

DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) BY SPENT MUSHROOM SUBSTRATES OF *Agaricus bisporus* AND *Lentinula edodes*

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Abstract. The cultivation of fungi is associated with the large production of spent mushroom compost (SCM), that have great ability to degrade lignin-like pollutants. The use of SMC to clean up contaminated soil is a promising alternative to other more expensive methods. A 12-week experiment with spent mushroom composts from *Agaricus bisporus* (champignon) and *Lentinula edodes* (shiitake) was carried out to compare their ability to degrade PAHs. The degradation of PAHs by *Agaricus bisporus* was in the following order: anthracene, pyrene, fluoranthene, and phenanthrene (87, 85, 83 and 79% of the control). The strongest degradation by *Lentinula edodes* was confirmed for anthracene (86% of the control), then for phenanthrene, fluoranthene and pyrene (78, 70 and 63% of the control, respectively). After a brief reduction of naphthalene content, a rapid increase was noted for both *Lentinula edodes* and *Agaricus bisporus* (170 and 149% of the control, respectively, at the end of the experiment).

Key words: anthracene, champignon, fluoranthene, naphthalene, phenanthrene pyrene, shiitake

INTRODUCTION

Mushroom cultivation involves large quantities of spent mushroom compost/substrate (SMC/SMS), which is an excellent source of enzymes, microorganisms, nutrients and organic matter. SMC addition to soil helps to change the physical and chemical soil properties such as pH, moisture and soil structure, and increases the activity of entering or already present soil microflora. Functioning as a nutritional supplement for crops and soil conditioner, the SMC can be directly supplemented into the environment [Semple et al. 2001, Ribas et al. 2009, Zebulun et al. 2011]. On the other

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hand, spent mushroom compost from white rot fungi possesses a great ability to degrade lignin-like pollutants. Non-specific extracellular ligninolytic enzymes secreted into the soil by white rot fungi are responsible for degradation of lignin and other structurally similar organic compounds, such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyl (PCBs), dioxins, pesticides, explosives, dyes, solvents, etc. [Joshi and Gold 2000, Baldrian 2003, Arun et al. 2008]. The oxidative enzymatic system of white rot fungi consists of lignin peroxidases (LiP), manganese peroxidases (MnP) and laccases (LAC) [Zheng and Obbard 2002].

High activity of ligninolytic enzymes in substrate after the harvest of fruit bodies and low specificity towards the oxidized substrate have led to them being regarded as a promising and alternative method of biological bioremediation, i.e. mycoremediation. The use of spent mushroom compost from *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes* in order to degrade organic pollutants present in soil and wastewater can be interesting due to its relatively low cost, environmental acceptability and possibility of utilizing a wide range of residues from the mushroom industry.

The aim of the study was to investigate and compare the ability and efficiency of spent mushroom compost from champignon (*Agaricus bisporus* (J. E. Lange) Imbach) and shiitake (*Lentinula edodes*) for the biodegradation of two-, three- and four-ring PAHs (naphthalene, fluoranthene, anthracene, phenanthrene and pyrene).

MATERIAL AND METHODS

Chemicals. Anthracene, fluoranthene, naphthalene, phenanthrene and pyrene, as representatives of PAHs, and acetone and petroleum ether were obtained from Sigma-Aldrich, Poland. EPA-525 fortification solution (acenaphthene d_{10} , phenanthrene d_{10} , chrysene d_{12} , perylene d_{12}) was obtained from Supelco.

Fungal material. Spent mushroom compost (SMC) originated from a mushroom farm (“MYCOMED”) located in Kościelna Wieś (Wielkopolska) as collected after the cultivation of *Lentinula edodes* on a substrate composed of a mixture of alder and oak sawdust and ending after the harvest of 3 flushes of fruit bodies.

Spent mushroom compost originating from “KOMPOSTPAL” S.C. located in Kościan (Wielkopolska) was collected after cultivation of *Agaricus bisporus* growing on a mixture of wheat straw and poultry manure according to generally accepted technology ending with the harvest of 3 flushes.

