CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Agrobiology, Food and Natural resources

Department of Agro-environmental Chemistry and Plant Nutrition

Monitoring of Selected Persistent Organic Pollutants (POPs) and Personal Care Products (PCPs) in the Sewage Sludge and their Bioremediation

Monitorování vybraných perzistentních organických polutantů (POP) a produktů osobní péče (PCP) v čistírenských kalech a jejich bioremediace

A Doctoral dissertation work

Author: **Abraham Demelash Chane, MSc.**

***Supervisor*: Prof. Ing. Pavel Tlustoš, CSc., dr. h. c.**

***Co-supervisor*: Ing. Zdeněk Košnář, Ph.D.**

Declaration

I declare that this diploma thesis work **Monitoring of Selected Persistent Organic Pollutants (POPs) and Personal Care Products (PCPs) in the Sewage Sludge and their Bioremediation** is my own work and all the sources cited here are listed in the references.

Prague, 2024

……..……..

Abraham Chane

Acknowledgement

First and foremost, I express my heartfelt gratitude to Almighty God for His constant guidance, blessings, and strength throughout my academic journey. I extend my deepest appreciation to my esteemed supervisor, Prof. Ing. Pavel Tlustoš, CSc, for his exceptional mentorship, critical comments, invaluable insights, and unwavering support. I am truly grateful for the opportunity he has given me to work with him as a Ph.D. student. I also extend my sincere appreciation to my co-supervisor, Ing. Zdeněk Košnář, Ph.D., for his guidance, encouragement, and expertise have profoundly shaped my research and academic growth. I am thankful to the staff members of the Department of Agro-Environmental Chemistry and Plant Nutrition at the Czech University of Life Sciences for providing a conducive environment for learning and research. Their support and resources have been indispensable in the successful completion of my Ph.D. Special thanks to Tatiana Robledo-Mahón (Ph.D.) for her mentorship and assistance during my internship, and my friend Niguss Solomon (Ph.D.) for his encouragement and invaluable insights.

I am grateful to my family for their love and inspiration, which have been my driving force. Finally, I extend my deepest thanks to my beloved wife, Mekdes Wondwossen, for her unwavering support, patience, and love throughout this journey.

Table of contents

1. Introduction

Sewage sludge is an organic waste material that, when appropriately processed, can serve as a cost-effective nutrient source, potentially replacing agricultural fertilizers. It is derived as a by-product of wastewater treatment, and its composition can vary considerably based on the source of the incoming wastewater to the treatment plant (Poluszyńska et al., 2017). The utilization of sewage sludge as a soil amendment not only enriches the soil with beneficial nutrients but also improves its overall quality. By incorporating sewage sludge into the soil, the structure can be enhanced, leading to improved stability and aggregation. This, in turn, can increase the soil's ability to retain water, allowing to better infiltration and reducing the likelihood of erosion (Chen et al., 2014; Lucid et al., 2014; Deselnicu et al., 2018).

One of the major challenges associated with the utilization of sewage sludge in agriculture is the presence of persistent organic pollutants (POPs), pharmaceuticals and personal care products (PCPs), per- and polyfluoroalkyl substances (PFAS), flame retardants, microplastics and surfactants. Municipal and industrial wastewater undergoes treatment processes to remove and eliminate undesirable and toxic compounds, which are produced as a result of the accumulation of pollutants from municipal and industrial activities (Tomasi Morgano et al., 2018). When sewage sludge is treated and subsequently disposed in the soil, it can introduce various contaminants into the environment. Another significant pathway of these contaminants to the soil is through irrigation with reclaimed wastewater (Wu et al., 2021; Kodešová et al., 2024). Reclaimed wastewater, which is often used for irrigation purposes, can contain traces of these compounds that find their way into the soil during the watering process. Additionally, the application of manure and compost can also contribute to contaminants accumulation in the soil (Kinney et al., 2008).

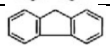
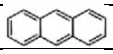
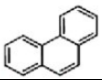
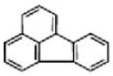
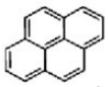
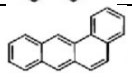
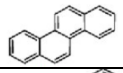
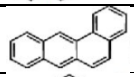
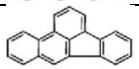
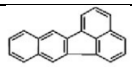
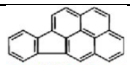
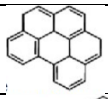
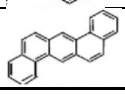
POPs and PCPs encompass a broad spectrum of compounds. POPs include polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) like dichlorodiphenyltrichloroethane (DDT), flame retardants (Polybrominated Diphenyl Ethers (PBDEs)), and polycyclic aromatic hydrocarbons (PAHs). PCPs encompasses fragrance compounds (including synthetic musk compounds (SMCs) and other fragrance compounds), preservatives (parabens, phenoxyethanol), UV filters, and antibacterial (triclosan, triclocarban) (Akhtar et al., 2021; Khalid and Abdollahi, 2021). The current study chose to focus on SMCs and PAHs among various contaminants due to their higher concentration in our sewage sludge samples and the significant environmental toxicity they present. These contaminants have garnered considerable attention in diverse environmental samples analyzed by researchers, primarily due to their prevalence and unique attributes. Their semi-volatile nature and physicochemical characteristics contribute to their mobility in the environment. Moreover, their inherent toxic properties, persistence, and potential for bioaccumulation underscore the necessity for comprehensive investigation. Therefore, SMCs and PAHs emerge as pivotal subjects for study, given their substantial impact on ecosystems and human health (Wong et al., 2019; Abdel-Shafy and Mansour, 2016).

Polycyclic aromatic hydrocarbons (PAHs) are a class of aromatic compounds characterized by two or more fused benzene rings. They could be produced through the thermal decomposition and subsequent recombination of organic molecules formed during the incomplete combustion of organic matter. These hazardous organic pollutants with higher stability, complex structure with high molecular weight can be found in sewage sludge and reclaimed wastewater (Table 1), where they readily adsorb onto the sludge during the wastewater treatment process (Suciu et al., 2015; Lv et al., 2024). The degradation of PAHs can occur through various mechanisms such as adsorption, volatilization, photolysis, chemical degradation, phytoremediation and microbial

degradation. The extent of PAH degradation depends on environmental conditions, the types and numbers of microorganisms present, as well as the nature and chemical structure of the specific PAH compound being degraded (Ward et al., 2003; Haritash and Kaushik, 2009; Kanungo et al., 2023).

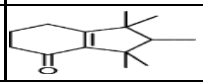
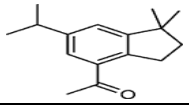
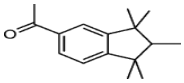
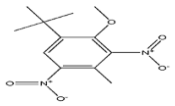
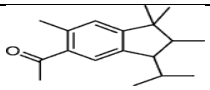
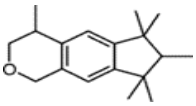
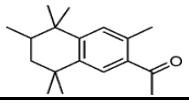
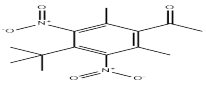
As previously mentioned, among personal care products, SMCs emerge as the most prevalent compounds. These can be categorized into two groups: polycyclic musk compounds (PMCs) and nitro musk compounds (NMCs). However NMCs are present in lower concentration than PMCs as their production were decreased significantly due to concerns about their adverse effects on both human health and the environment). NMs have similar physical–chemical properties to persistent organic pollutants such as organochlorine pesticides and polychlorinated biphenyls (Wong et al., 2019). For example, the octanol–water partition coefficients (Log K_{ow}) of musk ketone (MK) and musk ambrette (MA) are 4.3 and 3.7, respectively (Table 2). Synthetic musk compounds possess physio-chemical properties that make them environmentally persistent, bioaccumulative, and potential hormone disruptors (Pedersen et al., 2005).

Table 1: Basic characteristic of priority polycyclic aromatic hydrocarbons

Compound	Structure	Molecular formula	Molecular weight (g/mol)	Log K_{ow}	Water Solubility (µg/L)	Vapour pressure	Boiling point (◦C)
Naphthalene		$C_{10}H_8$	128.1	3.29	31.7	2.97	80.26
Acenaphthene		$C_{12}H_8$	154.2	3.98	1.93	3.66	95
Acenaphthylene		$C_{12}H_8$	152.2	4.07	3.93	1.4	265-275
Fluorene		$C_{13}H_{10}$	166.2	4.18	1.68–1.98	3.86	295
Anthracene		$C_{14}H_{10}$	178.2	4.45	76	4.15	218
Phenanthrene		$C_{14}H_{10}$	178.2	4.45	1.2	4.15	100
Fluoranthene		$C_{16}H_{10}$	202.26	4.9	200–260	4.58	375
Pyrene		$C_{16}H_{10}$	202.3	4.88	77	4.58	156
Benz[a]anthracene		$C_{20}H_{12}$	228.2	5.61	10	5.3	158
Chrysene		$C_{18}H_{12}$	228.3	5.9	2.8	5.3	254
Benzo[a]pyrene		$C_{20}H_{12}$	252.3	6.06	2.3	5.74	179
Benzo[b]fluoranthene		$C_{20}H_{12}$	252.3	6.04	1.2	5.74	168.3
Benzo[k]fluoranthene		$C_{20}H_{12}$	252.3	6.06	0.76	5.74	215.7
Indeno[1,2,3-cd]pyrene		$C_{22}H_{12}$	276.3	6.58	62	6.2	530
Benzo[ghi]perylene		$C_{22}H_{12}$	276.3	6.5	0.26	6.2	550
Dibenz[a,h]anthracene		$C_{22}H_{14}$	278.4	6.84	0.5	6.52	-

(Patel et al., 2020)

Table 2: Basic characteristic of selected synthetic musk compounds

Compound	Structure	Molecular formula	Molecular weight (g/mol)	Log K_{ow}	water solubility (Mg/L)	vapor pressure (Pa)	Boiling point (◦C at 760 mmHg)
Cashmeran (DPMI)		$C_{14}H_{22}O$	206	4.9	-	-	286.1
Celestolide (ADBI)		$C_{17}H_{24}O$	244	6.6	0.22	0.0192	309.1
Phantolide (AHMI)		$C_{17}H_{24}O$	244.4	6.7	0.25	0.0196	336.6
Musk ambrette (MA)		$C_{12}H_{16}N_2O_5$	268.3	3.7	-	-	369.3
Traseolide (ATII)		$C_{18}H_{26}O$	258	6.3	0.09	0.0091	350
Galaxolide (HHCB)		$C_{18}H_{26}O$	258.4	5.9	1.75	0.0707	326.3
Tonalide (AHTN)		$C_{18}H_{26}O$	258.4	5.7	1.25	0.0608	356.8
Musk ketone (MK)		$C_{14}H_{18}N_2O_5$	294.3	4.3	-	-	369

(Osemwengie and Steinberg, 2001; Aguirre et al., 2014)

Kow is n-Octanol/Water Partition Coefficient. Log Kow is useful for predicting the distribution of compound in the environment, that compounds with Log Kow 4.5 have higher bioaccumulation rates. Water solubility refers to the ability of a compound to dissolve in water. High water solubility often facilitates the dispersion and bioavailability of compounds. Vapor pressure is a measure of the tendency of a compound to evaporate or transition from the liquid (or solid) phase to the gas phase. It indicates how volatile a substance is and is influenced by temperature and the nature of the compound. The boiling point of a substance is the temperature at which its vapor pressure equals the external atmospheric pressure, causing it to transition from the liquid phase to the gas phase.

