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Evaluation of polychlorinated biphenyl degradation through refuse from *Pleurotus ostreatus*, *Lentinula edodes* and *Agaricus bisporus* production

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ABSTRACT

White rot fungi (WRF) are known to have the ability to degrade organic pollutants with a structure similar to lignin. Because of this, the degradation of polychlorinated biphenyls (PCBs) congeners no. 28, 52, 101, 138, 153 and 180 by substrate before fruiting (substrate) and/or after fruiting (SMS) from cultivated mushrooms *Pleurotus ostreatus*, *Lentinula edodes* and *Agaricus bisporus* was examined. The experiment was carried out in four replications for each treatment using a mixture of substrate/SMS and sandy soil with PCBs at a concentration of each congener at 50 and 100 μg kg^{-1} soil DW. The results indicate that degradation was dependent on substrate/SMS addition, the concentration of PCBs and time of incubation. The efficiency of PCB degradation was generally reduced with the number of chlorine atoms in the structure of congeners: 28, 52, 101, 138, 153 or 180. In all combinations, degradation increased with incubation time. Degradation by SMS was lower in comparison to degradation by a substrate of the same mushroom. The degree of degradation of a single PCB after 12 weeks of incubation for *A. bisporus* ranged from 31.32 ± 1.52 to 83.91 ± 1.07%, while for *P. ostreatus* it was between 37.88 ± 2.54 and 78.29 ± 1.41%; for *L. edodes* it ranged from 17.38 ± 1.06 to 75.30 ± 1.46%. The best average degradation was confirmed for 20% SMS of *A. bisporus* at 50 μg kg^{-1} PCB.

Key words: congener, mycoremediation, spent mushroom substrate, white rot fungi

INTRODUCTION

High toxic polychlorinated biphenyls (PCBs) are still a serious pollutant of the environment although their production and use was banned or their use is legal, but subject to strict conditions. They were used in industrial and commercial applications, e.g. heat transfer, electrical and hydraulic equipment. PCBs are toxic, highly resistant to oxidation, persistent in the environment and they can be accumulated in many species. Their decomposition is very expensive because of the cost of chemical reagents and the technological process. Thus, alternative methods such as bioremediation have become economically acceptable processes of PCB degradation. The transformation of PCBs is limited by their bioavailability and it is known that some microorganisms such as bacteria and fungi are able to decompose or transform PCBs (Fiebig et al. 1993, Rojas-Avelizapa 1999, Seto et al. 1999, Ruiz-Aguilar et al. 2002). However, very low concentrations of these organisms occurring in nature limit degradation (Ruiz-Aguilar et al.

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2002). The ability of PCB oxidation was also documented for white rot fungi (WRF), which secrete non-specific extracellular ligninolytic enzymes responsible for the degradation of lignin and organic compounds with similar structures. Consisting of laccases (LAC), peroxidases (LiP) and manganese peroxidases (MnP) (Zheng et al. 2002), the oxidative enzymatic system possesses the ability to degrade a variety of aromatic, recalcitrant pollutants, including polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCBs), dyes, dioxins, pesticides, explosives and solvents, etc. (Joshi and Gold 2000, Baldrian 2003, Arun et al. 2008, Gąsecka et al. 2012, 2013). *Phanerochaete chrysosporium* (a model fungus in studies of PCB degradation), *Trametes versicolor*, *Lentinula edodes* and *Pleurotus ostreatus* have proven to be effective in the degradation of PCBs. Some of them were reported to degrade even more than 70% of PCBs depending on the initial concentration of PCBs, time of incubation and the fungal species (Šašek et al. 1993, Kubátová et al. 2001, Ruiz-Aguilar et al. 2002).

The mushroom industry generates a high amount of compost, which is a good source of micronutrients, microorganisms and enzymes and has the ability to modify soil properties (pH, moisture and structure) and to change the activity of soil microflora. In consequence, it affects the bioavailability of pollutants. The effective use of spent mushroom compost in bioremediation has been confirmed for polychlorinated hydrocarbons, plasticizers, pesticides and herbicides (Brian et al. 2002, Sæbø and Ferrini 2006, Kadian et al. 2008, Chiu et al. 2009, Purnomo et al. 2010, Russo et al. 2012).

Our investigation focused on comparing the degradation of selected PCBs with 3, 4, 5, 6 and 7 chlorine atoms in their structure by substrate overgrown with mycelium before fruiting and/or by the substrate after fruiting (spent mushroom substrate) (SMS) as refuse from *P. ostreatus*, *L. edodes* and *Agaricus bisporus* production.

MATERIAL AND METHODS

Fungal material

Pleurotus ostreatus

Cut wheat straw chaff was used as a substrate for oyster mushroom production. The substrate was moistened with tap water to 70% of water content, pasteurized at 60°C for 48 hours, cooled and mixed with the mycelium of *P. ostreatus* cv. P80 (3% substrate, w/w). Then the mixture was placed in perforated plastic bags (5 dm^3) and incubated (25°C and relative air humidity of 80-85%). The complete overgrowth substrate was used in the experiment as the substrate before fruiting. The other part of the substrate was cultivated in the room (15-17°C, air relative humidity of 85-90%, light intensity of 6.75 μmol m^{-2} s^{-1} for 10 h). The room was ventilated during yielding to maintain the concentration of carbon dioxide in the atmosphere at a level of 0.06 -0.08%. The substrate was used in the experiment as SMS after two flushes of fruiting bodies (Gąsecka et al. 2013).

