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Uptake of perfluoroalkyl substances, pharmaceuticals, and parabens by oyster mushrooms (*Pleurotus ostreatus*) and exposure risk in human consumption

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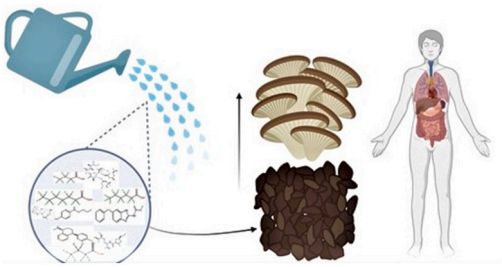
HIGHLIGHTS

- Uptake of organic micropollutants (MPs) by edible oyster mushrooms was studied.
- PFAS uptake was negatively correlated with perfluorocarbon chain length.
- Oyster mushrooms displayed potential to degrade MPs.
- Decrease of respiration was observed at the highest MP concentration tested.
- Potential risks to humans from MP uptake in mushrooms was evaluated.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic micropollutants (MPs) pose potential threats to environmental ecosystems and human health. This study investigated uptake of perfluoroalkyl substances (PFASs), pharmaceuticals, and paraben by edible oyster mushrooms (*Pleurotus ostreatus*), cultivated on spiked growth substrate. Concentrations of pharmaceuticals and paraben in substrate showed a decreasing trend over a 20-day harvesting period, whereas PFAS concentrations were variable over the harvesting period. However, only propylparaben, clarithromycin, and PFASs were detected in fruiting bodies of oyster mushroom. Uptake of PFASs by oyster mushroom fruit bodies was negatively correlated with perfluorocarbon chain length. An impact of MPs on fungal colonization was observed, with decreased respiration in treatments with the highest concentration of MPs, but production of fruiting bodies was not affected by exposure level. The potential human risk from ingestion of MPs was evaluated for oyster mushrooms exposed to the highest concentration of MPs in substrate, based on acceptable daily intake (ADI).

1. Introduction

With recent advances in the industrial sector, increasing global population, and changes in lifestyle, generation of food waste from agricultural, household, and industrial sectors has increased (Vijver, 2019). According to the Food and Agriculture Organization (FAO),[1]

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nearly one-third of all food produced annually is wasted, so better re-use of food waste is required. Food waste contains substantial amounts of carbohydrate, protein, and/or fat and has a high moisture content, which makes it suitable for treatment by processes such as anaerobic digestion, chemical hydrolysis, and aerobic composting (Arshadi et al., 2016; Du et al., 2018; Francavilla et al., 2016; Jordán et al., 2010; Sharma et al., 2020). Converting food waste into biofertilizer in order to close the nutrient loop delivers a variety of benefits to soils, such as providing organic material, enhancing pH, altering the carbon to nitrogen ratio (C/N), boosting water-holding capacity, reducing salinity, and improving aggregate stability (Sangamithirai et al., 2015; Wang et al., 2017). However, food waste feedstock has also been found to contain organic micropollutants (MPs), inorganic chemicals (e.g., heavy metals), biological impurities (e.g., plant or animal pathogens), and other foreign materials (e.g., glass or microplastics) (Alburquerque et al., 2012; Ali et al., 2019; Govasmark et al., 2011). Thus, presence of MPs in biofertilizer and their uptake by food crops need to be investigated, in order to assess the risk of human exposure (Sangamithirai et al., 2015; Sharma et al., 2020).

Micropollutants comprise a wide range of anthropogenic substances, including endocrine-disrupting compounds, pharmaceuticals and personal care products, perfluoroalkyl substances (PFASs), parabens, hormones and steroids, surfactants, flame retardants, siloxanes, industrial additives and agents, and gasoline additives (Bletsou et al., 2015; Luo et al., 2014). Most MPs are biologically active even at trace levels (a few ng/L) and can have detrimental impacts on human health and the environment (Luo et al., 2014; Pal et al., 2014; Sangamithirai et al., 2015; Sharma et al., 2020). For example, exposure to a non-lethal dose of antibiotics (such as clarithromycin and clindamycin) can result in development of antibiotic resistance genes in bacteria, which is an emerging concern for human health (Asif et al., 2017; García et al., 2013; Nazaret and Aminov, 2014; Pal et al., 2014; Rizzo et al., 2013). Parabens are considered endocrine-disrupting chemicals that have estrogenic effects. (Stuart et al., 2012) reported that this group of MPs can suppress the estrogenic activity and induce immunologically mediated, immediate systemic hypersensitivity reactions. It was shown PFASs exposure may cause multiple adverse effects such as hepatotoxicity, immunotoxicity, developmental toxicity, reproductive toxicity, neurotoxicity, and cancer (Das et al., 2015; Grandjean and Clapp, 2015; Song et al., 2016; Wang et al., 2019).