2. Literature review

2.1. Sewage sludge and its properties

Sewage sludge is a waste-product from wastewater treatment, a process designed to lessen risks to health and the environment. It is a nutrient-rich organic material (Bagheri et al., 2023). Sewage sludge has potential fertilizer properties and can be used to enrich agricultural soils due to high nitrogen, phosphorus and organic matter content. It contains microorganisms with the ability to degrade contaminants and provide valuable nutrients (Kinney et al., 2008). According to Lixandru (2005), one tone of dry sludge contains on average 200 kg of organic matter, 6 kg of N, 8 kg of P and 10 kg of different soluble salts. Due to the nutrient content and effective organic mass input, large amounts of sewage sludge can be used with potential positive effects on productivity and one of the most pressing issues is being solved: removing sewage sludge from waste water treatment plants in order to avoid incineration, high-cost processes and further pollution (Iticescu et al., 2018). In the 2017, 45 million tons of dry sewage sludge (MtDM) production was reported on a global scale (Gao et al., 2020). In the same year, 192,000 tonnes of SS DM were produced in the Czech Republic (Jadlovec et al., 2023). Total production of 11 million tons DM of sludge is reported in all Europe (EU-27), about 1 million tons dry matter (DM) of sludge are produced every year by French WWTPs, while Germany and UK produce 2.2 and 1.8 million tons, respectively (Kelessidis and Stasinakis, 2012).

Sewage sludge contains a large set of organic and inorganic contaminants including POPs and PCPs, that are water soluble, chemically or biologically sorbed (Phillips et al., 2012; Heidler and Halden, 2008). According to Inglezakis et al. (2014), concentrations of all pollutants in sewage sludge are affected mainly by the quality of the incoming wastewater,

type of treatment, and in some cases by meteorological conditions and of course they vary among WWTPs located in several parts of the world (Xia et al., 2005). For several years, the contamination of sludges by micropollutants has been documented. This contamination is attributed to the sorption of pollutants during sewage sludge treatments, primarily due to their hydrophobic characteristics or their tendency to be adsorbed onto particles (Mailler et al., 2014).

2.2. Sources and content of Polycyclic aromatic hydrocarbons and Synthetic musk compounds in the sewage sludge

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants either as natural component (i.e., as products of humus conversion by microorganism) or as pollutants (Sharma et al., 2007). PAHs in the environment originate from both natural and anthropogenic sources. Natural sources include activities such as seepage of petroleum, natural losses, coal deposits, and open burning of fossil fuels. On the other hand, anthropogenic sources involve human activities such as coal gasification, residential heating, liquefying plants, asphalt production, coal-tar pitch, carbon black production, petroleum refineries, and vehicle exhaust. PAHs are organic compounds that are mostly colorless, white, or pale-yellow solids (Thacharodi et al., 2023). PAHs, due to their complex aromatic structures, are difficult to treat completely and can accumulate in the sewage sludge, potentially impacting sewage treatment system operation (Jin et al., 2024). PAHs tend to accumulate in the sludge of wastewater treatment plants, effectively transforming the sludge into a repository for these substances, sequestering them from the water phase. The accumulation of PAHs in the sludge raises concerns regarding potential environmental contamination if the sludge is not properly handled or discarded. Therefore, it becomes crucial to

implement appropriate measures for the safe handling and disposal of the PAH-laden sludge to prevent any potential risks to ecosystems and human health (Amir et al., 2005).

Sun et al. (2019) investigated 75 sludge samples from different wastewater treatment plants in 23 cities across China. The investigation was aimed to measure the concentration of PAHs in the sludge samples, resulted with high concentrations of ∑16PAHs in sludge samples ranged from 0.56−230 mg/kg dw. Similarly, Poluszyńska et al. (2017) reported higher concentration of the ∑16 PAHs in raw sewage sludge ranging from 0.5 to 100.7 mg/kg dw. Another study by Ozcan et al. (2013) reported the concentration of PAHs lower than previous studies form two WWTPs in Turkey, ΣPAHs concentration ranging from 0.96 to 17.5 mg/kg dw. Several PAHs, such as naphthalene, acenaphthene, phenanthrene, and fluorene, are frequently found in the effluent from the municipal WWTPs (Katsou et al., 2015). Suciu et al. (2015) reported that countries set a limitation of priority PAHs concentration in the sewage sludge to use in agriculture. In this regard, Luxemburg, Czech Republic, Austria, Romania, and Sweden were set limits with concentration of 20, 10, 6, 5, 3 mg/kg dw of SS, respectively. Edokpayi et al. (2016) determines the PAHs in the effluents from wastewater treatment facilities that are considered as major anthropogenic contributions, resulted with the range of 13.2–26.4 mg/L. Another study by Liu et al. (2021) detected the total concentrations of PAHs, Cl-PAHs, Br-PAHs and O-PAHs ranged from 9–88.4, n.d.–5.7, n.d.–13.1 and 15.5–106.9 ng/L, respectively, in the effluents from wastewater treatment plants.

Synthetic musk compounds were mainly categorized into two groups, namely polycyclic musk compounds and nitro musk compounds. SMCs encompass a diverse range of chemical compounds intended to cleanse, hydrate, and rejuvenate the human body, thus becoming integral to our daily regimens (Saravanan et al., 2024). SMCs are high production volume substances, which are widely

used in everyday consumer products. Some have similar physical–chemical properties to persistent organic pollutants (Wong et al., 2019). The use and production of polycyclic musk compounds increased rapidly, especially the production volume of galaxolide in the United States (US) approximately one million kg/year in 2011 to 4 million kg/year during 2012–2015. However, the production of nitro musk compounds has been declining in Europe and the US markets (Wong et al., 2019). There were different sludge treatment technologies used in different countries to remove contaminants form the incoming waste to treatment plants.

The concentrations of personal care products were higher in the untreated raw sludge compared to treated sludge. In the primary sludge Ternes et al. (2004) found galaxolide and tonalide with the concentration of 187 mg/kg dw and 183 mg/kg dw, respectively. The fact that in the primary sludge treatment, there is fewer microorganisms and the sludge has higher lipid proportion and the concentration in secondary sludge is lower with a value of 131 and 10.2 mg/kg dw for galaxolide and tonalide, respectively (Verlicchi and Zambello, 2015). Sun et al. (2014) reported the contents of synthetic musk compounds from sludge samples at 40 WWTPs in the USA. Galaxolide and tonalide were found with higher concentration than Czech Republic ranging from 4.1 to 91 mg/kg dw and 0.65 to 15 mg/kg dw, respectively. According to Smyth et al. (2007), the concentrations of synthetic musk compounds in sewage sludge vary depending on the treatment methods employed in wastewater treatment plants. The study examined the impact of aerobic and anaerobic digestion of sewage sludge on the degradation of synthetic musk compounds. In the case of aerobic digestion, lower concentrations of musk compounds such as celestolide, phantolide, traseolide, galaxolide, tonalide, musk xylene, and musk ketone were observed, with values of 100, 79.6, 681, 12,000, 3,550, 64.6, and 115 μg/kg dw, respectively. In anaerobic digestion, the concentrations were slightly higher, measuring 209, 182, 1,400, 28,400, 8,020, 78.2, and 116 μg/kg dw for the

same compounds, respectively. The concentrations of galaxolide and tonalide in reclaimed wastewater effluents varied from 157 to 3,730 ng/L and 42 to 807 ng/L, respectively in USA and Europe (Reiner et al., 2007). Lv et al. (2010) showed in their study, the concentrations of synthetic musk compounds in the input wastewater were higher than leaving as effluent. It's noted that nitro musks has a better elimination tendency in the WWTPs. In a study by Sun et al. (2014), the presence of galaxolide and tonalide in WWTPs effluents in the United States was investigated. The concentrations of galaxolide ranged from 0.45 to 4.79 μg/L, while tonalide concentrations varied from <0.05 μg/L (LOQ – limit of quantification) to 0.44 μg/L.

2.3. Occurrence of persistent organic pollutants and personal care products in the soil

2.3.1. Irrigation by reclaimed wastewater

According to Haddaoui et al. (2016), reported ΣPAHs in the soil from wastewater irrigated field in which the concentration of PAHs were increased after 5 months of irrigation to 365.2 μg/kg dw at 0–10 cm depth from the initial concentration of 120.0 μg/ kg dw. However, according to Zhang et al. (2017), the total concentration of 16 PAHs in soils irrigated with wastewater ranged from 103.3 to 479.3 μg/kg dw, which was relatively similar values with the control soil irrigated with groundwater, where the values ranged from 140.5 to 418.3 μg/kg dw. Among these, the four most dominant PAHs were naphthalene (110.2 μg/kg dw), phenanthrene (30.7 μg/kg dw), fluorene (14.6 μg/kg dw), and benzo(b) fluorathene (25.1 μg/kg dw). Soils irrigated with wastewater ranged from 1,503 to 3,369 μg/kg dw, which was significantly higher than the control soil containing 1,304 μg/kg dw. At a different site, the control soil initially had 2,687 μg/kg dw of ∑16 PAHs. However, after irrigating with wastewater, the PAH concentrations increased to a range of 2,954 to 6,892 μg/kg dw.