Lentinula edodes

The substrates originated from a mushroom farm ("MYCOMED", Kościelna Wieś, Greater Poland region). A mixture of oak and alder sawdust (1:1, v/v) with the addition of wheat bran (20% in relation to 1 kg of dry weight of sawdust) was used as the substrate. The mixture was moistened with tap water to a water content of 65%. After pasteurization, the substrate was cooled and mixed with *L. edodes* spawn. The complete overgrown substrate was used in the experiment as the substrate before fruiting. The other part was cultivated in the hall (at 16-17°C, relative air humidity of 80-85%, light intensity of 6.75 μmol m^{-2} s^{-1} for 10 h). After three flushes of fruiting bodies the substrate was used in the experiment as SMS.

Agaricus bisporus

Spent mushroom substrate (phase II) originating from "KOMPOSTPAL" S.C. (Kościan, Greater Poland region) was collected after the cultivation of *A. bisporus* grown on a mixture of wheat straw and poultry manure according to generally accepted technology ending after the harvest of three flushes.

Experimental set-up

The experiment was carried out in four replications for each treatment in a mixture of PCBs, sandy soil and *P. ostreatus* or *L. edodes* substrates or SMS and *A. bisporus* SMS. The concentrations of each congener were 50 or 100 μg kg^{-1} soil DW. The PCBs were dissolved in 100 mL of acetone and added to the soil. After solvent evaporation to dryness, the contaminated soil was mixed with substrate or SMS at 0 (control sample), 10, or 20% w/w ratio. The mixture (2 kg per pot) was incubated for 12 weeks under controlled conditions of air humidity and temperature. The samples were collected every three weeks of incubation.

Chemicals

PCBs no. 28, 52, 101, 138, 153 and 180 as representatives of PCBs, acetone and n-hexane (Chromasolv Plus for HPLC) were obtained from Sigma-Aldrich.

Extraction of PCBs

The extraction of PCBs from a mixture of substrates and soil was performed according to the EPA 3550B protocol. Dried samples of 20 g were mixed with anhydrous Na_2SO_4 and a mixture of hexane/acetone (1:1 v/v) and sonicated for 15 min. Then the samples were shaken for six hours with an IKA KS 260 shaker (IKA-Werke GmbH & Co. Kg, Staufen, Germany). After filtration using Whatman no. 5 filters the extracts were mixed with copper powder and shaken for three hours. To remove polar impurities, the extracts were passed through a small column of Florisil Supelclean LC with 10 mL of hexane and then were evaporated under a stream of nitrogen.

GC-MS/MS analysis

Qualitative and quantitative analyses of the PCB residues were performed with a Varian 450 gas chromatograph (GC) (Varian BV, Middelburg, the Netherlands) coupled with a Varian 320 MS (Varian Inc., Walnut Creek, 94598 California, USA) mass detector using the method of external standard and single ion monitoring technique (SIM). The GC was equipped with a Varian VF5MS column (30 m × 0.32 mm, 0.25 µm) and helium was used as a carrier gas with a constant flow rate of 1.0 mL min^{-1}. The injection temperature was 250ºC; the injection volume was 1 µL (splitless). The analysis was performed according to the following temperature program: start 50°C for 1 min, 50 -250°C (20°C/min), 250°C for 20 minutes. For each of the PCBs the following ions (m/z) were chosen: PCB 28(256), PCB 52(292), PCB 101(324), PCB 138(358), PCB 153(360) and PCB 180 (394) (Sułkowski and Rosińska 1999).

pH measurement

The samples (10 g) were mixed with 10 mL of distilled water and after one hour the pH was measured (Hanna Instrument HI 2210 pH-meter, France).

Data analyses

The data were processed with Statistica 10.0 software (StatSoft Inc.). Two-factor analysis of variance was performed to examine the differences between degree of degradation of PCBs with regard to the levels of PCBs and substrates/SMC additions. Data were presented as mean values and standard deviations (SD). The average PBC degradation was analysed statistically using one way analysis of variance (ANOVA) followed by post hoc Tukey's test ($p = 0.05$).

RESULTS

The experiments confirmed a slow decrease of PCB content in soil (less than one degree every three weeks) in the controls, i.e. without the addition of *P. ostreatus, A. bisporus* or *L. edodes* substrate or SMS. After 12 weeks, the degradation of each congener in the control samples was slightly less than 2% for both concentrations of pollutants. It was included in the further calculation of the degree of degradation. The growth of fruiting bodies was not observed at any combination. The results of the PCB degradation are shown in Tables 1-9. In all of the combinations, PCB degradation increased during incubation.

Degradation of PCBs by P. ostreatus

At the end of the experiment the degradation of PCBs ranged from 37.88 ± 2.54% for congener 180 at 100 µg kg^{-1} and 10% SMS addition (Tab. 4), to 78.29 ± 0.24% for congener 52 at 50 µg kg^{-1} and 20% substrate addition (Tab. 5). The strongest degradation was confirmed for 50 µg kg^{-1} PCBs in a mixture of soil and 20% addition of substrate or SMS (Tabs 5 and 6). The use of SMS resulted in the reduction of PCB degradation in comparison to the substrate before fruiting (Tabs 1-8).

A higher addition of substrate/SMS at the same concentration of PCBs resulted in higher degradation of PCBs, while an increase of PCB concentration for the same substrate/SMS addition caused a reduction of degradation by up to 13%. The lowest degradation was confirmed at 100 µg kg^{-1} PCBs and 10% addition of substrate or SMS (Tabs 3 and 4). In all of the experiments, degradation of individual congeners after 12 weeks of incubation proceeded in the following order: 28 (~53-78%), 52 (~48-78%), 101 (~44-66%), 138 (~41-67%), 153 (~43-56%) and 180 (~38-53%).