Many MPs show low removal efficiency in wastewater treatment plants (Luo et al., 2014), and can thus be released into the aquatic environment (Golovko et al., 2021; Rostvall et al., 2018). As a consequence, MPs are frequently detected at concentration levels of ng/L to μg/L in the aquatic environment (Golovko et al., 2021; Rostvall et al., 2018). Re-use of polluted water for agricultural purposes can pose a risk of uptake of MPs by crops (Chen et al., 2013; Kim et al., 2007; Onesios et al., 2009; Wang et al., 2005).

Fungi of the *Pleurotus* genus have been extensively studied because they can colonize and degrade a wide range of lignocellulosic residues, have high gastronomic value, require less time to grow than other edible mushrooms, and their fruiting body is less frequently impacted by pathogens (Anasonye et al., 2014; Asif et al., 2017; Chen et al., 2015; Hai et al., 2006; Kulshreshtha et al., 2014). The best-known species in this genus is oyster mushroom (*Pleurotus ostreatus*), which can be harvested naturally from decaying wood or artificially cultivated, as it is highly adaptable and can grow on a wide variety of agro-industrial wastes (Pointing, 2001). Therefore *P. ostreatus* has the capability to synthesize relevant hydrolytic (celluloses and hemicelluloses) and unique oxidative (ligninolytic) extracellular enzymes that decompose wood and vegetal residues (Bonatti et al., 2004; Mikiashvili et al., 2005). Extracellular enzymes of white-rot fungi, including *P. ostreatus*, are distinguished by their capacity to degrade complex structures, enabling the fungi to digest a broad spectrum of resistant contaminants (Anasonye et al., 2014; Asif et al., 2017; Chen et al., 2015; Hai et al., 2006). However, there is a lack of research on whether *P. ostreatus* can degrade MPs and whether there is a risk to human health if oyster mushroom fruiting bodies produced on contaminated substrates are used for human consumption (Calderón-Preciado et al., 2013; Sabourin et al., 2012).

The aim of this study was to investigate uptake of selected MPs (PFASs, pharmaceuticals, and paraben) by oyster mushrooms grown on spiked substrate. Specific objectives were to i) evaluate the fate of MPs in mushroom substrate over time and uptake in the harvested fruiting bodies; ii) compare uptake of 18 MPs from two types of mushroom substrate, with and without addition of biofertilizer; iii) determine the impact of exposure to MPs on fungal growth and fruiting body production; and iv) assess the potential health risks of MPs following human consumption of oyster mushrooms produced on contaminated substrate.

2. Materials and methods

2.1. Chemicals and reference standards

A total of 18 compounds (13 PFASs, four pharmaceuticals, and one paraben) were investigated (see Table S1 in Supporting Information (SI)). All analytical standards used for analysis were of high purity grade (>95%) and purchased from Sigma-Aldrich (USA). Isotopically labeled standards (IS) (D_5-Oxazepam-, D_5-Diazepam, D_7-Propylparaben, $^{13}C_2$-PFHxA, $^{13}C_2$-PFDA, $^{13}C_2$-PFDoDA, $^{13}C_4$-PFOA, PFHxS-18O2, $^{13}C_5$-PFNA, $^{13}C_4$-PFOS, $^{13}C_2$-PFUnDA, $^{13}C_2$-PFBA, $^{13}C_8$-FOSA) were obtained from Wellington Laboratories (Canada), Teknolab AB (Kungsbacka, Sweden), Sigma-Aldrich (USA), and Toronto Research Chemicals (Toronto, Canada). Detailed information about the IS and native standards used can be found elsewhere (Rostvall et al., 2018).

Ultrapure water was generated by a Milli-Q Advantage Ultrapure water purification system and filtered through a 0.22 μm Millipak Express membrane and LC-Pak polishing unit (Merk Millipore, Billerica, MA). Methanol, acetonitrile, ammonium acetate, formic acid, ammonia, and ethyl acetate of high analytical grade were obtained from Sigma-Aldrich (USA).

2.2. Fungal strain and mushroom substrate

Grain spawn of the oyster mushroom strain *Pleurotus ostreatus* M2191 was obtained from Mycelia BVBA, Belgium, and used for inoculation of mushroom growth substrate. Two different mushroom substrates (A, B) were used in the experiment. Substrate A consisted of alder (*Alnus* spp.) sawdust (particle size 2–4 mm), fertilized with liquid anaerobic digestate (biofertilizer) obtained from a local Swedish biogas plant, where the biogas reactor was fed with organic household waste (37%), manure (31%), slaughter residues (19%), and other organic food waste (13%). The biodigestion process at the plant is certified according to SPCR 120 (Avfall Sverige, 2020), which requires all wastes used as feedstock to have their origin in the food and/or feed chain. The anaerobic digestate was filtered (0.8 mm) before use and added in a concentration of 0.5 L per kg dry weight (dw) of sawdust. The C/N ratio of substrate A was 99 ± 4.7 and the pH was 7.6 ± 0.2. Substrate B was designed as a standard mushroom substrate (Stamets, 2000), but with a lower concentration of wheat bran in order to obtain similar C/N ratio as in substrate A. Substrate B consisted of 87% alder sawdust (particle size 2–4 mm), 11% wheat bran, and 2% calcium sulfate. The C/N ratio of this substrate was 98 ± 3.5 and the pH was 7.5 ± 0.1.