Experimental set-up. To assess the abilities of spent mushroom compost to degrade the PAHs, the experiment was conducted on quartz sand in four replications for each treatment and carried out as follows. The PAHs (anthracene, fluoranthene, naphthalene, phenanthrene and pyrene) were dissolved in 100 mL of acetone and added to the quartz sand in the quantity of 120 mg each. After evaporating the acetone, the contaminated sand was mixed with spent mushroom compost in the ratio 4:1 (w/w). The mixture in quantities of 5 kg per plant pot was incubated for 12 weeks under controlled conditions.

Extraction and analysis of PAHs. The extraction procedure was based on the ISO 13877 norm with some modifications. The samples of sands and compost mixture were collected every 3 weeks and extracted with 20 mL of water, 40 mL of acetone and

20 mL of petroleum ether with addition of 16 g of NaCl. After 6 h of shaking, the organic fraction was collected and dried with CaSO₄. To the 900 µL of the extract, 100 µL of 2, 2, 4-trimethylpentane (isooctane) was added.

GC-MS/MS analysis. Quantitative analysis of PAH residues, with the lower limit of quantification 0.01 mg kg⁻¹ DM, was carried out with a Varian 450 gas chromatograph coupled with a Varian 320 MS mass detector working in the EI positive ion mode using the method of internal standard and single ion monitoring technique. Both transfer line and ion source temperature were maintained at 250°C.

Parameters of determination of PAH compounds corresponded to the values specified in the Regulation of the Minister of Environment of 9 September 2002 on standards of soil and land quality, i.e. naphthalene was quantified according to internal standard of acenaphthene d₁₀ (retention time: 4.84, 6.31 min, respectively, r² = 0.998), phenanthrene, anthracene and fluoranthene according to phenanthrene d₁₀ (retention time: 7.38, 7.66, 8.97, 7.68 min, respectively, r² = 0.996, 0.995, 0.999, respectively), and pyrene according to perylene d₁₂ (retention time: 9.26, 14.14 min, respectively, r² = 0.993). The GC was equipped with VF -1 ms 30 m × 0.25 mm i.d. 0.25 µm film thickness column and helium was used as carrier gas with flow 1.0 mL min⁻¹. Injection temperature was 250°C, injection volume was 1 µL (splitless). The following temperature program was used: from 60°C to 240°C for 7 min at 30°C min⁻¹, and 240 to 300°C for 20 min at 10°C min⁻¹.

Data analyses. The data were processed using Microsoft Excel 2003. Statistical analysis was done using STATISTICA 6 with two-way ANOVA and post hoc Tukey test at $\alpha = 0.05$.

RESULTS

In our investigation we confirmed a significant influence of cultivar of fungus and the time of the experiment on PAHs (anthracene, fluoranthene, naphthalene, phenanthrene and pyrene) contents (table 1).

Table 1. Two-factor analysis of variance (two-way ANOVA) for the PAH content in sand/substrate mixture

Table 1. Dwuczynnikowa analiza wariancji dla zawartości WWA w mieszaninie piasku i kompostu

Effect Efekt	Df Stopień swobody	Empirical p-value – p empiryczne					pH
		anthracene antracen	fluoranthene fluorenten	naphthalene naftalen	phenanthrene fenantren	pyrene piren	
Fungus (F) Grzyb	1	0.0001	0.000	0.0096	0.0000	0.0000	0.0000
Time (T) Czas	4	0.0000	0.000	0.000	0.0000	0.0000	0.0000
Interaction (F × T) Interakcja	4	0.0024	0.000	0.0414	0.0000	0.0000	0.0000

The 12-week experiments also confirmed some differences in patterns (kinetics) of degradation of PAHs by *Agaricus bisporus* and *Lentinula edodes* (figs. 1 and 2).