Irrigation with reclaimed wastewater plays a crucial role in reducing the demand for fresh water and facilitating the recycling of nitrogen, phosphorus, and other nutrients present in the reclaimed water. Soils irrigated with reclaimed wastewater showed higher concentrations of tonalide (6.2 µg/kg dw) compared to galaxolide (2.0 µg/kg dw). However, other synthetic musk compounds, namely cashmeran, celastolide, and phantolide, were detected in trace concentrations, with values of 0.7, 1.2, and 1.6 µg/kg dw, respectively (Chase et al., 2012). To reduce the risk associated with irrigation of reclaimed water, Qadir et al. (2015) suggested some strategies such as primary and secondary treatments and aerobic and anaerobic digestion of wastewater to a desired effluent quality, on-farm wastewater treatment options and farm-based measures to reduce risks in areas irrigated by untreated or partially treated wastewater.

2.3.2. Sewage sludge application

Several studies have examined the accumulation of PAHs in agricultural soil following the application of sewage sludge. The impact of sewage sludge on soil PAHs content indicates that depending on the PAHs content in the sludge and the initial PAHs level in the soil, the addition of sewage sludge can lead to a significant increase in soil PAH content. Pulkrabová et al. (2019) studied the long-term effects of sewage sludge application on agricultural soils, they showed the presence of PAHs with concentrations ranging from 54.1 to 1443 µg/kg dw in the soil samples.

According to Tomczyk et al. (2020), the addition of sewage sludge from various WWTPs to control soil led to an increase in the content of Σ16 PAHs, ranging from 32% to 94%. The initial PAH concentration in the control soil was measured at 216 µg/kg dw.

Additionally, the concentration of PAHs in agricultural soil after five years of repeated sludge application was investigated. The content of 16 PAHs was found to be higher in the amended soil, with values reaching up to 2.5 mg/kg dw compared to the non-amended soil, which had a concentration of 1.7 mg/kg dw (Liu et al., 2017). Cai et al. (2008) conducted a pot experiment by

applying sludge with different rates of 10, 20 and 40 g/kg dw to the soil. After a period of 64 days, the soil samples were collected and analysed, resulting with 53 to 304 μg/kg dw of total PAHs concentration, which were significantly higher than the control soil, which had PAHs concentration only 8 μg/kg dw.

The accumulation of personal care products in the soil can stem from various sources. Among these, the disposal of treated sewage sludge has been identified as a significant pathway for contaminants, as noted by Chase et al. (2012). The concentration of compounds in the soil following sludge application can be influenced by several factors, such as the rate of sludge application, the biochemical properties of the soil, and the specific compounds present (Jones et al., 2014; Verlicchi and Zambello, 2015).

Studies have reported that synthetic musk compounds in the soil amended with biosolids can be detected in relatively high concentrations. Kinney et al. (2008) reported the concentration of galaxolide and tonalide in the biosolid amended soils within the range of 1,050−2,770, to 287−773 μg/kg dw, respectively. Ramos et al. (2021a) also observed the content of synthetic musk compounds in sewage sludge amended soils within the range from 1 to 341 μg/kg dw. Galaxolide and tonalide were found in concentrations 341 and 88 μg/kg dw, respectively. However, other polycyclic musk compounds such as DMBI (cashmeran), ADBI (celestolide), AHMI (phantolide) and ATII (traseolide) were detected in lower concentrations and ranged from 1.0 to 2.3 μg/kg dw. All nitro musk compounds are not usually detected in sewage sludge amended soil.

A study conducted by Domínguez-Morueco et al. (2018) and Wong et al. (2019) examined the presence of all polycyclic and nitro musk compounds in different soil types, including industrial, urban areas, and agricultural soils. The concentrations of these compounds varied across the samples, with values ranging from 0.1 to 5.2 μg/kg dw, not detected to 2.9 μg/kg dw, and <0.1 to

7.2 μg/kg dw, respectively. However, when considering musk compounds such as galaxolide and tonalide in soils amended with biosolids, higher concentrations were observed. The concentrations of galaxolide ranged from 2.4 to 67.5 μg/kg dw, while tonalide concentrations ranged from 0.7 to 29.0 μg/kg dw.

Repeated sewage sludge soil application showed an increase in the accumulation of synthetic musk compounds compared to one application. Chen et al. (2014) reported that the reason for this phenomenon may be that the pore structure of the soil changed due to these repeated applications developing a better adsorption capacity.

2.4. Assessment of the effects of SMCs and PAHs on soil, plant and human health

Soil plays an important and irreplaceable role in the biosphere, controlling primary biomass productivity, organic matter degradation, and biogeochemical cycles. Although the soil has unreplaceable role in several economic and cultural activities (e.g. agriculture, forestry, horticulture) depending on its quality and health, is also a final sink of contaminants. Some emerging contaminants can persist and accumulate in the soil and soil biota, causing potentially harmful effects on ecosystem's health. Studies on the toxicity of contaminants proved that SMCs and PAHs could be toxic to terrestrial organisms (Balk and Ford, 1999; Chen et al., 2012; Nam et al. 2015), microorganisms in the soil (LüZe et al., 2017; Arruda et al., 2022; Panneerselvam et al., 2022) and plants (An et al., 2009; Wang et al., 2013; Liu et al., 2020; Gawryluk et al., 2022).

2.4.1. The effect of PAHs and SMCs on soil macro-organisms

PAHs in soil are generally not expected to have toxic effects on terrestrial invertebrates unless the soil is heavily contaminated. However, when exposure is significant, adverse effects on these organisms can include issues such as tumors, reproduction, development, and immunity (Abdel-

Shafy and Mansour, 2016). Earthworms are common and important inhabitants of the soil environment. Their role in the soil is inevitable by decomposing organic matter, in biomagnification in food chain and known to accumulate large amounts of inorganic and organic contaminants. The application of biosolids on the land can have an impact on the living organisms, the environment, and the quality of food or feedstuff. Even if earthworms are found in low trophic level, they could facilitate the contaminants to higher trophic level by eating soil particles. They are known to be consumed by many bird species, which representing up to 90 % by weight of diet of some other species (Kinney et al., 2008). Nam et al. (2015) conducted an acute toxicity test on the earthworm *Eisenia fetida* using several PAHs, including fluorene, anthracene, phenanthrene, fluoranthene, and pyrene. Among the tested PAHs, only two, fluorene and phenanthrene, exhibited toxicity. The LC_{50} (median lethal concentration) and LC_{95} (lethal concentration for 95%) values for fluorene after 72 hours of exposure were 394.1 μg/L and 789.7 μg/L, respectively. For phenanthrene, the corresponding LC_{50} and LC_{95} values after 72 hours of exposure were 114.0 μg/L and 176.1 μg/L, respectively.

To perform the risk assessment of SMCs for soil organisms, Homem et al. (2017), found that PNEC (Predicted No Effect Concentration) of tonalide, galaxolide and musk ketone were $PNEC_{Tonalide} = PNEC_{Galaxolide} = 71.0$ μg/kg dw, and $PNEC_{musk\ ketone} = 320$ μg/kg dw in the sediments and soil. The HQ_{mean} (hazard quote) were 0.037 and 0.032 for galaxolide and tonalide (Homem et al., 2017). Based on these values, Zheng et al. (2019) reported the HQ_{sum} for galaxolide and tonalide was less than 0.1 of farm land soil samples, which means the concentration of SMCs could pose a low risk to soil organisms.

The study on *Eisenia fetida* and *Achatina fulica* shows that galaxolide caused the toxicity effect in both soil organisms. The non-observed effect concentration (NOEC) values for cocoon

production and the number of juveniles of *Eisenia fetida* were 9000 μg/kg dw and 6000 μg/kg dw, respectively.

2.4.2. The effect of PAHs and SMCs on soil microorganisms

Soil microorganisms play an essential role in degradation processes and biogeochemical cycles in soil. They decompose organic matter, recycle nutrients, and develop healthy soil structure (Ho and Chambers, 2019).

Panneerselvam et al. (2022) reported that PAHs disrupt the development, morphology, and metabolism of soil microorganisms, resulting in functional disturbances, protein denaturation, destruction of cell membrane integrity, and disruption of the soil's vital system and support of its biological productivity. The presence of high levels of PAHs in soil can adversely affect total bacterial and fungal species, microbial metabolic processes, and enzyme activities, leading to an impact on microbial community composition by putting pressure on sensitive soil microorganisms.

Study by LüZe et al. (2017) investigated the joint effects of galaxolide and cadmium on soil microbial community, too. The results indicate that under contamination of galaxolide and the galaxolide-cadmium mixture, the ability of soil microorganisms to use carbon sources has improved and the utilization efficiency of esters was the highest, while sugars and acids have a lower utilization efficiency compared to 6 other types of carbon sources. The abundance of bacteria increased with increasing concentrations of galaxolide. However, the abundance of fungi and actinomycetes decreased with increasing concentrations of galaxolide. Actinomycetes were more sensitive to galaxolide than bacteria and fungi. Similarly, when galaxolide was added to sediment at 300 mg/kg dw, the bacterial community was not directly affected (i.e., structure, richness, and diversity) by this concentration (Peng et al., 2019).

2.4.3. Bioaccumulation and toxicity to humans through food chain

Human exposure to PAHs is unavoidable in the current situation. PAH exposure occurs mainly *via* three routes, i.e., inhalation, ingestion, and dermal contact (Burchiel and Luster, 2001). For many people, the primary exposure of PAHs occurs at the workplace, e.g., workers in coke manufacturing factories and food processing industries, traffic police through inhalation of vehicle exhaust and road dust containing PAHs (Lee, 2010).

The problems can be acute or chronic on human health, in which acute effect depends mainly on the extent of exposure (e.g., length of time), the concentration of PAHs during exposure, the toxicity of the PAHs, and the route of exposure. However, chronic effect can be identified by long term exposure to PAHs, food consumption, exposure via dermal contact could be some of the potential reason for long term health effect of PAHs on human being (Srogi, 2007; Diggs et al., 2011).