2.3. Mushroom production

The substrates were packed in suitable boxes for mushroom production (TP1600 #30 WH, Sac O2, Nevele, Belgium), 210 g dw of substrate was used for each box. Distilled water (160 mL) was added to reach a moisture content of 43% in each box. The boxes were pasteurized at 65 °C for 8 h and, after the substrate had cooled down, sterile distilled water (230 mL) spiked with different concentrations of the MPs solution was added to reach a final moisture content of 65% in the

substrate. The MPs were added in two concentration levels to the substrate, with nominal values of 10 ng/g wet weight (ww) and 100 ng/g ww for individual compounds. These values correspond to a concentration of 29 ng/g dw and 286 ng/g dw, respectively.

The substrate in each box was thoroughly mixed to ensure a homogeneous distribution of the MPs in the substrate. After this step, spawn of oyster mushroom was added in a concentration of 10% dw to the substrate. In this step the substrate in each box was once again thoroughly mixed. The boxes were incubated in a climate chamber at 22 °C and 65% relative humidity for 20 days, by which time the substrate was densely colonized with mycelium. The boxes were then incubated at 10 °C for three days to induce fructification, followed by incubation at 22–24 °C and 85% relative humidity until harvest of the first flush of oyster mushroom fruiting bodies.

2.4. Experimental set-up

The experiments included the two substrate types (A, B), each treated with two different MP concentration levels (10 ng/g ww and 100 ng/g ww), and one control (substrate without addition of MPs) (Table S2 in SI). Substrate samples (~60 g) were taken immediately before the start of the experiments (day 0), and after 10 days and 20 days of mycelial growth. Fruiting bodies were harvested six days after emergence of the pins. All samples were frozen directly after sampling and lyophilized. All harvested fruiting bodies from a replicate (one box) were then milled as one sample. The MP concentrations in the substrate and fruiting bodies were determined in the lyophilized biomass as described below. For chemical analysis, a simple random sampling strategy was applied. Each sample of substrate and fungi was thoroughly mixed with a metal spatula, and an amount appropriate for analysis was taken from the center. The amount and quality (total protein and chemical composition) of the fruiting bodies produced were also recorded. In substrate B, representing standard mushroom substrate, respiration was monitored during fungal colonization in the control treatment (without addition of MPs) and in the treatment with the high exposure to MPs (100 ng/g ww).

2.5. Analysis of MPs

2.5.1. Sample preparation of substrate and oyster mushroom samples for MP analysis

The substrate samples were extracted by a validated in-house method based on the previous studies (Klement et al., 2020; Kodešová et al., 2019b). A blank and a post-extraction spiked matrix sample were prepared for each substrate type, and additional spiked samples were prepared for determination of absolute recovery (for details, see text in SI).

The oyster mushroom samples were extracted by a validated in-house method (Kodešová et al., 2019a, 2019b). A blank and a post-extraction spiked matrix sample were prepared for each mushroom batch, and additional spiked samples were prepared for determination of absolute recovery (for details, see text in SI).

2.5.2. Instrumental analysis of MPs

Extracts were analyzed for the presence of the 18 MPs using a DIONEX UltiMate 3000 ultra-high performance liquid chromatography (UPLC) system (Thermo Scientific, Waltham, MA, USA) coupled to a triple quadrupole (TSQ) mass spectrometer (TSQ QUANTIVA, Thermo Scientific, Waltham, MA, USA) (for details, see text in SI).

2.5.3. Quality assurance and quality control for MP analysis

The performance of the method was assessed with regard to its linearity in calibration, blanks, limit of quantification (LOQ), absolute recovery, precision, and matrix effect. The blank levels were subtracted from the values obtained for the samples.

The LOQ ranged from 2 ng/g ww to 12 ng/g ww in fruit bodies (except PFBA with LOQ 53 ng/g ww) and from 5 ng/g ww to 24 ng/g ww in substrate (except PFUnDA with LOQ 24 ng/g ww), while absolute recovery ranged between 26% and 76%. Yielded LOQ values were in the vast majority of cases lower than 10 ng/g ww and, thus, fit the purpose of the study. Relative standard deviation (RSD) for substrate samples was between 9% and 28%, and for fruit bodies between 33% and 104% for quadruplicate samples. For details, see Tables S3, S4, and S5 and text in SI.