Degradation of PCBs by L. edodes

The results indicate that PCB degradation after 12 weeks of incubation ranged from 17.38 ± 1.06% for congener 180 and 10% SMS addition at 100 µg kg^{-1} PCBs (Tab. 3) to 75.30 ± 1.46% for congener 52 and 20% substrate addition at50 µg kg^{-1} PCBs (Tab. 5). The *L. edodes* SMS caused the extenuation of

Table 1. Degradation of 50 μg kg^{-1} PCBs (%) by 10% substrate (before fruiting) addition to growth medium

Mushroom	Time of incubation (weeks)	28	52	101	138	153	180
P. ostreatus	3	19.45^{i}±2.49	20.16^{i}±0.82	16.95^{i}±0.86	18.56^{i}±1.20	18.31^{i}±1.03	14.98^{i}±1.07
P. ostreatus	6	36.26^{fg}±0.98	35.46^{fg}±0.21	34.91^{fg}±1.55	31.21^{g}±1.01	25.72^{h}±2.78	25.79^{h}±4.47
P. ostreatus	9	57.74^{b}±1.36	49.26^{cd}±0.57	45.39^{de}±2.09	43.52^{e}± 0.65	40.09^{ef}±2.14	35.62^{b}±2.63
P. ostreatus	12	67.08^{a}±1.21	64.95^{a}±0.75	55.42^{b}±1.37	54.17^{bc}±0.27	56.34^{b}±1.04	52.84^{bc}±0.96
L. edodes	3	24.39^{kl}±0.37	28.24^{j}±1.56	25.48^{jk}±1.01	20.95^{m}±0.77	24.83^{kl}±1.34	22.17^{lm}±0.31
L. edodes	6	37.07^{i}±1.09	48.24^{e}±1.05	43.66^{de}±0.49	35.39^{i}±0.17	40.85^{efg}±1.35	37.36^{hi}±0.41
L. edodes	9	45.66^{d}±1.30	61.21^{a}±0.59	49.72^{bc}±0.51	37.80^{ghi}±0.70	45.36^{d}±0.52	40.37^{fgh}±1.69
L. edodes	12	50.79^{bc}±1.19	62.10^{a}±0.69	52.49^{b}±0.81	42.88^{def}±0.83	49.63^{bc}±1.56	44.22^{d}±0.52

Mean values ± standard deviations (n = 4); identical superscripts denote no significant ($p < 0.05$) differences between mean values for each mushroom according to Tukey's HSD test (ANOVA)

Table 2. Degradation of 50 μg kg^{-1} PCBs (%) by 10% SMS addition to growth medium

Mushroom	Time of incubation (weeks)	28	52	101	138	153	180
P. ostreatus	3	17.28^{ij}±1.71	17.54^{ij}±0.81	15.61^{j}±2.59	15.43^{j}±2.04	12.93^{j}±0.92	12.46^{j}±1.99
P. ostreatus	6	32.34^{fg}±2.82	32.79^{f}±0.58	25.69^{h}±1.69	27.11^{gh}±1.28	21.76^{hi}±0.52	21.52^{hi}±3.29
P. ostreatus	9	46.16^{bc}±0.81	41.33^{cde}±1.51	35.68^{ef}±0.82	37.62^{def}±2.11	36.14^{def}±2.12	34.59^{f}±2.27
P. ostreatus	12	62.07^{a}±1.64	60.76^{a}±1.26	47.05^{b}±0.97	46.43^{bc}±1.64	45.53^{bc}±1.57	41.62^{bcd}±2.48
L. edodes	3	23.06^{kl}±0.36	21.59^{klm}±1.30	24.21^{k}±0.97	19.89^{lm}±0.73	21.21^{klm}±1.14	19.11^{m}±1.05
L. edodes	6	35.03^{hi}±1.03	36.21^{ghi}±2.41	42.43^{de}±2.01	33.61^{ij}±0.21	34.88^{hi}±1.15	31.35^{j}±0.34
L. edodes	9	43.14^{d}±1.23	56.43^{b}±0.58	47.23^{c}±0.48	37.62^{fgh}±0.87	39.51^{efg}±0.79	32.93^{ij}±0.61
L. edodes	12	47.99^{c}±1.13	63.00^{a}±0.67	49.86^{c}±0.77	40.72^{def}±0.79	42.39^{de}±1.32	37.11^{gh}±0.41
A. bisporus	3	36.42^{i}±0.56	34.82^{ij}±0.89	31.39^{ijk}±1.25	30.26^{jk}±1.12	29.77^{jk}±1.61	28.86^{k}±2.41
A. bisporus	6	55.34^{ef}±1.63	52.61^{fg}±2.73	55.01^{ef}±2.59	51.13^{fgh}±0.25	48.97^{gh}±1.62	46.52^{h}±0.51
A. bisporus	9	68.15^{bc}±1.94	64.99^{cd}±1.97	61.24^{d}±0.62	53.17^{fg}±1.49	50.79^{fgh}± 2.94	49.86^{fgh}±2.51
A. bisporus	12	75.81^{a}±1.78	72.85^{ab}±2.54	64.64^{cd}±0.99	61.93^{d}±1.21	59.51^{de}±1.86	55.07^{ef}±0.65

Mean values ± standard deviations (n = 4); identical superscripts denote no significant ($p < 0.05$) differences between mean values for each mushroom according to Tukey's HSD test (ANOVA)