Many PAHs are mutagenic, carcinogenic, teratogenic, and immunotoxic to living organisms, including microorganisms, animals, and humans (Bolden et al., 2017). Extensive studies have demonstrated that PAHs can potentially exert negative impacts on various organisms due to their ability to bioaccumulate and their high mutagenic potential (Kleinteich et al., 2018). Inhalation exposure to PAHs, in particular, is associated with an increased risk of lung cancer, making it a critical health concern (Kim et al., 2013). Bolden et al. (2017) also reported different health problems to humans that arise form PAHs exposure like; non-cancer reproductive effects in both males and females, DNA damage in oocytes, reproductive disease, breast and ovarian damage.

With improvements in living standards, it is expected that the global market demand for personal care products commodities will continue to grow. Large quantities of SMCs are continuously released into the environment during the production and use of these commodities (Liu et al., 2021; Tavera Busso et al., 2018). Through the food chain, synthetic musk compounds

have the potential to bioaccumulate, leading to an escalation in their detrimental impact on organisms (Luo et al., 2023). Synthetic musks have been reported to induce oxidative and genetic damage to organisms, and cause hepatotoxicity, enzymatic and reproductive toxicity to various creatures. SMCs, have been linked to neurotoxicity and potential carcinogenic effects in humans. The decline in production of musk ambrette is attributed to its ability to induce limb weakness, as well as central and peripheral demyelination (Pinkas et al., 2017; Parolini et al., 2015). Additionally, study by Ayuk-Takem et al. (2014) suggests that tonalide and galaxolide may inhibit polyisoprenylated methylated protein methyl esterase, potentially increasing the risk of degenerative disorders.

2.4.4. Bioaccumulation and toxicity to plants

One of the main failures in PAH remediation in soil using plant species was the negative impact on a germination and seedling growth (Gawryluk et al., 2022). According to their study, the germination energy was decreased for the grass species: tall fescue (*Festuca arundinacea* Schreb), red fescue (*Festuca rubra* L.), perennial ryegrass (*Lolium perenne* L.) and common meadow-grass (*Poa pratensis* L.) were decreased as the PAHs concentration increased. Similarly, the root and seedling height were decreased. Wei et al. (2014) examined the impact of phenanthrene on higher plants by investigating its effects on wheat seed germination and various physiological changes in seedlings. The findings revealed that phenanthrene hindered seed germination and influenced the growth and chlorophyll levels of wheat seedlings. Moreover, phenanthrene increased the levels of lipid peroxidation (LPO) and triggered H_2O_2 accumulation in leaf tissues in a dose-dependent manner, accompanied by alterations in the antioxidant status.

Wheat (*Triticum aestivum* L.), one of the most important agricultural crops in the world, is frequently used as a model plant in ecotoxicological studies. Chen et al. (2010) have studied single

and joint effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (*Triticum aestivum* L.). The study revealed that galaxolide showed similar toxicity to wheat as tonalide did in terms of seed germination and seedling growth, with the sequence of effects being germination rate > shoot elongation > root elongation. This suggests that wheat root and shoot elongation could serve as reliable indicators of polycyclic musk contamination in soil. However, the elongation of wheat shoot was more susceptible to polycyclic musks than wheat root elongation. The study also reported that the EC_{10} of root elongation and shoot elongation of *Triticum aestivum* to galaxolide and tonalide exposure were 55.7 and 25.0 mg/kg dw, respectively. Tonalide could result in 50 % inhibition rates of wheat root and shoot elongation at concentrations of 2257 and 945 mg/kg dw. However, the concentration of galaxolide inducing 50 % inhibition of root growth was lower than that of tonalide. The EC_{10} (Effective concentration at 10%) values of galaxolide and tonalide were nearly two orders of magnitude lower than the EC_{50} (half maximal effective concentration) values, which were generally considered as the toxicity threshold.

Another study reported that galaxolide and tonalide concentration did not appear to be toxic to wheat plants. Depending on the test parameters employed, the half maximal effective concentration (EC_{50}) analyses showed that galaxolide and tonalide in the tested soils varied from 85 to 2444 mg/kg dw and 187 to 4322 mg/kg dw, respectively (Wang et al., 2013).

2.5. Bioremediation of PAHs and SMCs in the soil

Different types of pollutants, which limit the soil fertility, impacting our world ecosystem. Among these pollutants, the presence of polycyclic aromatic hydrocarbons (PAHs) stands as a particularly enormous global concern due to their detrimental effects. Addressing PAHs pollution has become a crucial priority, prompting the implementation of diverse remediation strategies,

with bioremediation being one of the promising approaches used to restore the environment (Patel et al., 2020).

Feng et al. (2019) reported the degradation of PAHs in the soil contaminated from 10, 25 and 50 % of sludge and compost amendment. After 126 days of experiment, the highest removal rates of 16 PAHs were 53 % and 54.4 % at 50 % sludge amendment and 25 % compost amendment, respectively. Low molecular weight PAHs showed a better degradation from the soil than high molecular weight PAHs. Tomczyk et al. (2020) reported a 35 % degradation of Σ16 PAHs in the soil. The highest reductions, ranging from 51 % to 54 %, were noted for PAHs with 2, 5, and 6 rings. Among these, the most significantly degraded PAHs were those with 2 rings, owing to their high susceptibility to biological degradation.

A study by Litz et al. (2007) indicated the dissipation of galaxolide and tonalide in the soil amended with sewage sludge. In 8 weeks of experiment there was no observed relevant degradation of musk compounds by microbial activity. However, after 32 weeks, 53 % of the substance dissipated in the Cambisol substrate, while 50 % dissipated in the Luvisol and 47 % in the gleyic Podzol after 37 weeks. The degradation occurred slowly with elimination rates after 37 weeks of approx. 50 % and 25 % for galaxolide and tonalide, respectively. The results showed that tonalide is more persistent compared to galaxolide degradation. Based on this, half-life degradation was calculated for both compounds and resulted in 10-17 months for galaxolide, and 2-24 years for tonalide. Soil properties such as organic carbon play an important role to limit the bioavailability of chemicals and reduce their biodegradability (Wu et al., 2015; Ghahari et al., 2021).

2.5.1. The role of microorganisms in the degradation of SMCs and PAHs

Microorganisms in the soil environment have been known to play a major role in the biodegradation of organic compounds. Soil bioremediation employs the use of microorganisms that are naturally occurring or introduced into the soil to degrade pollutants. This technology is attracting increasing attention as a less expensive and more environmentally friendly alternative (McErlean et al., 2006).

In recent years, the practice of mycoremediation for PAHs has gained considerable attention, involving various fungal species. Thus, mycoremediation is a biological instrument for pollutant degradation, transformation, or immobilization in the environment. It has been discovered that fungi naturally have the ability and efficacy to breakdown PAHs. Unlike bacteria, not all fungi use PAHs as their sole carbon source. Instead, they engage in co-metabolism, breaking down PAHs while producing a diverse array of oxidized products, including CO_2. This degradation process by fungi is facilitated by monooxygenase enzymes (Gupta and Pathak, 2020).

Ligninolytic fungi, which can also to be more effective than some bacteria, because this fungi possess special enzymes (lignin peroxidase, manganese peroxidase, laccase) which have an important role in the initial attack towards PAHs with a high molecular weight present in the soil (Cristaldi et al., 2017). The level and the rate of biodegradation of PAHs by fungal enzymes depends on several factors: oxygen, accessibility of nutrients, pH, temperature, chemical structure of the compound, cell transport properties and chemical breakdown in the soil (Zafra and Cortés-Espinosa, 2015). Fungal manganese peroxidases facilitate the co-oxidation of PAHs by utilizing enzyme-mediated lignin peroxidation. On the other hand, fungal lignin peroxidases directly oxidize several PAHs. Notably, white-rot fungi (WRF) were found to convert anthracene into anthraquinone during their metabolic processes (Ansari et al., 2023). WRF have been suggested as effective agents for promoting the biodegradation of PAHs, particularly those with high

molecular weights. In comparison to bacteria, certain white-rot fungi species demonstrate superior abilities in soil colonization and competition with the native microflora (Novotný et al., 2000). Many soil fungi showed the ability to thrive, develop, and reproduce while also possessing the capability to degrade PAHs. These fungi utilize specific enzymes, such as manganese peroxide, lipases, proteases, and dioxygenases, to break down the PAH compounds (Zafra et al., 2017).

Wang et al. (2009) studied the degradation of three polycyclic aromatic hydrocarbons (PAHs) phenanthrene, pyrene, and benzo[a]pyrene in soils was investigated using *Phanerochaete chrysosporium.* The enzyme activities of lignin peroxidase and manganese peroxidase produced during the degradation process were analyzed. The findings revealed that the 19-day degradation was 72.8 % for phenanthrene, 51.2 % for pyrene and to 25.5 % for benzo[a]pyrene.

In the presence of the fungus *Aureobasidium pullulans* (ATCC 66657) about 80 % of tonalide was removed within 3 weeks. A similar study reported the degradation of tonalide and galaxolide in the cultures of white rot fungus *Phanerochaete chrysosporium* in 6 and 3 days, respectively (Balk and Ford, 1999). Rodarte-Morales et al. (2011) studied the degradation potential ability of three terrestrial WRF strains on polycyclic musk compounds. The tested WRF were an anamorph species of *Bjerkandera* sp. R1, *Bjerkandera adusta* and *Phanerochaete chrysosporium* inoculated on malt extract plates containing musk compounds. The results showed that celestolide, galaxolide and tonalide were not detected at the end of experiment. Suggesting that volatilization and indigenous microorganisms could also have a role in their removal since they were not found in the control treatments as well.

2.5.2. Phytoremediation and plant accumulation of SMCs and PAHs

Plants usually take up SMCs through passive absorption of soil water through the epidermal layer of the root and the cortex of root cells (Kinney and Heuvel, 2020). This pathway is

considered as the main way of the uptake (Hurtado et al., 2016). For both neutral and charged compounds, evapotranspiration was correlated with the uptake of organic chemicals into roots and leaves (Dodgen et al., 2015).