2.6. Impact of MP exposure on production of oyster mushrooms

Fungal respiration during the mycelial colonization phase (only for 0 ng/g ww and 100 ng/g ww MPs in substrate B) was measured using carbon dioxide loggers (Extech CO210, Nashua, USA), which were placed directly above the gas exchange filter on each inoculated box. The boxes were placed at a distance of 2 m from each other and each box was enclosed in a plastic cone with height 45 cm, a closed base, and an open top with diameter 25 cm. Carbon dioxide emissions were measured once every hour for 20 days.

The amount of mushrooms (fresh and dry weight) produced in the first flush was determined for each treatment. The dry weight was recorded after lyophilization. Mushroom production (fresh weight) was related to the amount of substrate (dry weight), in order to determine the biological efficiency (BE) of the substrate (Table S6 in SI), calculated as:

$$BE = \left[\frac{(Amount\ of\ mushroom\ (fresh\ weight)}{Amount\ of\ substrate\ \ (dry\ weight)}\right] * 100\%$$

Total protein content in the fruiting bodies was analyzed by the Dumas method (Bellomonte et al., 1987), using a Vario Max CN and a conversion factor of 4.38 for total nitrogen (Barros et al., 2008).

To determine the chemical composition of the harvested mushrooms, the dried samples were wet-combusted in nitric acid (65%) using a microwave (CEN Mars 5) and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). The elements Al, B, Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Si, and Zn were included in this analysis.

2.7. Calculation of potential human health hazard of MPs

Exposure to MPs associated with human consumption of the mushrooms was examined using the acceptable daily intake (ADI) value for individual substances (if available). The ADI values were calculated for two types of subject, a 25 kg child and a 70 kg adult, based on European regulations and therapeutic doses (Bull et al., 2011), and then compared with the concentrations of MPs determined in fruiting bodies. These data enabled calculation of the amount of mushrooms that needed to be consumed in order to exceed the ADI for a particular group (for details, see text in SI). It should be noted that the substrate was spiked with MPs, which means that the results represented high human exposure, but still within the range of possible exposure.

2.8. Statistics

All experiments were set up with four replicates, and mean values and standard deviation are reported. The data were analyzed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test (Minitab, version 19). Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Concentrations of MPs in substrates over time

The concentrations of the 18 MPs in the two types of spiked mushroom substrate, at three different spiking levels (0, 10, and 100 ng/g ww

substrate), with and without addition of biofertilizer (substrate A vs. B), were monitored over 20 days of mushroom harvesting (Table S7 in SI). In the non-spiked substrates, none of the target MPs was detected except for clarithromycin in substrate A (Table S7 in SI). The latter reflected the presence of clarithromycin in the biofertilizer liquid used for preparation of substrate A. This is not unexpected as previous studies have demonstrated a wide range of MPs in digestate containing food waste (Ali et al., 2019).

The concentrations of clarithromycin, clindamycin, sertraline and fenbendazole decreased over time in both substrates spiked with 10 ng/g ww (Table S7 in SI) and it was below LOQ after 10 day of exposure. In this concentration we were unable to detect propylparaben in both substrates and PFBS in substrates A, since these compounds were below LOQ (Table S7 in SI). It should be mentioned that LOQs of some studied compounds were close or above the spiked concentration of 10 ng/g ww. Analytes groups targeted in this work were relatively heterogeneous with a wide range of physicochemical properties and the sample matrix composition was complex; thus, some of the investigated compounds had higher LOQ values.

The concentrations of pharmaceuticals (clarithromycin, clindamycin, sertraline, and fenbendazole) and propylparaben decreased over time in both substrates spiked with 100 ng/g ww (Fig. 1). After 10 days, the concentrations of most pharmaceuticals had decreased below their LOQ. However, clarithromycin only showed an approximately 35–60% reduction in the initial concentration after 10 days and the concentration did not decrease further until day 20 for both substrate types. Propylparaben was detected only at the beginning of the experiments, in the substrate groups with the highest spiking concentrations, and was not detectable after 10 and 20 days. On the other hand, PFAS concentration did not decrease over 20 days (Fig. 1).

Persistent presence of PFASs in the substrate was expected, as chemicals in this group are very persistent in natural environments and show strong potential for sorption to organic matter, soil, or other material (Beans, 2021; Campos Pereira et al., 2018; Sáez et al., 2008). For some of the PFASs even slight increases in substrate concentration were observed. This finding can be explained by the fact that oyster mushrooms are heterotrophs, and degradation of organic carbon during the growth process can reduce the initial dry mass by up to 32% (Chen et al., 2020). Overall, no significant changes were observed for PFASs in the substrates ($p > 0.05$), which indicate no or a low uptake of PFASs by the oyster mushroom. Even the short-chain PFASs showed no significant reduction in the substrates although they have a high mobility and can be taken up by plants (Gobelius et al., 2017).