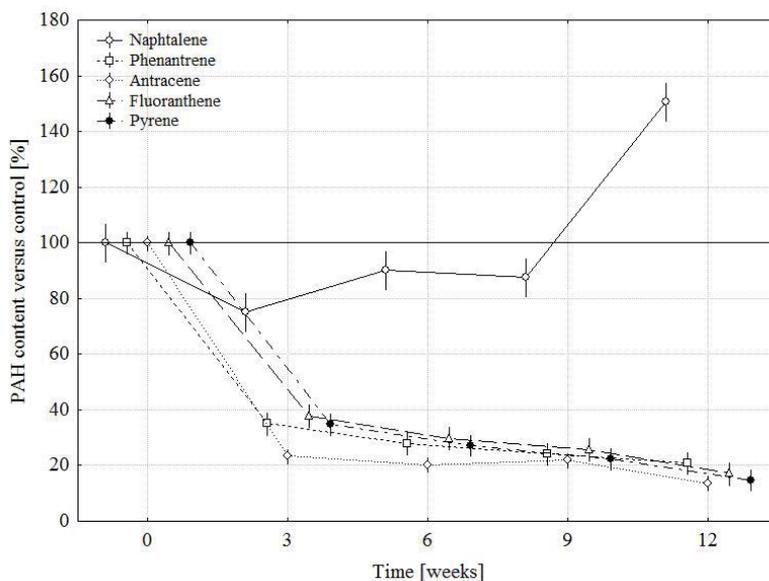


Fig. 1. PAH degradation by champignon (*Agaricus bisporus*). Vertical bars show 95% confidence level

Ryc. 1. Degradacja WWA przez pieczarkę dwuzarodnikową *Agaricus bisporus*. Linie pionowe wskazują przedział ufności 95%

The strongest and similar degradation of anthracene, phenanthrene, fluoranthene and pyrene versus control by *Agaricus bisporus* was detected after 3 weeks (fig. 1, tab. 2). The anthracene, phenanthrene, fluoranthene and pyrene contents rapidly decreased to 23, 35, 38 and 35% (respectively) of the control. During the next weeks of the experiment the decreases were very slow. Further incubation indicated stable degradation of the PAHs, and at the end of the experiment (after 12 weeks) anthracene, phenanthrene, fluoranthene and pyrene contents were 13, 21, 17 and 15% of the control. The naphthalene content was also reduced after 3 weeks of the experiment, but only to 75% of the control. However, during the next weeks the content increased to 149% of the control by the end of the experiment.

The degradation of phenanthrene and anthracene by *Lentinula edodes* was stable and nearly linear (especially for phenanthrene) during the experimental period (fig. 2, tab. 2). After 12 weeks the contents were 22% and 14% of the PAHs content at the beginning of the experiment, respectively. The fluoranthene and pyrene contents slowly decreased during 6 weeks and a rapid drop was confirmed afterwards. At the end of the experiment the contents were 30 and 37% of the initial content, respectively. The naphthalene content was reduced to 74% of the control after three weeks, but increased to 170% of the control by the end of the experiment.

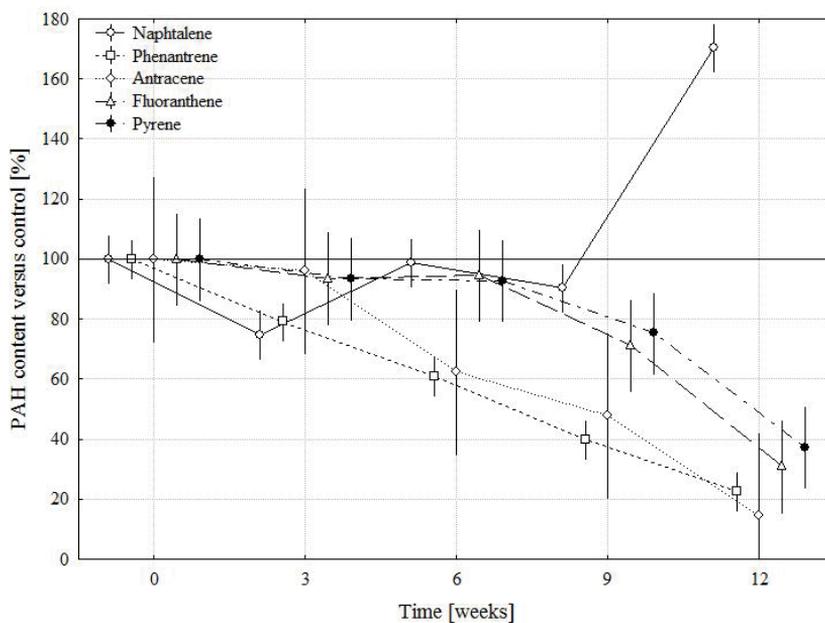


Fig. 2. PAH degradation by shiitake (*Lentinula edodes*). Vertical bars show 95% confidence level