Table 3. Degradation of 100 μg kg^{-1} PCBs (%) by 10% substrate (before fruiting) addition to growth medium

Mushroom	Time of incubation (weeks)	28	52	101	138	153	180
P. ostreatus	3	15.21^{nop}±0.71	16.61^{mno}±1.41	14.49^{nop}±1.81	14.07^{op}±1.87	11.73^{p}±0.56	12.21^{op}±2.59
P. ostreatus	6	33.69^{h}±0.63	34.97^{gh}±1.53	27.39^{j}±0.51	22.79^{kl}±1.63	19.89^{lm}±1.67	18.97^{lmn}±0.18
P. ostreatus	9	49.59^{bc}±1.21	38.68^{fg}±1.54	36.31^{fgh}±0.55	32.27^{hi}±1.53	28.33^{ij}±0.57	25.97^{jk}±1.79
P. ostreatus	12	57.61^{a}±0.64	53.38^{ab}±1.22	44.07^{ef}±2.68	43.47^{ef}±0.87	47.44^{de}±0.48	39.61^{ef}±2.03
L. edodes	3	19.94^{hijk}±0.66	12.69^{lm}±0.87	17.26^{jkl}±0.81	12.63^{lm}±0.75	12.65^{lm}±0.28	10.63^{m}±0.63
L. edodes	6	26.91^{ef}±3.67	20.54^{ghijk}±1.37	25.64^{efgh}±0.97	17.89^{b}±0.65	18.62^{ijk}±0.25	15.31^{klm}±0.19
L. edodes	9	35.68^{bc}±3.55	34.62^{cd}±1.46	29.60^{ef}±2.97	22.15^{fghij}±1.09	23.38^{ghij}±0.65	18.76^{ijk}±0.77
L. edodes	12	41.25^{b}±2.66	51.54^{a}±1.09	34.21^{cd}±2.09	24.85^{efgh}±1.56	25.83^{efg}±0.98	20.92^{ghijk}±1.29

Mean values ± standard deviations (n = 4); identical superscripts denote no significant ($p < 0.05$) differences between mean values for each mushroom according to Tukey's HSD test (ANOVA)

Table 4. Degradation of 100 μg kg^{-1} PCBs (%) by 10% SMS addition to growth medium

Mushroom	Time of incubation (weeks)	28	52	101	138	153	180
P. ostreatus	3	11.47^{nop}±0.68	11.61^{nop}±1.01	13.01^{mno}±1.12	11.77^{nop}±1.28	9.14^{op}±0.19	8.69^{p}±0.52
P. ostreatus	6	30.02^{g}±1.03	26.83^{ghi}±0.81	23.89^{ijk}±1.45	20.09^{kl}±1.77	17.09^{lm}±1.81	15.46^{mn}±1.67
P. ostreatus	9	41.81^{cde}±0.98	34.99^{f}±2.61	30.25^{g}±1.19	28.67^{gh}±1.21	25.31^{hij}±1.35	21.33^{jkl}±1.46
P. ostreatus	12	53.06^{a}±0.13	49.39^{ab}±1.67	45.39^{bc}±0.55	41.02^{cde}±1.09	43.53^{de}±1.35	37.88^{ef}±2.54
L. edodes	3	18.84^{hijk}±0.62	12.09^{lmn}±0.51	15.27^{klm}±0.51	12.28^{lmn}±1.19	11.32^{mn}±1.08	8.67^{n}±0.26
L. edodes	6	28.76^{def}±0.41	15.68^{e}±0.36	22.92^{gh}±0.49	17.72^{ijk}±0.73	15.86^{kl}±0.26	12.67^{lmn}±0.21
L. edodes	12	38.93^{b}±2.39	45.83^{a}±0.42	31.58^{cd}±1.93	24.92^{fg}±0.86	21.78^{ghi}±1.33	17.38^{jk}±1.06
A. bisporus	3	29.05^{hi}±0.99	24.26^{ij}±0.37	27.01^{hi}±0.60	19.28^{jk}±1.68	15.67^{k}±0.99	13.12^{k}±0.45
A. bisporus	6	38.87^{ef}±3.47	43.27^{cde}±1.27	31.41^{gh}±0.92	24.24^{ij}±2.29	22.79^{ij}±0.67	19.08^{jk}±0.87
A. bisporus	9	46.41^{bcd}±2.73	51.80^{b}±2.03	36.35^{fg}±0.99	28.41^{hi}±1.24	32.28^{gh}±0.38	24.25^{ij}±1.13
A. bisporus	12	60.29^{a}±2.74	51.81^{b}±5.79	47.23^{bc}±3.31	39.98^{def}±2.53	37.78^{efg}±0.72	31.32^{gh}±1.52

Mean values ± standard deviations (n = 4); identical superscripts denote no significant ($p < 0.05$) differences between mean values for each mushroom according to Tukey's HSD test (ANOVA)

Table 5. Degradation of 50 μg kg^{-1} PCBs (%) by 20% substrate (before fruiting) addition to growth medium