The decreasing trend in concentrations of the pharmaceuticals and propylparaben can be explained by uptake of selected MPs by the oyster mushrooms. Enzymatic activity of the oyster mushrooms, which are white-rot fungi, is another factor that can potentially explain the decreasing concentration of pharmaceuticals and propylparaben in the substrates over time (Asgher et al., 2008; Chang et al., 2018). Several factors can influence the degradation potential of white-rot fungi, including: i) non-specific enzyme systems, which ensures applicability to break down a wide range of MPs; ii) extracellular enzymes, which enable the fungi to degrade MPs with low water solubility; iii) rapid hyphal growth, which allows the fungi to colonize quickly and get access to pollutants; and iv) capability (of selected white-rot fungi species) to degrade MPs in nutrient-deficient environments over a wide range of pH

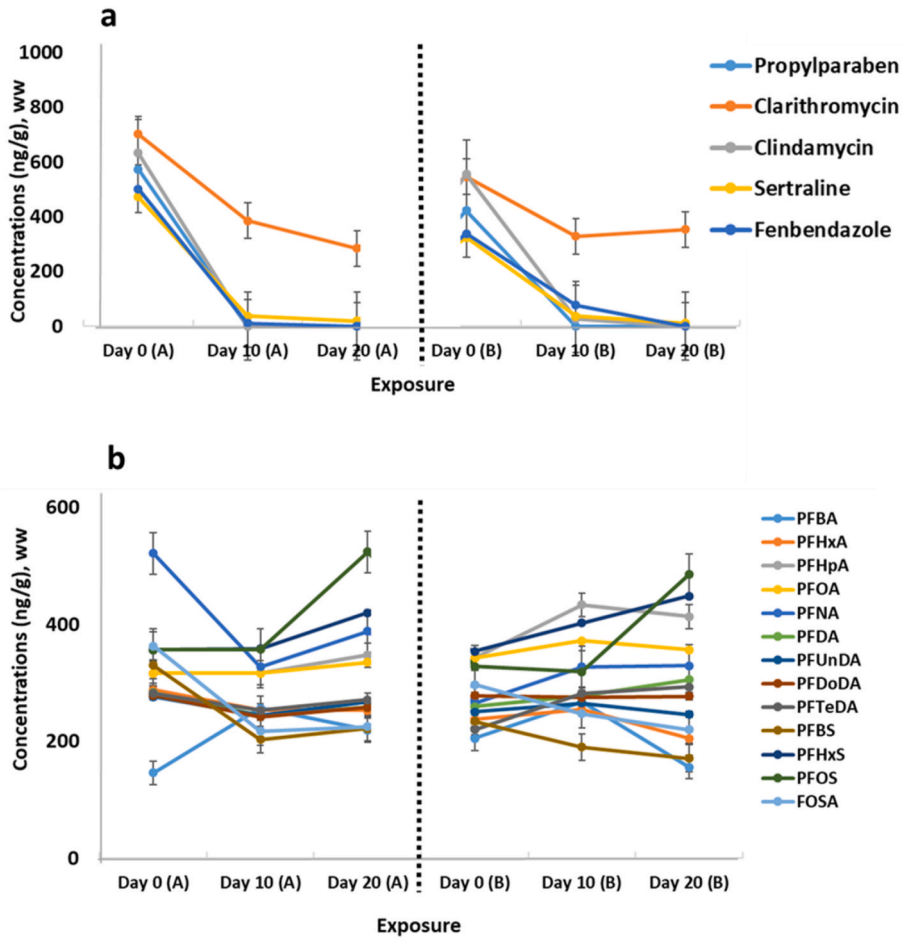

Fig. 1. Average measured concentrations (ng/g wet weight, ww) of a) pharmaceuticals and paraben, and b) perfluoroalkyl substances (PFASs) in spiked (100 ng/g, ww) mushroom growth substrates A and B over time ($n = 4$).

(Pointing, 2001). It is important to note, however, that the breakdown of MPs by white-rot fungi is a co-metabolic process, meaning that it takes place in the presence of a readily degradable substrate. White-rot fungi secrete three distinct extracellular ligninolytic enzymes: lignin peroxidases, laccase, and manganese-dependent peroxidases. Laccase is the key enzyme in the ligninolytic enzyme system, which has been found to remove many MPs that are difficult to decompose, such as endocrine disruptors (Asif et al., 2017). In addition to the extracellular enzymes, the intracellular cytochrome P450 enzyme system can catalyze a variety of reactions to degrade MPs, including heteroatom oxygenation, dehalogenation, and hydroxylation (Bernhardt, 2006; Golan-Rozen et al., 2011; Marco-Urrea et al., 2006, 2008). Taking the degradation abilities of white-rot fungi into consideration, it is highly plausible that the decrease in substrate MP concentrations observed in this study did not originate solely from uptake by the fungi, but also from the complex enzymatic system in the fungi.