Ryc. 2. Degradacja WWA przez shiitake (*Lentinula edodes*). Linie pionowe wskazują przedział ufności 95%

Table 2. PAH content in the sand/substrate mixture during the experiment (n = 4)

Tabela 2. Zawartość WWA w mieszaninie piasku i kompostu po uprawie grzybów w trakcie doświadczenia (n = 4)

Time (weeks) Czas (tydzień)	PAH content, $\mu\text{g} \times \text{kg}^{-1}$ d.w. – Zawartość WWA, $\mu\text{g} \times \text{kg}^{-1}$ s.m. (Mean values \pm SD – Średnia arytmetyczna \pm odchylenie standardowe)				
	anthracene antracen	fluoranthene fluorenten	naphthalene naftalen	phenanthrene fenantren	pyrene piren
control kontrola	5499 \pm 203	4765 \pm 166	1245 \pm 104	4074 \pm 166	5458 \pm 178
<i>Agaricus bisporus</i>					
3	1288 \pm 96	1811 \pm 75	935 \pm 89	1427 \pm 189	1910 \pm 125
6	1115 \pm 103	1421 \pm 106	1123 \pm 93	1141 \pm 81	1488 \pm 166
9	1246 \pm 80	1229 \pm 122	1092 \pm 137	985 \pm 76	1220 \pm 190
12	738 \pm 11	812 \pm 84	1855 \pm 30	854 \pm 72	812 \pm 84
<i>Lentinula edodes</i>					
control kontrola	5944 \pm 198	4864 \pm 112	1396 \pm 98	4133 \pm 136	5546 \pm 190
3	5738 \pm 145	4508 \pm 138	1033 \pm 95	3226 \pm 233	4992 \pm 125
6	3744 \pm 103	4573 \pm 122	1369 \pm 136	2477 \pm 133	5063 \pm 189
9	2953 \pm 112	3394 \pm 114	1256 \pm 76	1622 \pm 106	4109 \pm 129
12	811 \pm 55	1472 \pm 149	2373 \pm 177	917 \pm 78	2039 \pm 120

Table 3. pH of the mixture of SMC and quartz sands during the experiment
 Tabela 3. pH mieszaniny kompostu pogrzybowego i piasku w trakcie doświadczenia

Times (weeks) Czas (tydzień)	<i>Agaricus bisporus</i>	<i>Lentinula edodes</i>
Control – Kontrola	7.42	4.15
3	7.56	6.19
6	7.51	6.86
9	7.53	7.43
12	7.27	7.48

During the experiment, pH of the mixture of quartz sands and SMC from *Agaricus bisporus* was nearly stable and ranged from 7.27 to 7.56 (tab. 3). In contrast, pH of the mixture of quartz sands and SMC from *Lentinula edodes* rapidly increased from 4.15 at the beginning to 7.48 at the end of the experiment (tab. 3).

DISCUSSION

Mycoremediation seems to be a feasible alternative to clean up soil contaminated with PAHs, which are often found in waste effluents as mixtures. The experiment focused on the ability of spent mushroom compost from *Agaricus bisporus* (phase II mushroom compost) and *Lentinula edodes* to degrade selected PAHs during a 12-week experiment. The fungi reduced the concentration of fluoranthene, anthracene, phenanthrene and pyrene by a considerable amount. Although phenanthrene is not generally degradable by lignin-modifying enzymes such as those of white rot fungi, because of its high ionization potential (8.19 eV) [Bezalel et al. 1996], our results were very satisfactory. A considerable reduction was confirmed for *Lentinula edodes*, belonging to white rot fungi, and also for *Agaricus bisporus*, demonstrating the ability of the investigated fungi to oxidize persistent organic pollutants. Phenanthrene was degradable both by *Agaricus bisporus* and *Lentinula edodes* down to 20 and 22% of the control. The results were similar to those obtained for *Bjerkandera* BOL13 [Terrazas-Siles et al. 2005] and more considerable to those observed for *Agaricus bisporus* (Phase II mushroom compost) [Brian et al. 2002]

The degradation of anthracene by both fungi was very similar to other studies with *Pleurotus ostreatus* [Zebulun et al. 2011]. It was earlier confirmed that the release of ligninolytic enzymes such as lignin peroxidase, laccase, and manganese by *Pleurotus ostreatus* [Zebulun et al. 2011] was associated with anthracene degradation.