Mushroom	Time of incubation (weeks)	28	52	101	138	153	180
P. ostreatus	3	22.09^{no}±0.78	21.61^{no}±1.49	21.28^{no}±1.51	21.63^{no}±1.53	19.55^{o}±0.64	18.82^{o}±1.18
P. ostreatus	6	39.57^{ij}±1.25	38.05^{ij}±0.97	36.44^{jk}±0.44	33.15^{kl}±2.19	28.81^{kl}±0.71	25.09^{mn}±2.81
P. ostreatus	9	58.18^{c}±0.31	52.34^{de}±0.76	46.89^{fg}±0.36	41.43^{hi}±1.12	44.41^{gh}±3.01	40.41^{hij}±1.41
P. ostreatus	12	78.25^{a}±0.24	78.29^{a}±1.41	66.51^{b}±1.79	67.39^{b}±0.36	54.49^{cd}±1.11	49.98^{ef}±1.29
L. edodes	3	32.23^{i}±0.49	39.62^{h}±1.09	33.67^{i}±1.34	27.67^{j}±1.02	23.79^{k}±0.28	28.62^{ij}±0.98
L. edodes	6	48.97^{g}±1.44	61.25^{d}±1.41	57.67^{e}±0.64	46.76^{g}±0.23	42.29^{c}±1.10	49.35^{g}±0.54
L. edodes	9	60.31^{de}±1.72	67.33^{bc}±1.52	65.69^{c}±0.67	54.29^{f}±1.21	48.62^{h}±0.94	54.22^{f}± 2.04
L. edodes	12	68.02^{bc}±0.07	75.30^{a}±1.46	69.81^{b}±0.37	59.36^{de}±0.64	57.26^{ef}±0.51	58.69^{de}±0.79

Mean values ± standard deviations (n = 4); identical superscripts denote no significant ($p < 0.05$) differences between mean values for each mushroom according to Tukey's HSD test (ANOVA)

Table 6. Degradation of 50 μg kg^{-1} PCBs (%) by 20% SMS addition to growth medium

Mushroom	Time of incubation (weeks)	28	52	101	138	153	180
P. ostreatus	3	19.28^{l}±1.07	18.14^{lm}±2.41	19.05^{lm}±1.32	17.43^{lm}±0.99	14.60^{lm}±2.13	17.91^{m}±1.55
P. ostreatus	6	37.33^{fg}±1.15	36.14^{gh}±0.26	32.67^{hi}±0.59	31.27^{ij}±0.36	27.51^{jk}±1.94	24.38^{k}±1.23
P. ostreatus	9	49.27^{d}±1.12	48.47^{d}±1.47	42.78^{e}±1.48	40.71^{ef}±0.74	40.82^{ef}±2.99	35.39^{ghi}±0.54
P. ostreatus	12	77.69^{a}±2.41	70.61^{b}±0.73	62.69^{c}±1.66	60.05^{d}±0.13	49.12^{d}±1.72	48.49^{d}±0.71
L. edodes	3	30.46^{jk}±0.47	35.08^{i}±1.31	31.99^{ij}±1.27	26.28^{l}±0.99	28.01^{kl}±1.51	27.02^{l}±1.01
L. edodes	6	46.28^{fg}±1.36	50.39^{de}±2.01	49.05^{ef}±0.94	44.41^{gh}±0.22	46.08^{fg}±1.52	41.42^{h}±0.45
L. edodes	9	56.99^{c}±1.62	57.42^{c}±0.55	62.41^{b}±0.63	52.51^{d}±0.72	51.79^{de}±0.69	46.04^{fg}±0.75
L. edodes	12	64.28^{ab}±0.06	66.44^{a}±0.37	66.32^{a}±0.35	56.19^{c}±1.11	56.31^{c}±1.42	49.26^{def}±0.66
A. bisporus	3	48.11^{klm}±0.74	46.00^{lmn}±1.17	41.46^{mno}±1.65	39.98^{no}±1.48	39.33^{no}±2.12	38.13^{o}±1.34
A. bisporus	6	63.11^{gh}±2.16	69.51^{defg}±3.61	56.99^{hi}±0.99	47.54^{klm}±0.34	49.36^{jkl}±2.61	41.47^{mno}±2.97
A. bisporus	9	73.37^{cde}±3.25	75.86^{bcd}±2.59	70.22^{cdef}±1.79	70.23^{cdef}±1.98	53.77^{ijk}±1.87	55.86^{ij}±1.07
A. bisporus	12	81.12^{ab}±0.68	83.91^{a}±1.07	75.39^{bcd}±1.31	76.82^{bc}±2.86	67.42^{efg}±4.87	65.28^{fg}± .46

Mean values ± standard deviations (n = 4); identical superscripts denote no significant ($p < 0.05$) differences between mean values for each mushroom according to Tukey's HSD test (ANOVA)

Table 7. Degradation of 100 μg kg^{-1} PCBs (%) by 20% substrate (before fruiting) addition to growth medium

Mushroom	Time of incubation (weeks)	28	52	101	138	153	180
P. ostreatus	3	16.94^{lm}±0.82	17.62^{lm}±1.13	16.45^{lm}±0.83	18.04^{lm}±0.11	13.91^{m}±1.04	16.24^{lm}±1.35
P. ostreatus	6	36.51^{ef}±0.52	36.77^{ef}±0.79	29.11^{hi}±0.99	26.78^{ij}±2.13	23.26^{jk}±2.93	19.33^{kl}±2.13
P. ostreatus	9	51.36^{bcc}±2.16	48.21^{bcd}±1.00	38.19^{e}±1.26	34.21^{efg}±1.04	33.48^{fgh}±1.63	31.06^{ghi}±1.59
P. ostreatus	12	71.38^{a}±5.81	57.17^{b}±3.22	51.61^{bc}±4.78	52.17^{bc}±5.11	49.15^{bcd}±3.06	45.37^{d}±2.91
L. edodes	3	24.82^{jklm}±0.79	17.67^{pr}±0.85	20.84^{nop}±1.08	14.22^{rs}±0.86	14.69^{rs}±0.73	12.12^{s}±0.50
L. edodes	6	35.72^{e}±1.31	34.41^{ef}±0.65	30.19^{ghi}±0.33	22.84^{lmn}±1.56	22.02^{mno}±0.91	18.09^{opr}±0.71
L. edodes	9	46.28^{c}±2.05	48.94^{bc}±0.82	38.28^{de}±1.57	27.53^{ghij}±0.84	26.55^{ijkl}±2.65	23.11^{klm}±0.65
L. edodes	12	51.66^{b}±0.42	56.01^{a}±0.38	41.18^{d}±2.52	31.24^{fg}±0.48	30.69^{efg}±1.88	26.85^{hijk}±1.35