Several authors showed that the increased soil organic matter can decrease the transfer of target compounds and increase the sorption capacity of the compounds, to decrease their bioavailability for plant uptake (Macherius et al., 2012). The uptake and translocation of synthetic musk compounds in tomato plants after 180 days of growth was assessed. Concentration of polycyclic musk compounds cashmeran, celestolide, phantolide, traseolide, and tonalide were ranged from not detected to 5 µg/kg dw in tomato fruits and grew up in compost amended soil. Galaxolide was found comparatively with a higher concentration of 68 µg/kg dw. Only musk ketone were found from nitro musk compounds with the concentration of 1.3 µg/kg dw in the tomato fruit samples (Ramos et al., 2021b). In some studies, tonalide was not detected in the above-ground parts of plants, whereas galaxolide was predominantly found. This could be due to the higher water solubility of galaxolide (with a solubility of 0.5 mg/L at 25 °C) compared to tonalide. The higher water solubility of galaxolide enables the compound to enter the plant vascular system more readily and, consequently, facilitates its translocation to other parts of plant. Additionally due slightly lower log K*ow* of galaxolide, it would not be easy to bind it to root lipids as tonalide (Fernandes et al., 2022).

Synthetic musks were detected in wheat above ground biomass (cashmeran: 0.3 µg/kg dw, celestolide: 0.02–0.05 µg/kg dw, galaxolide: 3.7– 4.6 µg/kg dw, tonalide: 0.4–0.6 µg/kg dw, musk xylene: 0.1 µg/kg dw) and in sugar beet leaves (cashmeran: 0.1–0.2 µg/kg dw) (Fussell et al., 2014).

Galaxolide was detected at concentrations of up to 346 µg/kg dw, while tonalide was detected at a concentration of up to 283 µg/kg dw in pea root samples in soils amended with biosolids at a rate of 30 t/ha. In the above ground peanut parts galaxolide was detected in all treatments up to 10.5 µg/kg dw as well as detected in the seeds with a concentration up to 4.5 µg/kg dw. While in the other study by Calderón-Preciado et al. (2011), tonalide and galaxolide were detected in apple tree leaves with concentration of 0.04 and 0.057 µg/kg dw, respectively. Alfalfa accumulated 0.024 and 33.8 µg/kg dw for tonalide, and galaxolide, respectively. The used irrigation water for both apple and alfalfa plants contained the compounds tonalide and galaxolide with concentration of 0.15 and 0.31 µg/L, respectively.

Tonalide and galaxolide were detected at concentrations of 0.9 and 0.5 µg/kg dw, respectively, in barley grain cultivated at soil amended with 60 t/ha of fresh sewage sludge (Rivier et al., 2019). In this study concentration of musks in the grain from sewage sludge amended soils was not significantly different from the non-amended control treatments. During a three-month experiment, the degradation of the polycyclic musk compounds galaxolide and tonalide ranged from 75 % to 93 % and 66 % to 83 %, respectively, in soil amended with 20 and 60 t/ha of biosolid, whether planted with barley or left as unplanted bare soil (Rivier et al., 2019).

Zhang et al. (2017) reported the concentration of all priority PAHs in the tissue of maize plant, except indeno[1,2,3-cd] pyrene and benzo[g,h,i]perylene. The 3-ring PAHs were dominant in the maize tissues with 71 % of total PAHs, others 2, 4, 5 and 6 rings of PAHs were found with percentage of 14.8 %, 12.2 %, 1.2 % and 0.4 %, respectively. Li and Ma (2016) investigated the concentration of PAHs in the wheat from sewage sludge amended soil at varying rates (5, 10, 20, and 40 t/ha/year), resulted in concentration increase of sum of 16 PAHs in both wheat roots and shoots. The values ranged from 166 to 700 µg/kg dw for roots and 110 to 260 µg/kg dw for shoots.

However, interestingly, the PAH contents in the grains (81.2–95.2 µg/kg dw) did not show any statistically significant differences among the different application rates. In a study conducted by Paraíba et al. (2010), the bioconcentration of 16 PAHs in plants was investigated, specifically in corn grains. The bioconcentration factor (BCF) of these PAHs in corn grains varied within a range of 1.57 to 10.97.

2.5.3. Remediation of PAHs and SMCs by composting

Composting municipal solid waste and sewage sludge provides an effective strategy for waste reduction in densely populated areas. The benefits of sewage sludge composting include, cost effective, reduces the volume of waste that would otherwise occupy landfill space, minimum maintenance, easy operation and converting waste into valuable products (Meng et al., 2019). According to Lü et al. (2021), it was found that a significant majority, approximately 82 %, of composting activities were carried out at the laboratory scale. In contrast, only a relatively small proportion, accounting for approximately 18 %, was conducted at the pilot or plant scale. This suggests that most of the composting research and experimentation has primarily taken place in controlled laboratory environments, while a smaller fraction has been implemented on a larger scale in pilot or plant settings. The treatment of sewage sludge is an essential step in all wastewater treatment plants. This process involves the use of microorganisms and can be performed in either open system heaps or specialized bioreactors. Its primary objectives are to reduce the volume and mass of raw materials also producing high-quality organic fertilizer (Poluszyńska et al., 2017). Moreover, the agronomic utilization of composted manure brings forth numerous benefits. Compost, which contains a wealth of organic matter and vital nutrients, can be introduced into the soil to augment its fertility and structure. This approach facilitates the recycling of ample nitrogen (N), phosphorus (P), potassium (K), and organic matter present in sewage sludge, achieved through

the application of composted sludge products to land (Lu et al., 2019a). The bioavailability of compounds and microorganisms are some of the factors that plays a role in the degradation of contaminants in the sewage sludge composting (Lu et al., 2019; Guo et al., 2020).

Kinney et al. (2006) reported that the concentration of tonalide in composted sludge was 3,500 µg/kg dw compared to 5,000 µg/kg dw in the dried sludge. Composting is becoming more important than the direct use of sewage sludge in Czech Republic and Slovakia in the agricultural land to reduce the negative effects of contaminants (Kelessidis and Stasinakis, 2012). Biel-Maeso et al. (2019) reported the removal of synthetic musk compounds during the sewage sludge composting, resulted with higher degradation of galaxolide, tonalide, traseolide, celestolide with the values of 97, 87, 98 and 100 %, respectively.

Poluszyńska et al. (2017) investigated the degradation of sum of ∑16 PAHs in the sludge composting with sawdust for 30 days, resulted with a significance reduction with average from 26.07 to 4.01 mg/kg dw (84.6 %). From which two ring PAHs were removed 100 %, 3 rings (88 %) and 6 aromatic rings in the molecule (86.9 %). Lu et al. (2019) also reported the degradation of sum of six PAHs (∑PAHs) after 39 days from different kinds of sludge composting namely acenaphthylene, fluorene, phenanthrene, anthracene, chrysene and benzo(k)fluoranthene was 58.7 % in raw sludge, 58.5 % in chemical conditioned with polyacrylamide (PAM) sludge, 76.4 % in bioleached sludge, and 60.4 % in Fe[III]/CaO-conditioned sludge, respectively. Lü et al. (2021) reported after reviewed different studies that the removal of PAHs form sludge composting ranging from less than 50 % to even more than 90 %.

Siebielska (2014) studied the removal of naphthalene and benzo(a)pyrene during the thermophilic phase of sludge composting with rate of 98.2 and 40 %, respectively. The results shows that higher molecular weight PAHs were decomposed with a slower rate than lower ring

PAHs. Oleszczuk (2009) reported PAH removal during sewage sludge composting significantly correlated with the PAH bioavailability. The fact that bioavailability of organic contaminants is the main factor determining their fate, toxicity and losses in the environment. A study by Cai et al. (2007) focused on the removal of total polycyclic aromatic hydrocarbons (∑PAHs) from sewage sludge composting with straw, the findings indicated a significant reduction in ∑PAH levels. Over a period of 56 days of composting, the initial concentration of 28.6 mg/kg dw decreased to a range of 1.8−10.2 mg/kg dw, representing a remarkable removal rate of 64−94 %.

3. Scientific hypotheses and objectives

3.1. Hypotheses

- The concentration of selected persistence organic contaminants (POPs) and personal care products (PCPs) in sewage sludge will be affected by WWTP parameters and digestion technology.
- Phytoremediation, mycoremediation, and their combined application significantly remove POPs and PCPs from sewage sludge-amended soil.
- Composting of sewage sludge will degrade/reduce selected POPs and PCPs present in the sewage sludge.

3.2. Objectives

- Investigate the concentration of POPs and PCPs in sewage sludge from different WWTPs based on WWTP parameters and digestion technology.
- Evaluate the degradation of POPs and PCPs in the sewage sludge amended soil through the application of phytoremediation, mycoremediation, and their combination.
- Determine the efficiency of contaminants removal from sewage sludge by using composting.

4. Published Papers

4.1. Košnář et al. (2023). Concentration of the main persistent organic pollutants in sewage sludge in relation to wastewater treatment plant parameters and sludge stabilisation

Authors: Zdeněk Košnář, Filip Mercl, Lorenzo Pierdonà, **Abraham Demelash Chane**, Pavel Míchal, Pavel Tlustoš

Published in the journal: Environmental Pollution, 333, 122060
Information about the magazine: IF = 8.9 (2023)

Environmental Pollution 333 (2023) 122060

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Concentration of the main persistent organic pollutants in sewage sludge in relation to wastewater treatment plant parameters and sludge stabilisation☆

Zdeněk Košnář *, Filip Mercl, Lorenzo Pierdonà, Abraham Demelash Chane, Pavel Míchal, Pavel Tlustoš

Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 165 00, Praha, Suchdol, Czech Republic

ARTICLE INFO

Keywords:
Wastewater management
Biosolids
Screening
Organic contaminants
Concentration

ABSTRACT

Concentration of 16 polycyclic aromatic hydrocarbons (PAHs), 7 polychlorinated biphenyls (PCBs), and 11 organochlorine pesticides (OCPs) in sewage sludge from 40 wastewater treatment plants (WWTPs) was investigated. Relationship between pollutant sludge contents, main WWTP parameters and type of sludge stabilisation was carefully evaluated. Average load of PAHs, PCBs, and OCPs in different sludges from Czech Republic was 3096, 95.7 and 76.1 μg/kg dry weight, respectively. There were moderate/strong correlations among the individual tested pollutants in sludge (r = 0.40–0.76). Relationship between total pollutant contents in sludge, common WWTP parameters and sludge stabilisation was not evident. Only individual pollutants such anthracene and PCB 52 correlated significantly ($P < 0.05$) with biochemical oxygen demand ($r = -0.35$) and chemical oxygen demand removal efficiencies ($r = -0.35$), suggesting recalcitrance to degradation during wastewater treatment. When sorted according to the design capacity, a linear correlation between WWTP size and pollutant contents in sludge was evident with growing WWTP capacity. Our study indicated that WWTPs with anaerobic digestion are prone to accumulate a statistically higher content of PAHs and PCBs ($P < 0.05$) in digested sludges compared to aerobically digested ones. The influence of anaerobic digestion temperature of treated sludge on tested pollutants was not evident.