3.2. *Micropollutant uptake by oyster mushrooms grown on substrate with and without added biofertilizer*

The concentrations of the 18 studied MPs in fruiting bodies grown on the two types of the spiked substrates A and B (with and without addition of biofertilizer) were compared (Fig. 2). Apart from propylparaben, which was detected in fruiting bodies harvested from the 0 ng/g ww and 10 ng/g ww substrate groups (Table S8 in SI), none of the MPs studied was found in mushrooms harvested from the 0 ng/g ww and 10 ng/g ww substrate groups. On the other hand, 11 compounds were detected in fruiting bodies harvested from substrate spiked with MPs at 100 ng/g ww level (Fig. 2, Table S8 in SI). Currently, commercial production of oyster mushrooms within EU is mainly performed on substrates based on straw. In this production, a maximum residue level for the commonly used plant growth regulator chlormequat in the straw has been set at 0.9 mg/kg (EFSA, 2019). As part of the development of circular food production systems there is an increasing interest in recirculating waste sources for mushroom production (Girotto and Piazza, 2021). Therefore, the risk for contamination of a wider range of MPs need to be taken into account. In our study, the measured concentrations in the substrate, of the MPs detected in the fruiting bodies, were in the range of 0.3–0.7 mg/kg (Table S7 in SI) which is comparable to maximum residue level of chlormequat in mushroom substrates. Thus, in future work defining maximum residue levels in mushroom substrates considerably lower levels than for chlormequat need to be considered.

There was no difference between the substrate types with and without added biofertilizer at any MP spiking level. This indicates that substrate composition might not influence the extent of uptake of specific MPs by oyster mushroom.

The above results indicate that uptake of MPs by oyster mushroom is dependent on the concentration of MPs in the substrate. A number of studies have reported potential uptake of pharmaceuticals by plants and fungi from polluted soil, and from polluted water used for irrigation of crops (Kodešová et al., 2019b; Reichl et al., 2018; Zhang et al., 2019). Thus, presence of pharmaceuticals in food sources is a concern, due to their potential adverse health effects (Kodešová et al., 2019b; Zhang et al., 2019). Accumulation of the antidepressant sertraline in roots and leaves of garden cress (*Lepidium sativum*) has been reported, at concentrations of 4.3 μg/g and 0.52 μg/g, respectively, after three days of growth on liquid growth medium containing 10 mg/L of this compound (Reichl et al., 2018). Uptake of clindamycin by carrot roots has also been reported, resulting in a concentration of 53 ng/g in roots after irrigation with water containing 1000 ng/L of the native compound (Jones-Lepp et al., 2010).

Uptake of PFASs by oyster mushroom was found to be correlated with perfluorocarbon chain length and decreased with each additional CF_3 moiety for perfluorocarboxylates (PFCAs) and perfluorosulfonates (PFSAs) (Fig. 3). Uptake of PFASs by oyster mushroom fruiting bodies was also dependent on the functional group, with on average 10% higher uptake of PFSAs compared with PFCAs. Previous studies have mainly reported higher uptake of PFSAs compared to PFCAs, however, the uptake can be influenced by plant species, soil characteristics (e.g. organic carbon) and other parameters (Lesmeister et al., 2021). This has been observed in previous studies investigating the uptake of PFASs by different plant species (Gobelius et al., 2017). The higher uptake of shorter-chain PFASs and PFCAs can be explained by their higher water solubility (Campos Pereira et al., 2018), and thus high mobility and availability to the oyster mushrooms.

Uptake of trace MPs by plants has been predicted previously based on four critical parameters, lipophilicity (log P), molecular weight, hydrogen bond (H-bond) donors, and H-bond acceptors (Kumar and Gupta, 2016; Lipinski et al., 2001). Those studies proposed dividing MPs into two groups, Group A and Group B. Group A includes MPs for which uptake is expected to be enhanced when their molecular weight is less than 300 Da, log P is less than 3, the number of hydrogen bond acceptors is less than 3, or the number of hydrogen bond donors is less than 3. It has been noted that MPs in Group A are of high concern in terms of human health exposure. Group B includes MPs for which uptake is expected to be limited if the compounds have molecular weight between 300 and 500 Da, their log P is between 3 and 5, the number of hydrogen bond acceptors is between 6 and 10, or the number of hydrogen bond donors is between 3 and 5. This could potentially explain the higher uptake of PFASs in comparison with pharmaceuticals in the present study, as the PFASs studied have no more than one H-bond donor and no more than three H-bond acceptors (Group A MPs), while the pharmaceuticals studied here generally feature a higher number of H-bond donors and acceptors (Group B MPs). It could also explain why perfluorobutane sulfonate had the highest uptake in the oyster mushroom