Mean values ± standard deviations (n = 4); identical superscripts denote no significant ($p < 0.05$) differences between mean values for each mushroom according to Tukey's HSD test (ANOVA)

Table 8. Degradation of 100 μg kg^{-1} PCBs (%) by 20% SMS addition to growth medium

Mushroom	Time of incubation (weeks)	28	52	101	138	153	180
P. ostreatus	3	13.98^{no}±0.35	15.96^{no}±1.07	15.69^{no}±0.71	13.76^{no}±1.69	11.35^{o}±0.55	12.21^{no}±2.15
P. ostreatus	6	34.32^{gh}±0.59	22.08^{lm}±0.98	26.86^{jkl}±1.67	24.29^{klm}±2.31	21.49^{m}±2.48	16.47^{n} ±1.66
P. ostreatus	9	42.39^{cde}±1.32	38.39^{efg}±1.19	35.53^{fgh}±0.86	31.69^{hij}±2.17	31.88^{hi}±2.13	28.49^{ijk}±1.86
P. ostreatus	12	66.56^{a}±4.80	47.82^{cd}±4.71	50.73^{b}±4.67	49.01^{bc}±3.78	45.61^{cd}±3.36	41.91^{def}±3.97
L. edodes	3	23.22^{ij}±0.43	25.78^{hi}±1.04	18.98^{k}±0.58	13.34^{lm}±1.09	13.06^{lm}±0.35	10.28^{m}±0.16
L. edodes	6	33.94^{e}±0.94	37.61^{d}±1.35	28.12^{gh}±0.51	21.19^{jk}±0.65	19.09^{k}±0.32	15.65^{l}±0.53
L. edodes	9	42.92^{c}±1.15	48.99^{b}±1.32	33.87^{e}±1.69	29.09^{fg}±0.32	22.68^{ij}±2.26	19.09^{k}±0.51
L. edodes	12	47.93^{b}±1.14	55.24^{a}±0.39	38.01^{d}±2.31	32.41^{ef}±0.03	27.22^{gh}±0.13	21.93^{jk}±0.45
A. bisporus	3	34.29^{ijk}±1.22	29.21^{klm}±0.45	24.89^{mn}±0.38	21.21^{nop}±0.33	18.06^{op}±0.28	15.39^{p}±0.23
A. bisporus	6	52.10^{de}±3.98	44.38^{fg}±1.31	32.21^{jkl}±0.96	33.88^{jk}±1.68	27.44^{lm}±0.81	23.38^{mno}±0.69
A. bisporus	9	64.16^{b}±4.23	51.99^{de}±2.87	37.31^{hij}±1.85	37.23^{hij}±0.78	33.79^{jk}±0.97	28.79^{klm}±0.82
A. bisporus	12	71.37^{a}±2.87	59.68^{bc}±3.39	54.17^{cd}±5.86	46.56^{ef}±1.32	41.67^{fgh}±1.13	40.11^{ghi}±2.21

Mean values ± standard deviations (n = 4); identical superscripts denote no significant ($p < 0.05$) differences between mean values for each mushroom according to Tukey's HSD test (ANOVA)

degradation of PCBs in comparison to the substrate before fruiting (Tabs 1-8). In all of the combinations, the highest degradation was confirmed for PCB no. 52 and the lowest for PCB no. 180.

The increase of the substrate/SMS addition enhanced the degree of degradation at the same concentration of PCBs. The increase of concentration of PCBs at the same level of substrate/SMS addition resulted in a decrease of degradation by more than 20%. In all of the experiments, the degradation of individual congeners after 12 weeks of incubation proceeded in the following order: 52 (~46-75%), 28 (~41-68%), 101 (~32-70%), 138 (~25-59%), 153 (~22-57%) and 180 (~17-59%).

Degradation of PCBs by A. bisporus

A. bisporus was able to degrade from 31.32 ± 1.52 for congener 180, 10% SMS at 100 μg kg^{-1} PCBs (Tab. 4) to 83.91 ± 1.07% for congener 52, 20% SMS and 50 μg kg^{-1} PCBs (Tab. 6). As it was documented for the other mushroom species, the increase of SMS addition enhanced the degradation about several per cent. However, the higher the concentration of PCBs applied, the lower the degradation that was observed. Congeners 28 and 52 were the most degradable, while congener 180 was the least degradable. The degradation rate ranges as follows: 52 (~60-84%), 28 (~60-81%), 101 (~47-75%), 138 (~40-77%), 153 (~38-67%) and 180 (~31-65%).

