Heidelberg: Springer-Verlag; 2010.
[9] Gadd G.M. Interaction of fungi with toxic metals. New Phytol. 1993;124:25-60.
[10] Olumuyiwa S, Oluwatoyin F, Olanrewaja OA, Steve RA. Chemical composition and toxic trace element composition of some Nigerian edible wild mushroom. Inter J Food Sci Technol. 2007;43:24-29. DOI: 10.1111/j.1365-2621.2006.01375.x.
[11] Uzun Y, Genccelep H, Kaya A, Akcay ME. The mineral contents of some wild edible mushrooms. Ekoloji. 2011;20(80):6-12.
[12] Yilmaz F, Isiloglu M, Merdivan M. Heavy metal levels in some macrofungi. Turk J Bot. 2003;27:45-56.
[13] Godyń P, Dołhańczuk-Śródka A, Ziembik Z, Moliszewska E. Estimation of the commited radiation dose resulting from gamma radionuclides ingested with food. J Radioanal Nuclear Chem. 2014;299(3):1359-1364. DOI: 10.1007/s10967-014-2926-3.
[14] United Nations. 2000. Report, Annex C: Exposures to the public from man-made sources of radiation. http://www.unscear.org/docs/ reports/annexc.pdf.
[15] Akahane K, Yonai S, Fukuda S, Miyahara N, Yasuda H, Iwaoka K, et al. The Fukushima Nuclear Power Plant accident and exposures in the environment. Environmentalist. 2012;32(2):136-143. DOI: 10.1007/s10669-011-9381-2.
[16] Aleksakhin RM, Sanzharova NI, Fesenko SV. Radioecology and the accident at the Chernobyl nuclear power plant. Atomic Energy. 2006;100(4):257-263.
[17] United Nations. 2008. UNSCEAR 2008 Report Vol. I. Sources of ionizing radiation. United Nations Scientific Committee on the Effects of Atomic Radiation UNSCEAR 2008 Report to the General Assembly, with scientific annexes. Annex D. http://www.unscear.org/docs/reports/2008/11-80076_Report_2008_ Annex_D.pdf.
[18] Bishnoi NR, Garima A. Fungus - an alternative for bioremediation of heavy metal containing wastewater: a review. J Sci Ind Res. 2005;64:93-100.
[19] Kalač P, Svoboda L, Havlíčková B. Contents of cadmium and mercury in edible mushrooms. J Appl Biomed. 2004;2:15-20.

BIOAKUMULACYJNA I BIOINDYKACYJNA ROLA GRZYBÓW W ŚRODOWISKU

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Abstrakt: Grzybnia, szczególnie grzybów wielkoowocnikowych, dzięki zdolności do akumulowania metali ciężkich stanowi potencjalne źródło informacji o zanieczyszczeniu środowiska. Wśród nich należy wymienić boczniaka (*Pleurotus osteratus*), grzyba zarówno uprawnego, jak i występującego naturalnie. W pracy wykonano analizę zdolności bioindykacyjnych i biokumulacyjnych tego gatunku w stosunku do izotopów gamma-promieniotwórczych. Próbki *P. osteratus* w różnym wieku (młode, dojrzałe, starzejące) zebrano w naturalnym stanowisku, w centrum Opola. Aktywności izotopu K-40 były podobne we wszystkich badanych próbkach, przy czym najniższa koncentracja tego izotopu została stwierdzona w najmłodszych owocnikach. Izotop Cs-137 najliczniej występował w najstarszych owocnikach. Radioizotop Pb-210 znaleziono w młodych owocnikach, a izotop Pb-212 stwierdzono w starych owocnikach.

Słowa kluczowe: grzyby, radionuklidy, indykacja zanieczyszczeń środowiska