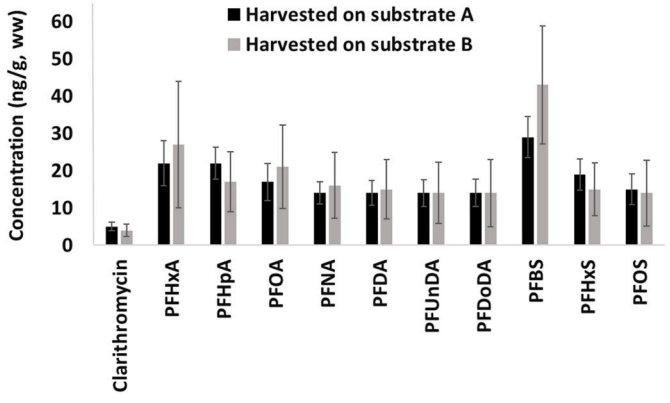

Fig. 2. Average concentrations of micropollutants (MPs) found in oyster mushroom fruiting bodies harvested after 20 days from two types of substrate (A (biofertilized) and B (standard)), spiked at 100 ng MP/g ww ($n = 4$).

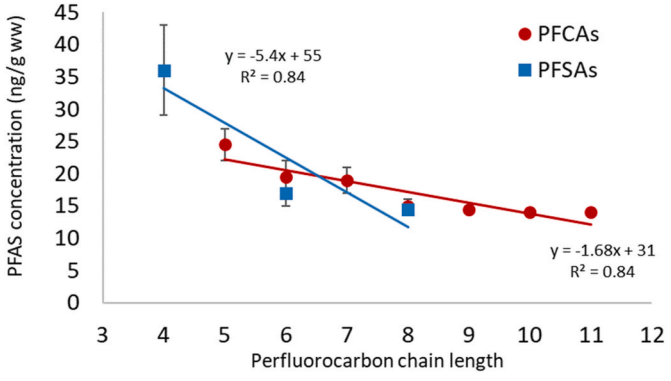

Fig. 3. Uptake of perfluoroalkyl substances (PFASs) by oyster mushroom fruiting bodies as a function of perfluorocarbon chain length.

fruiting bodies, considering its low molecular weight (~300 Da) and low log P (<3), which was lower than that of most of the other PFASs investigated.

It was shown that oyster mushrooms have a capacity to remove of some organic pollutants due to the synergetic effects of extracellular, intercellular, and/or mycelium bound enzymes (Asif et al., 2017). These effects can lead to a complete mineralization of the compounds or just to the excretion of metabolites. Therefore, more efforts are required to identify and elucidate the formation of degradation/intermediate products for future studies.

A human exposure assessment was performed only for oyster mushrooms grown on substrates with the highest spiking concentration (100 ng/g ww of each MP), as these were the only groups showing detectable concentrations of most MPs (Table S7 in SI). Eleven MPs (10 PFASs and one pharmaceutical) were included in the assessment, since the other MPs were not detected in the fruiting bodies. Exposure was assessed based on the calculated ADI values for detected MPs, which are presented in Table S9 in SI. The concentrations of MPs found in fruiting bodies were compared against these ADI values. This information was used to determine the number of grams of oyster mushrooms that needed to be consumed to reach the ADI. The daily consumption of mushrooms needed to reach the ADI for a 25 kg child and 70 kg adult is shown in Table S10 in SI. The daily consumption values obtained indicated that consumption of oyster mushrooms produced on substrates contaminated with MPs can pose a threat to human health, especially for children. As regards exposure to pharmaceuticals, consumption of the oyster mushrooms did not pose a very serious threat, as a person would need to ingest at least 25 kg to exceed the ADI. For PFASs the situation was more concerning, as consumption of just 15 g of contaminated oyster mushrooms would result in ADI being exceeded for all PFASs detected in fruiting bodies. However, it should be noted that our study represented a high risk scenario considering human exposure, since the substrate was spiked with considerable amount of MPs. However, as mentioned above, the concentration in the substrate of the MPs detected in fruiting bodies was comparable to existing threshold levels of organic pollutants in mushroom substrate (EFSA, 2019).

3.3. Impact of MP exposure on yield of oyster mushroom fruiting bodies

Between day 2 and day 8, a faster increase in release of carbon dioxide (reflecting mushroom respiration) was observed in the unexposed treatment (0 ng/g ww MPs) compared the treatment where the oyster mushrooms were exposed to a high concentration of MPs (100 ng/g ww) (Fig. 4), resulting in a significant difference between these treatments. After day 8, no significant differences were observed between the treatments.