Average degradation of PCBs

The results of the average degradation of PCBs (a mean value from the degradation of all PCBs at the end of the experiment) ranged from 74.99 to 30.07% (Tab. 9). The strongest ability to oxidase PCBs was confirmed for 20% SMS of *A. bisporus*

Table 9. Average degradation of PCB (%) after 12 weeks of incubation

Combination	Degradation	Combination	Degradation
AbSMS 3	74.99^{a}	Le 1	50.35^{bcdefg}
Po 3	65.82^{ab}	PoSMS 4	50.27^{bcdefg}
AbSMS 1	64.97^{abc}	Po 2	47.60^{bcdefgh}
Le 3	64.74^{abc}	LeSMS 1	46.85^{cdefgh}
PoSMS 3	61.44^{abcd}	PoSMS 2	45.05^{defgh}
LeSMS 3	59.80^{abcd}	AbSMS 2	44.73^{defgh}
Po 1	58.47^{abcde}	Le 4	39.60^{efgh}
Po 4	54.47^{bcdef}	LeSMS 4	37.12^{fgh}
AbSMS 4	52.26^{bcdefg}	Le 2	33.10^{gh}
PoSMS 1	50.58^{bcdefg}	LeSMS 2	30.07^{h}

Identical superscripts denote no significant ($p < 0.05$) differences between mean values according to Tukey's HSD test (ANOVA)

Table 10. pH of soil and growth medium

Sample	pH	Sample	pH
S 1	7.0	AbSMS1	6.4
S 2	7.0	AbSMS2	6.3
C 1	6.8	AbSMS3	6.7
C 2	6.4	AbSMS4	6.9
Po 1	5.9	PoSMS 1	6.7
Po 2	6.0	PoSMS 2	6.8
Po 3	6.7	PoSMS 3	7.2
Po 4	6.7	PoSMS 4	7.9
Le 1	5.9	LeSMS 1	6.7
Le 2	6.0	LeSMS 2	7.3
Le 3	6.5	LeSMS 3	7.0
Le 4	6.9	LeSMS 4	7.9

at 50 μg kg^{-1} PCBs, while the weakest for 10% SMS of *L. edodes* at 100 μg kg^{-1} PCBs. The high degradation (more than 60%) was also obtained for substrates before fruiting and SMS at 50 μg kg^{-1} PCBs.

Changes of pH

During the experiment, pH changes were observed in the control (soil without substrate/SMS addition) and mixtures of soil and substrate/SMS contaminated by PCBs at both levels (Tab. 10). Soil pH was neutral (7.0). At the end of the experiment (after 12 weeks), a reduction of pH was observed in most of the combinations besides several exceptions (Tab. 10).

Abbreviations:

S 1 – soil at the beginning of the experiment; S 2 – soil at the end of the experiment; C 1 – mixture of soil and 50 μg kg^{-1} of each PCB; C 2 – mixture of soil and 100 μg kg^{-1} of each PCB; Po 1 – mixture of soil, *P. ostreatus* substrate (10%) and 50 μg kg^{-1} of each PCB; Po 2 – mixture of soil, *P. ostreatus* substrate (10%) and 100 μg kg^{-1} of each PCB; Po 3 – mixture of soil, *P. ostreatus* substrate (20%) and 50 μg kg^{-1} of each PCB; Po 4 – mixture of soil, *P. ostreatus* substrate (20%) and 100 μg kg^{-1} of each PCB; PoSMS 1 – mixture of soil, *P. ostreatus* SMS (10%) and 50 μg kg^{-1} of each PCB; PoSMS 2 – mixture of soil, *P. ostreatus* SMS (10%) and 100 μg kg^{-1} of each PCB; PoSMS 3 – mixture of soil, *P. ostreatus* SMS (20%) and 50 μg kg^{-1} of each PCB; PoSMS 4 – mixture of soil, *P. ostreatus* SMS (20%) and 100 μg kg^{-1} of each PCB; Le 1 – mixture of soil, *L. edodes* substrate (10%) and 50 μg kg^{-1} of each PCB; Le 2 – mixture of soil, *L. edodes* susbtrate (10%) and 100 μg kg^{-1} of each PCB; Le 3 – mixture of soil, *L. edodes* substrate (20%) and 50 μg kg^{-1} of each PCB; Le 4 – mixture of soil, *L. edodes* substrate (20%) and 100 μg kg^{-1} of each PCB; LeSMS 1 – mixture of soil, *L. edodes* SMS (10%) and 50 μg kg^{-1} of each PCB; LeSMS 2 – mixture of soil, *L. edodes* SMS (10%) and 100 μg kg^{-1} of each PCB; LeSMS 3 – mixture of soil, *L. edodes* SMS (20%) and 50 μg kg^{-1} of each PCB; LeSMS 4 – mixture of soil, *L. edodes* SMS (20%) and 100 μg kg^{-1} of each PCB; AbSMS 1 – mixture of soil, *A. bisporus* SMS (10%) and 50 μg kg^{-1} of each PCB; AbSMS 2 – mixture of soil, *A. bisporus* SMS (10%) and 100 μg kg^{-1} of each PCB; AbSMS 3 – mixture of soil, *A. bisporus* SMS (20%) and 50 μg kg^{-1} of each PCB; AbSMS 4 – mixture of soil, *A. bisporus* SMS (20%) and 100 μg kg^{-1} of each PCB

DISCUSSION

The degradation of PCBs by *P. ostreatus*, *A. bisporus* and *L. edodes* was dependent on substrate/SMS addition, the concentration of PCBs and the time of incubation. The results of the degradation of a single PCB in a mixture of soil and substrate/SMS of *P. ostreatus*, *L. edodes* and *A. bisporus* ranged between 17 and 84%, while the total degradation was between 30 and