1. Introduction

Sewage sludge is considered a by-product of wastewater treatment plants (WWTPs). It comes from the mechanical, chemical, and biological treatment of both domestic and industrial wastewater (Shen et al., 2007). Apart from being considered waste, sewage sludge can be seen as a valued sink of organic matter and nutrients, especially nitrogen and phosphorus as it has been shown that its application to soil can significantly improve the nutrient status and soil fertility (Dijk et al., 2016; Singh and Agrawal, 2008).

Unfortunately, WWTP processes lead to the accumulation of undesirable microorganisms (Goberna et al., 2018), inorganic contaminants (Jones et al., 2014), and especially a wide range of persistent organic pollutants (POPs) in the sludge which can pose a serious threat to the environment (Dai et al., 2007; Stevens et al., 2003; Suciu et al., 2015; Fijalkowski et al., 2017).

POPs are a group of organic compounds that have been intentionally or inadvertently produced and introduced into the environment. Major concerns about POPs are connected to their environmental persistence, long-distance transportability, and accumulation in plants and animals, including in top species of the food chain (Xu et al., 2013). Nowadays, POPs are ubiquitous in the environment, with possible adverse effects on biota because of their toxicity (Roig et al., 2012). Among the main POPs are polycyclic aromatic hydrocarbons (PAHs) derived from the incomplete combustion of organic matter. Also, there are industrially produced polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) including a wide range of compounds (Blanchard et al., 2004; Jaspers et al., 2014). Due to their lipophilic properties, these compounds have been found in many highly fatty foods such as meat, eggs, and milk products, and the carcinogenic risks of these compounds have been described (Passuello et al., 2010). The recycling potential of the produced sludge in agriculture is weakened by the high content of PAHs,

☆ This paper has been recommended for acceptance by Charles Wong.

* Corresponding author.
E-mail address: kosnarz@af.czu.cz (Z. Košnář).

https://doi.org/10.1016/j.envpol.2023.122060
Received 15 February 2023; Received in revised form 12 June 2023; Accepted 14 June 2023
Available online 15 June 2023

PCBs, and OCPs often found in this material, which clearly poses a threat to the environment, as has been reported in several soil application experiments (Bright and Healey, 2003; Harrison et al., 2006; Olofsson et al., 2013; Sun et al., 2019. Legislative limits of PAHs, PCBs, and OCPs in sludge for soil application are set differently in many countries (Wiśniowska et al., 2019).

A number of previously published research articles have shown the flow of persistent pollutants across the whole WWTP system and described that selected POPs are removed from wastewater only due to the sorption of compounds by the particulate matter present in sewage during wastewater treatment (Aemig et al., 2006; Katsoyiannis and Samara, 2005; Wang et al., 2013). Novel approaches to the removal of resistant organic pollutants from wastewater have been intensively studied. For instance, Ighalo et al. (2022) summarised using nano-adsorbents for the elimination of POPs from wastewater using adsorption processes, and Samuel et al. (2022) reviewed the oxidation of organic contaminants from oily wastewater using photocatalytic membrane reactors, Derco et al. (2015) showed significant removal of organochlorine pesticides from wastewater by the combined process of ozone with zero-valent iron nanoparticles and Feng et al. (2021) described the role of enzymes and many enzymatic applications for POP removal in different types of wastewater treatment. Nevertheless, the relationship between the technological parameters used for WWTPs under real conditions and the content of organic pollutants in the resulting sewage sludge remains unclear.

This work hypothesises that the accumulation of PAHs, PCBs, and OCPs in the sludge can be affected by the applied WWTPs parameters and the technology of sewage sludge stabilisation. The main objectives of this work were to determine the current PAH, PCB, and OCP concentrations in the sewage sludge from 40 different WWTPs and to investigate if the contents are influenced by the current technological parameters of WWTPs.

2. Material and methods

2.1. Description of sampled wastewater treatment Plants and sludge samples

Sewage sludge (n = 40) was sampled during the summer of the years 2017–2019 from 40 different Czech wastewater treatment plants. In this study, the designed capacity of respective WWTPs described by the population equivalent (PE) was grouped as follows: small (<50,000; n = 21), medium (50,000–100,000; n = 11), and large (>100,000; n = 8). According to the technology of sludge stabilisation, they were divided into two main groups, aerobic digestion (n = 27) and anaerobic ones (n = 13), to evaluate the applied WWTP technology on the concentration of PAHs, PCBs, and OCPs in sludges. Further, the sludges were divided according to the temperature of anaerobic digestion into mesophilic and thermophilic sub-classes. Sludge stabilisation times (age) of sampled facilities were <1–50.2 days for aerobic digestion, 10–48 days for mesophilic anaerobic digestion, and 26.3–60 days for thermophilic anaerobic digestion. The investigated WWTPs' parameters, stabilisation technology and their properties given by the operators of WWTPs are summarised in Tables S1–a. The raw influent of the sewer system of investigated WWTPs usually was a mix of sewage (domestic and municipal) and surface runoff water. If the sewer system of the WWTP was connected to some specific type of industry, it is shown in Tables S1–b. Sewage sludge samples and WWTP technological parameters were provided by WWTP operators only under the condition of anonymity; therefore, the exact locations of individual WWTPs in the Czech Republic cannot be shown. Data provided by WWTP operators and WWTP locations are subjected to anonymity. Therefore, only 29 WWTPs provided the main information on the wastewater treatment processes (Tables S1–b).

Sludge samples (~10 kg fresh weight) were obtained from each WWTP by thoroughly mixing randomly taken subsamples (n = 10) and transporting them to the laboratory (~4 °C). From the homogenised sludge samples, two sample replications were made for further investigation. Samples were then dried by a freeze dryer (Labconco Corporation, USA), milled to a fine powder with an analytical mill (IKA®-Werke GmbH & Co. KG, Germany), and stored in a freezer before further analyses. The physicochemical characteristics of 40 tested sludge samples are shown in Table S2.

2.2. Chemicals and materials

The name, CAS number, and molecular and structural formulas of the individual 16 PAHs, 7 PCBs, and 11 OCPs are given in Tables S3, S4, and S5. Analytical standards of PAHs (PAH Mix 16), PCBs (PCB Mix 7), OCPs (Pesticides Mix 10), and hexachlorobenzene each at 100 μg/mL in cyclohexane, were purchased from Neochema (Bodenheim, Germany). Internal standards (Table S6) of naphthalene-d_8, acenaphthene-d_8, phenanthrene-d_{10}, chrysene-d_{12}, perylene-d_{12} (PAH Mix deuterated), alpha hexachlorocyclohexane-d_6 (alpha HCH-d_6), 2,3,4,5,6-pentachlorobiphenyl-d_5 (PCB-d_5), and 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane-d_8 (DDT-d_8) each at 100 μg/mL in cyclohexane, and a surrogate standard (Table S7) of *p*-terphenyl-d_{14} at 1000 μg/mL in dichloromethane were purchased from Restek (B/N surrogate mix, USA). Analytical graded *n*-hexane and dichloromethane were used (95+ Pestapur, Chromservis, Czech Republic). Solid-phase silica extraction cartridges (Strata SI-1, Phenomenex, USA) were used for the extract purification.

2.3. Extraction of PAHs, PCBs, and OCPs

The PAHs, PCBs, and OCPs were extracted from 1.00 g of sludge samples using a surrogate standard (Table S7) with 20 mL acetone/n-hexane (1:1, *v/v*) by the ultrasonication method previously described by Košnář et al. (2021). The collected extracts were concentrated to near dryness using a Büchi rotavapor (Switzerland) at 40 °C, then re-dissolved with 1 mL of *n*-hexane for further purification using silica SPE cartridges. First, the SPE cartridges were conditioned using 10 mL of hexane–dichloromethane (3:1, *v/v*). After loading with 1 mL of sample extract, the cartridge was washed with 10 mL of hexane followed by elution with 10 mL of *n*-hexane–dichloromethane (3:1, v/v). The eluate was evaporated to a near dryness and reconcentrated to 1 mL with *n*-hexane. After the addition of internal standard (Table S6) solution at 500 μg/L, it was analysed for PAHs, PCBs, and OCPs.

2.4. GC-MS/MS analysis

Analysis of 16 PAHs, 7 PCBs, and 11 OCPs was carried out using the 7000D Triple Quadrupole GC-MS/MS (Agilent Technologies, USA) coupled with PAL RTC (Switzerland). The tested compounds were separated by a 30 m-long chromatography capillary column with 0.25 mm inner diameter and 0.25 μm film thickness (Restek-XLB, Bellefonte, USA). A volume of 2 μL of sample extract was injected in the splitless mode regime. More detailed conditions are described in previous study by Košnář et al. (2021). The dynamic multiple reaction monitoring mode was used to quantifying of target compounds. MS/MS transitions, collision energies, and the internal standard used for the quantification of the respective compounds are shown in Table S8. The eight-point calibration curve of individual compounds in *n*-hexane ranged from 0.5 ng/mL to 1000 ng/mL with R^2 higher than 0.992 for all analytes. Detection limits of PAHs, PCBs, and OCPs (1–2 μg/kg dw) are shown in Table S9 as well as analyte recoveries from spiked blank samples (43–108%) and spiked sewage sludge samples (70–107%) at three concentration levels (100–300 ng/ml). The average recoveries of tested pollutants from sewage sludge were 90 ± 10% of 16 PAHs, 88 ± 5% of 7 PCBs, and 83 ± 6% of 11 OCPs. Alongside each sample batch, calibration, procedural blanks, and check samples in *n*-hexane were done. All analytes in the blanks were below the detection limit. In this study,

average recovery of *p*-terphenyl-d_{14} from spiked sludge samples was 94% and the results were adjusted for efficiency.