It was documented that high molecular weight PAHs sometimes were degraded after low molecular weight PAHs had been utilized [Haritash and Kaushik 2009]. The low reduction of the tetracyclic PAHs such as pyrene and benzo(a)anthracene is attributed to their structural complexity and associated physical properties, i.e. high molecular weight and insolubility [Tekere et al. 2005]. Our results were different and pointed to considerable degradation of pyrene. Degradation of pyrene by *Agaricus bisporus* was the strongest at the beginning of the experiment (3 weeks) and at the end the concentra-

tion was only 17% of the control. The pyrene degradation by *Lentinula edodes* was very weak until 6 weeks and then a drop of its contents was noted.

It is known that the optimum level of PAH degradation usually occurs in environments that are acidic and rich in organic carbon. Some experiments on *Pleurotus ostreatus* [Pozdnyakova et al. 2006] and *Ganoderma lucidum* [Ting et al. 2011] showed that optimum PAH degradation and laccase production occurred at pH of 4.0. In our experiment a rapid change of pH was detected only for *Lentinula edodes*, while for *Agaricus bisporus* pH was nearly stable. Generally the strongest oxidation was noted at pH > 7. However, pH seems to influence the rate of degradation of some PAHs such as pyrene and fluoranthene, because their rapid decrease was confirmed for *Lentinula edodes* at pH 6.86–7.48. Naphthalene degradation was observed after three weeks of the experiment for both *Lentinula edodes* and *Agaricus bisporus*, then the concentration increased. We suppose this was the result of degradation of other PAHs containing three or four rings in the molecule. The product of degradation of anthracene, pyrene, phenanthrene and fluoranthene was probably two-ring naphthalene, so a rapid increase of the concentration (up to 150 and 170% of the control) was reported at the end of the experiment.

CONCLUSIONS

The experiment confirmed strong and unique possibility of PAH degradation by both *Agaricus bisporus* and the white rot fungus *Lentinula edodes*. The level and kinetics of PAH degradation were dependent not only on the cultivar of fungus, but also on the structure of the PAH molecule. The results are promising and indicate the applicability of SMC in bioremediation technologies designed to treat soil contaminated with PAHs.

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DEGRADACJA WIELOPIERŚCIONIOWYCH WĘGLOWODORÓW AROMATYCZNYCH PRZEZ KOMPOST PO UPRAWIE *Agaricus bisporus* I *Lentinula edodes*

Streszczenie. Produkcja grzybów związana jest z wytwarzaniem dużej ilości kompostu, który wykazuje możliwości degradacji ligninopodobnych zanieczyszczeń. Wykorzystanie kompostu do oczyszczania skażonych gleb jest obiecującą alternatywą w stosunku do innych droższych metod. Dwunastotygodniowe doświadczenie z użyciem kompostu po-grzybowego uzyskanego z uprawy *Agaricus bisporus* (pieczarka dwuzarodnikowej) i *Lentinula edodes* (shiitake) przeprowadzono w celu porównania ich zdolności do degradacji WWA. Degradacja WWA przez *Agaricus bisporus* przebiegała w następującej kolejności: antracen, piren, fluorenten, i fenantren (87, 85, 83 i 79% zawartości w próbce kontrolnej). Najsilniejszą degradację przez *Lentinula edodes* potwierdzono dla antracenu (86% zawartości w próbce kontrolnej), następnie dla fenantrenu, fluorentenu i pirenu (odpowiednio 78, 70 i 63% zawartości w próbce kontrolnej). Po krótkotrwałej redukcji zawartości naftalenu stwierdzono gwałtowny jego wzrost zarówno dla *Agaricus bisporus* jak i *Lentinula edodes* (170 i 149% zawartości w próbce kontrolnej).

Słowa kluczowe: antracen, fenantren, fluorentaen, naftalen, piren, pieczarka, shiitake

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