75%. The highest degradation was observed when 20% substrate/SMS and 50 μg kg^{-1} were used (the highest degree was confirmed for *A. bisporus*). The degradation of PCBs by both substrate and SMS was quite satisfactory, although the efficiency for SMS was weaker. Other results indicate different degradation of PCBs (Asther et al. 1987, Šašek et al. 1993, Kubátová et al. 2001). It was previously documented that the degradation by different WRF was from 0 (Asther et al. 1987) to 73% (Ruiz-Aguilar et al. 2002), depending on the initial concentration of PCBs and mushroom species. *P. ostreatus* was documented to degrade 0% (Ruiz-Aguilar et al. 2002), 29% (Šašek et al. 1993) and even 40% of PCBs (Delor 103 over two months) (Kubátová et al. 2001). *Phanerochaete chrysosporium* and *Trametes versicolor* did not show the ability to degrade PCBs in real soil (Kubátová et al. 2001), but the efficiency of degradation was improved by surfactants. *T. versicolor* was able to remove from 29 to 70%, *P. chrysosporium* from 34 to 73% and *L. edodes* from 0 to 33% of PCBs in the presence of a non-ionic surfactant (Tween 80) (Ruiz-Aguilar et al. 2002). Additionally, Ruiz-Aguilar et al. (2002) documented that the degradation of a mixture of PCBs in different concentrations in the presence of non-ionic surfactants was dependent on the initial concentration of PCBs and fungal species. In our experiment, only low concentrations of PCBs were used (50 and 100 μg kg^{-1} of each congener), while in the mentioned studies the concentration of PCBs was up to 3000 μg kg^{-1}.

It was confirmed that the efficiency of PCB degradation increased with a lower chlorination grade (Kubátová et al. 1996, 2001, Koeller 1999). The chlorination grade affected the translocation of PCBs into fruit bodies as well (Moeder et al. 2005). The translocation of congeners was also not observed, since no fruiting bodies were formed. Additionally, the formation of PCB metabolites was not investigated. The preferred degradation of congeners is as follows: *ortho* (chlorine atoms at 2, 2', 6, 6' positions in the biphenyl ring), *meta* (chlorine atoms at 3, 3', 5, 5' positions in the biphenyl ring) and *para* (chlorine atoms at 4, 4' positions in the biphenyl ring) (Kubátová et al. 2001). The efficiency of PCB congener degradation in our experiment generally was as follows: 28 ≈ 52 > 101 > 138 > 153 > 180. In some cases, the degradations of congeners were comparable. The degradation efficiency of PCBs increased with the number of *ortho* positions substituted by chlorine atoms (Moeder et al. 2005). Although congener 28 has one chlorine atom substituted at the *ortho* position and two chlorine atoms at *para* positions, the degradation was nearly the highest (up to 81% for 20% *A. bisporus* SMS addition at 50 μg kg^{-1} PCB), while another study confirmed that congener no. 28 was resistant to degradation (Zeddel et al. 1993). The 52 congener with two chlorine atoms at *ortho* and *para* positions is also described as highly resistant (Zeddel et al. 1993), but we proved degradation up to 84% (for 20% *A. bisporus* SMS addition at 50 μg kg^{-1} PCB). Other selected congeners have two or three chlorine atoms at the *ortho* position, two or three chlorine atoms at *meta* and one or two chlorine atoms at *para* positions. Because degradation did not increase with *ortho* chlorinated PCBs, we suppose there is a strong effect of other factors on degradation, e.g. low initial concentration of PCBs or activity of microflora in the substrate/SMS.

The degradation of PCBs resulted from the activity of ligninolytic enzymes and the optimum pH for laccase activity for the most efficient removal of different organic pollutants was confirmed from 3 to 7 (Mayer and Stamples 2002, Keum and Li 2004). During the experiment, the acidification of the growth medium could be a result of the decomposition of mycelium or wheat straw chaff. The drop of pH probably has a positive effect on enzyme activity and in consequence on the degradation of PCBs.

The differences between PCB degradation by substrate and SMS of the selected mushrooms were very weak (with some exceptions), so we suggested that it is possible to use SMS in the decontamination of PCB polluted soil. According to Council Directive 1999/31/EC, each European Union country should reduce the amount of organic refuse by 50% by 2050 (Sæbø and Ferrini 2006). Because Poland is one of the biggest producers of *P. ostreatus* and *A. bisporus*, annually generating over a thousand tons of spent mushroom substrate and compost, mycoremediation could solve the problem of disposal of the refuse. Additionally, the use of the SMS allows to dose its quantity and adapt the dose to the concentration or environmental conditions. It is particularly useful especially as the most important factor limiting the bioremediation of PCBs is a low concentration of microorganisms in the soil and sediments being able to remove the pollutants (Providenti et al. 1993, Robinson and Lenn 1994, Verstraete and Devliegher 1996/1997). Furthermore, SMS not only decontaminates the soil, but is also a high value fertilizer.

CONCLUSIONS

The efficiency of PCB degradation was dependent on the species of fungus, type of substrate, PCB structure and their initial concentration. A high degradation was obtained for substrates before fruiting and SMS at a low initial concentration of PCBs. The experiment confirmed the possibility of the application of substrates and SMS to remove PCBs from contaminated soil with average degradation exceeding 30% for all combinations.

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AUTHOR CONTRIBUTIONS

M.G. – designed and performed experiment, performed analytical measurements, wrote manuscript; K.D. – designed and performed experiment, performed statistical analysis; M.S. – designed experiment, prepared and described fungal material; K.S. – designed experiment, prepared and described fungal material; all authors discussed the results and implications and commented on the manuscript at all stages.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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