2.5. Data evaluation and statistical analyses

The group of 16 PAHs represents the total sum of 16 US EPA PAHs (Table S3). The 7 PCB are the sum of 7 US EPA PCBs (Table S4), and 11 OCPs is the sum of 11 individual OCPs (Table S5). To elucidate the effect of WWTP technologies on compounds with similar physicochemical properties, the total PAHs were divided into three fractions according to their molecular weight and number of rings: i) low molecular weight (LMW) PAHs (the sum of naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene); ii) medium molecular weight (MMW) PAHs (the sum of fluoranthene, pyrene, benzo[a]anthracene, and chrysene); and iii) high molecular weight (HMW) PAHs (the sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo [a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene). The OCPs were sorted into groups of HCH isomers and DDT congeners (isomers/metabolites) according to the sum of respective compounds.

Normal distribution of data was checked by Kolmogorov–Smirnov, Lilliefors, and Shapiro–Wilk tests. When the data followed the assumptions of normality, a paired samples (PS) *t*-test, to observe significant differences ($P < 0.05$) between two groups, and a one-way ANOVA followed by a post hoc Tukey's HSD test was used to determine significantly differences ($P < 0.05$) between three or more groups. When the data were not normally distributed, Mann–Whitney U (M-W U) and Kruskal–Wallis H (K–W) tests ($P < 0.05$) were applied. Pearson product-moment correlation was conducted to elucidate the effect of the main WWTP technologies on the content of PAHs, PCBs, and OCPs in sludge samples. Sample size requirements n = ≥ 25 for estimating Pearson correlations according to Bonett and Wright (2000) met the criteria as the correlation analyses were performed from 40 sludge samples. The strength of the correlation was evaluated according to Evans (1996) as follows: 0.00–0.19 very weak, 0.20–0.39 weak, 0.40–0.59 moderate, 0.60–0.79 strong, and 0.80–1.00 very strong. SigmaPlot 14.5 (Systat Software, USA), Statistica 12.0 (StatSoft, USA) and Excel 2010 (Microsoft Corporation, USA) were used for statistical analysis and figures.

3. Results and discussion

3.1. Current state of persistent pollutants in sewage sludge in the Czech Republic

The occurrence of the total sums of PAHs, PCBs, and OCPs present in the 40 samples from different WWTPs can be seen in Table 1. The average PAH content sorted by the molecular weight and quantity of benzene molecules in the compound occurred in sewage sludge samples in the following order: HMW PAHs = MMW PAHs > LMW PAHs. This was expected as PAHs with a low molecular weight can volatilise from sludge more easily than those of medium and high molecular weight, which contain more than three benzene rings (Nas et al., 2020). The total amount of the 16 PAHs in sewage sludge was in the range 526–9718 μg/kg dw which was consistent with many other studies where the sludges were collected in WWTPs using similar wastewater treatment processes such as activated sludge in secondary treatment with further anaerobic/aerobic sludge stabilisation (Dai et al., 2007; Jones et al., 2014; Nas et al., 2020; Peréz et al., 2001; Shen et al., 2007). Much smaller amounts of the 16 PAHs in biologically treated sewage sludge (n = 35) were reported by Suciu et al. (2015). Contradictory to our results, higher amounts of the 16 PAHs have also been previously reported (Cai et al., 2007; Ning et al., 2014; Stevens et al., 2003; Sun et al., 2019) and Hua et al. (2008) found a PAH content in Chinese sludges from 33,000 to 83,000 μg/kg dw.

An evaluation of individual PAHs, PCBs, and OCPs found in sewage sludge is shown in Table S10. All 16 PAH compounds were present in each sewage sample tested. They were within a wide range, from 2.10 μg/kg dw of acenaphthene to 3077 μg/kg dw of benzo[a]anthracene. Our average content of carcinogenic benzo[a]pyrene of 190 μg/kg dw was lower than that described elsewhere (Mailler et al., 2014; Vácha et al., 2006). Only the congeners PCB 138 and PCB 153 were observed in all 40 sludge samples tested but the average amount did not exceed 22.0 μg/kg dw. Each of the OCPs was also found in small amounts and the detection of alpha HCH in sludge samples was very rare.

Table 1
Evaluation of PAHs, PCBs, and OCPs present in 40 sewage sludge samples from different WWTPs.

Compounds	n > MDL	Average (μg/kg dw)	SD	Median (μg/kg dw)	Min (μg/kg dw)	Max (μg/kg dw)	CV (%)
LMW PAHs	40	137	138	90.0	26.7	697	101
MMW PAHs	40	1592	1135	1308	301	4486	71
HMW PAHs	40	1367	1153	1023	173	4688	84
16 PAHs	40	3096	2328	2454	527	9718	75
7 PCBs	40	95.7	104	72.7	10.5	588	109
HCHs	23	14.6	9.20	14.1	1.20	35.0	63
DDTs	40	62.4	63.0	47.4	12.9	419	101
11 OCPs	40	76.1	70.6	66.3	17.9	481	93

n > MDL = number of samples in which the compound was detected; **MDL** = method detection limit; **SD** = standard deviation; **CV** = coefficient of variation; **LMW PAHs** (low molecular weight PAHs) = sum of naphthalene, acenaphthylene, acenaphthene, and fluorene; **MMW PAHs** (medium molecular weight PAHs) = sum of phenanthrene, anthracene, fluoranthene, and pyrene; **HMW PAHs** (high molecular weight PAHs) = sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene; **16 PAHs** (total sum PAHs) = sum of LMW, MMW, and HMW PAHs; **7 PCBs** = sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180; **HCHs** = sum of alpha, beta, gamma, and delta hexachlorocyclohexane isomers (HCH); **DDTs** = sum of 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 2,4′-DDT, 4,4′-DDD, and 4,4′-DDT congeners; **11 OCPs** = sum of HCH isomers, DDT congeners, and hexachlorobenzene (HCB).

The Stockholm Convention on Persistent Organic Pollutants is an international environmental treaty signed in 2001 which eliminates or restricts the production and use of 12 selected POPs; it became effective in the Czech Republic in 2004. Despite the banning of many compounds driven by the Stockholm Convention, relatively high amounts of most PCBs and OCPs can still be found nowadays in sewage sludge. Average amounts of the 7 PCBs and 11 OCPs did not exceed 100 μg/kg dw; the highest content was 481 and 587 μg/kg dw, respectively, which was unexpected as the use of PCBs was restricted worldwide from the middle of 1980s (US EPA, 2022). Many previous studies (Katsoyiannis and Samara, 2005; Mailler et al., 2014; Olofsson et al., 2013; Stevens et al., 2003) reported similar amounts of PCBs, but reports of lower amounts of indicator PCBs in sewage sludges have also been published by many other authors (Aparicio et al., 2009; Guo et al., 2009). The contents of hexachlorobenzene (HCB) and selected congeners of HCH and DDT in this study were comparable with those reported by Katsoyiannis and Samara (2005). In this study, the average total amount of the 11 OCPs was 76.1 μg/kg dw; almost 82% were DDT congeners, and the rest were HCB and HCH isomers.

Among the determined POPs, the threshold values given by the proposed Czech directive (Public Notice, 2016; Vácha et al., 2007) were exceeded twice in the case of the 16 PAHs (>10,000 μg/kg dw) and once in the case of the 7 PCBs (>500 μg/kg dw) which indicated that most of the sludges could be recycled in agriculture. When comparing the total amounts of PAHs in Czech sludge over time within the gap of nearly 20 years, the average amount of the 16 PAHs decreased from 6827 μg/kg dw (n = 45) published by Vácha et al. (2005) to 3095 μg/kg dw (n = 40) in the current study. This might be related to the fact that forming of PAHs in the Czech environment decreased with time, probably due to the introduction of a low-emission technology in industry. Therefore, a lower amount could reach the sewerage. Further, the content of PCBs

and OCPs was not dramatically changed when compared to the report of Vácha et al. (2005), which supports their persistence in the environment.

3.2. Correlation analysis among persistent organic pollutants in sludge

The correlation analysis among PAHs in Table S11 shows a very strong correlation among most of the individual PAHs in sewage sludge ($P < 0.001$), except for naphthalene and acenaphthene, with MMW and HMW PAHs. The same was true for the 7 indicator PCBs except for congener PCB 101, as presented in Table S12. Comparable correlation coefficients were obtained in many previous other studies (Hua et al., 2008; Sun et al., 2019). In the case of the 11 OCPs in Table S13, the correlation between HCB and selected DDT congeners was very strong ($r = 0.86$, $P < 0.001$). Pearson correlation coefficients (r) of PAHs, PCBs, OCPs, and respective pollutant groups of 40 sludges are given in Table 2. PAHs which were sorted according to their molecular weight and number of benzene rings, were correlated in sewage sludge, with correlation coefficients in the range from 0.43 ($P < 0.01$) to 0.95 ($P < 0.001$). This suggests that individual PAHs with different molecular weights are accumulated equally in sewage sludge, as also indicated elsewhere (Hua et al., 2008). A moderate positive correlation was found between the total sum of PAHs and PCBs ($r = 0.54$, $P < 0.001$), and the correlation between PAHs and the total sum of DDT congeners ($r = 0.40$, $P < 0.05$) indicated the wide extent of the tested contaminants across the environment. Moreover, a strong correlation was found between the total sum of DDT congeners and the 7 PCBs ($r = 0.76$, $P < 0.001$). That significant correlation was not expected as PCBs were used more frequently in areas with heavy industry than in agriculture or rural regions. This could support their ubiquity in the environment even though they have been restricted for decades.