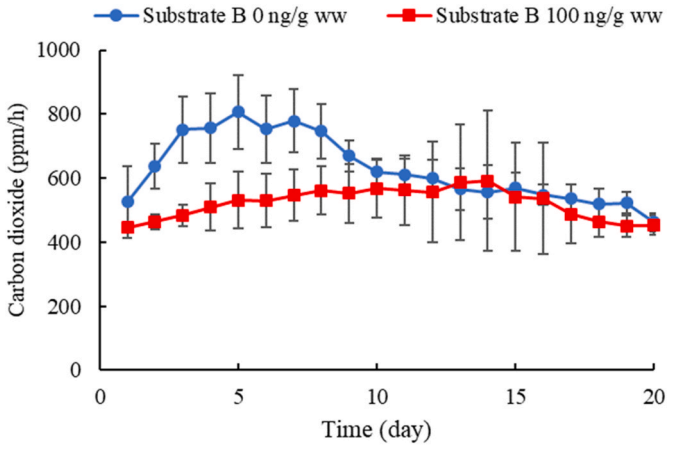

Fig. 4. Release of carbon dioxide during colonization by oyster mushroom (*Pleurotus ostreatus*) of standard growth substrate B without added MPs (0 ng/g wet weight, ww) and spiked with a high concentration of MPs (100 ng/g ww).

Fungal organisms are heterotrophs, so the decreased emissions of car-bon dioxide in the treatment with the highest concentration of MPs suggest impaired metabolisation of the substrate (Loeffen and Bakker, 1998). Thus, the mycelial colonization phase, or spawn run, was negatively affected by the exposure to MPs. The need for an acclimatization phase by the fungi when exposed to a complex chemical surrounding has been reported in other studies (Mohd Hanafiah et al., 2019). The effect of the antibiotic oxytetracycline on mycelial growth rate of *P. ostreatus* was studied in detail by Migliore et al. (2012). They demonstrated a similar pattern as observed in the present study, that the fungus had the capability to adapt and achieve a growth rate comparable to the unexposed control after a period of slower growth during the first 7 days. However, despite this difference in initial colonization, no difference was observed between the treatments in production of fruiting bodies, or in protein or moisture content of the fruiting bodies (Table S2 in SI). Furthermore, analysis of the chemical composition of the fruiting bodies revealed only minor differences (Figure S1 in SI). The fruiting bodies in all cases had numerous clusters in shades of grey and appeared typical of *P. ostreatus*. These results suggest that high exposure to MPs affects fungal growth, but that production of fruiting bodies is not compromised.

4. Conclusions and outlook

This study provided insights into the uptake of MPs by oyster mushrooms from spiked substrate and potential risks from human consumption. The concentrations of MPs over time depended on the physicochemical properties of the MP. Paraben and pharmaceutical concentrations in substrate exhibited a clear decreasing trend over time, with a notable difference after only 10 days of fungal colonization. Substrate concentrations of PFASs, on the other hand, did not change over time or decreased depending on the compound.

It was shown that oyster mushrooms are capable of uptake of some MPs, with higher uptake of PFASs than of paraben or pharmaceuticals. Addition of biofertilizer seemed not to have a distinct influence on uptake concentrations of MPs, even though the biofertilizer itself contained trace amounts of clarithromycin. No impact of MP exposure (substrate spiking level) on production and quality of fruiting bodies was observed.

An exposure assessment was performed for oyster mushroom exposed to the highest concentration of MPs in the growth substrate (100 ng/g ww substrate), based on ADI values. Consumption of just 15 g of the oyster mushrooms in that treatment would result in ADI values being exceeded for all PFASs detected.

Overall, uptake of MPs in *Pleurotus* species such as oyster mushrooms has not been widely studied, even though this species has potential for use as a significant food source in the future. Thus, more studies are needed to estimate the uptake and risk of MPs in *Pleurotus* species.

This study showed that oyster mushrooms have the potential to degrade pharmaceuticals and parabens. Thus, more studies are needed on use of oyster mushrooms for remediation purposes through uptake and degradation of MPs.

Author statement

Oksana Golovko: Conceptualization, Methodology, Formal analysis, Validation, Data curation, Writing – original draft preparation. **Michał Kaczmarek**: Methodology, Formal analysis, Validation, Data curation, Writing – original draft preparation. **Håkan Asp**: Methodology, Formal analysis. **Karl-Johan Bergstrand**: Methodology, Formal analysis. **Lutz Ahrens**: Project administration, Conceptualization, Methodology, Writing- Reviewing and Editing. **Malin Hultberg**: Project administration, Conceptualization, Methodology, Formal analysis, Validation, Data curation, Funding acquisition, Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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