3.3. Relationship between persistent organic pollutants content in sludge and WWTP parameters and sludge stabilisation

Pearson correlation analysis was also conducted to understand the effect of basic WWTP technological parameters on the concentration of POPs in the 40 sewage samples tested (Tables S14–S17). Coefficients (r) derived from these analyses (Table 3) showed only a weak negative correlation between acenaphthylene and wastewater load percentage and a positive one with the wastewater hydraulic retention time of WWTPs. Prior to the data evaluation, it could be assumed that wastewater hydraulic retention time in WWTP and the retention time of sewage sludge in the stabilisation segment of WWTP would be crucial parameters to influence the occurrence of POPs in sewage sludge. Unfortunately, the correlation tests revealed no relationship between investigated parameters and POPs sludge concentration. Man et al. (2017) suggested that microbial degradation of PAHs was not prominent in secondary treatment and removal of individual POPs was governed by the sorption of suspended particulate matter. Nevertheless, Komolafe et al. (2021) reported that a long hydraulic retention time in a WWTP is not a suitable mitigation removal strategy for POPs and personal care products in WWTP with activated sludge in secondary treatment, and for the prediction of effluent POPs concentration is almost impractical. Dubey et al. (2021) stated that the sorption of hydrophobic compounds depends on the molecular weight of PAHs and the intramolecular hydrophobic environment for chlorinated compounds such as PCBs.

A negative, weak correlation ($r = -0.38$) among anthracene and BOD and, COD was found at $P < 0.05$. This could suggest that the microbial treatment of wastewater partly influences anthracene concentration. Further, a statistically significant negative correlation ($r = -0.52$) between PCB 52 congener and COD was observed at $P < 0.05$. This could indicate that the possible degradation of these pollutants during the wastewater treatment processes is very difficult. Negative correlation coefficients could be explained by the dilution of compounds in sewage sludge because, with increasing the COD removal rate, higher efficiency of organic matter conversion into sludge is expected. No significant correlation ($P > 0.05$) was observed between the 11 OCPs and

Table 3
Pearson product-moment correlation coefficients (r) for selected persistent organic pollutants, total sum of 16 PAHs, 7 PCBs, 11 OCPs, and main technological parameters of WWTPs derived from Pearson's correlation analysis of 40 sludge samples.

Variables	ACY	ANT	PCB 52	16 PAHs	7 PCBs	11 OCPs
WWTP design capacity	ns	ns	ns	ns	ns	ns
Wastewater load percentage	−0.39*	ns	ns	ns	ns	ns
Wastewater hydraulic retention time in WWTP	0.32*	ns	ns	ns	ns	ns
BOD removal efficiency	ns	−0.38*	−0.36*	ns	ns	ns
COD removal efficiency	ns	−0.35*	−0.52*	ns	ns	ns
Retention time of sewage sludge in the stabilisation	ns	ns	ns	ns	ns	ns

Significance of observed Pearson's product-moment coefficients (r): * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; **ns** = not significant at α = 95%. Abbreviations: **16 PAHs** (total sum PAHs) = sum of LMW, MMW, and HMW PAHs; **7 PCBs** = sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180; **11 OCPs** = sum of HCH isomers, DDT isomers, and hexachlorobenzene (HCB); **ACY** = acenaphthylene; **ANT** = anthracene.

Table 2
Pearson product-moment correlation coefficients (r) for LMW PAHs, MMW PAHs, HMW PAHs, 16 PAHs, 7 PCBs, 4 HCH isomers, 6 DDT isomers, and 11 OCPs derived from Pearson's correlation analysis of 40 sludge samples.

Variables	LMW PAHs	MMW PAHs	HMW PAHs	16 PAHs	7 PCBs	HCHs	DDTs	11 OCPs
LMW PAHs	1	0.53***	0.43**	0.53***	0.74***	ns	0.32*	0.34*
MMW PAHs		1	0.95***	0.99***	0.57***	ns	0.45**	0.43**
HMW PAHs			1	0.98***	0.44**	ns	0.32*	0.30*
16 PAHs				1	0.54***	ns	0.40*	0.38*
7 PCBs					1	ns	0.76***	0.75***
HCHs						1	ns	ns
DDTs							1	0.99***
11 OCPs								1

Significance of observed Pearson's product-moment coefficients (r): * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; **ns** = not significant at α = 95%. Abbreviations: **LMW PAHs** (low molecular weight PAHs) = sum of naphthalene, acenaphthylene, acenaphthene, and fluorene; **MMW PAHs** (medium molecular weight PAHs) = sum of phenanthrene, anthracene, fluoranthene, and pyrene; **HMW PAHs** (high molecular weight PAHs) = sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene; **16 PAHs** (total sum PAHs) = sum of LMW, MMW, and HMW PAHs; **7 PCBs** = sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180; **HCHs** = sum of alpha, beta, gamma, and delta hexachlorocyclohexane isomers (HCH); **DDTs** = sum of 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 2,4′-DDT, 4,4′-DDD, and 4,4′-DDT congeners; **11 OCPs** = sum of HCH isomers, DDT congeners, and hexachlorobenzene (HCB).

WWTP parameters, as shown in Table 3.

In his review, Stasinakis (2012) published that another group of organic pollutants, endocrine disruptors in sewage sludge, are often correlated with the concentration of respective compounds in influent wastewater and could be affected by the physicochemical properties of target compounds, sludge characteristics, and the operational parameters of WWTP. In a comprehensive view, our correlation analyses used the technology of 40 different WWTPs and PAHs, PCBs, and OCPs demonstrated no relevant correlations at $P < 0.05$. This lack of correlations could reflect the general resistance of these compounds to be transformed or degraded by the physicochemical/biological processes during wastewater treatment in WWTPs. Therefore, the concentration of these pollutants in the resulting sewage sludge is not mainly affected by the technological parameters of WWTPs investigated in Table 3. A previous study of antibiotic residues in sewage sludge by Dubey et al. (2021) also found no correlation between the occurrence of these micropollutants and WWTP capacity, processing volume, and PE. Jones et al. (2014) suggested that the majority of POPs are adsorbed onto dissolved solid particles of wastewater, and they accumulate in sludge as the difference between pollutants concentrations in the influent/effluent. POPs in sludge could persist for a long time. The environmental persistence of studied POPs varies with their physicochemical characteristics, molecular weight, or degree of halogenation in the case of PCBs. According to the Annex D of the Stockholm Convention, POPs degradation half-life of 60 days (water) or 180 days (soil, sediment) is presumed. In general, POPs could have a half-life of years or decades in soil/sediment (Scheringer et al., 2012). Włodarczyk-Makuła (2012) determined half-life of PAHs up to 2048 days for benzo(a)pyrene in digested sludge from a municipal WWTP.

3.4. Changes of persistent organic pollutants in sludge in relation to the designed WWTP capacity

Despite to no correlation being found between the concentration of selected POPs and WWTP design capacity, as shown in Table 3, when the latter is compared with the content of POPs in sewage sludge a clear increasing trend can be seen. Except for naphthalene, the average content of other PAHs differed according to the suggested WWTP design capacity groups (Fig. S1). Statistically significant ($P < 0.05$) differences in the content of every given PAH compound, except for naphthalene, were found when comparing WWTPs capacities. The content of some MMW and HMW PAHs such as benzo[a]anthracene, chrysene, dibenzo [a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene was significantly higher ($P < 0.05$; Tukey's HSD/K–W test) in the sewage sludge from medium and large WWTPs.

Similar trends to those described above were observed for the 7 indicator PCBs (Fig. S2). All of the individual HCH congeners including HCB, 2,4′-DDE, and 4,4′-DDD were distributed similarly in sludge samples with the designed WWTP capacity (Fig. S3) as there were no significantly different results ($P > 0.05$; K–W test). The highest content ($P < 0.05$; Tukey's HSD/K–W test) of 4,4′-DDE, 2,4′-DDD, 2,4′-DDT, and 4,4′-DDT congeners (Fig. S3) in sludge from small or medium WWTPs could reflect the fact that DDT was frequently used in agricultural areas in the past where small or medium WWTPs now operate.

When PAHs were plotted against the PEs of each WWTP by Stevens et al. (2003), they found no relationship. In our study the content of LMW, MMW, and HMW PAHs, and 16 PAHs (Fig. 1) and 7 PCBs (Fig. 2) in sewage sludge gradually increased as the design capacity of WWTPs enlarged. This correlation may suggest that the concentration of these organic pollutants in sewage sludge tends to be higher in WWTPs in urban areas than in non-urban areas, as was also proposed by Wluka et al. (2021). The contents of all PAH groups (Fig. 1) were statistically higher ($P < 0.05$; K–W test) in sludges from large treatment plants in comparison to the small ones, but when compared to medium WWTPs, the PAH amounts were comparable (K–W test). The same was true for the total sum of the 7 PCBs in sewage sludge (Fig. 2). There were no

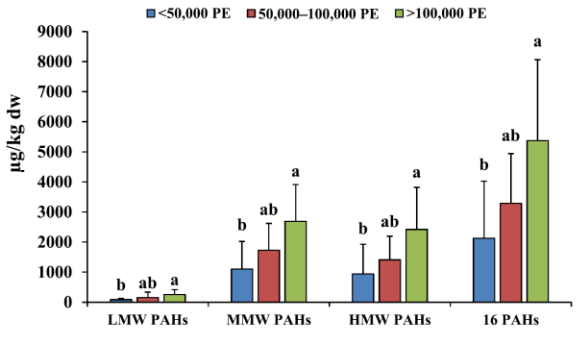


Fig. 1. Average content of LMW PAHs, MMW PAHs, HMW PAHs, and 16 PAHs in sewage sludge samples in relation to the designed capacity of WWTP expressed by population equivalent (PE).
PE – population equivalent: <50,000 (small WWTP; n = 21), 50,000–100,000 (medium WWTP; n = 11), and >100,000 (large WWTP; n = 8). Different letters indicate significant differences ($P < 0.05$) between tested groups of WWTPs for each respective PAH. Lowercase letters indicate difference by K–W test. Abbreviations: **LMW PAHs** (low molecular weight PAHs) = sum of naphthalene, acenaphthylene, acenaphthene, and fluorene; **MMW PAHs** (medium molecular weight PAHs) = sum of phenanthrene, anthracene, fluoranthene, and pyrene; **HMW PAHs** (high molecular weight PAHs) = sum of benzo [b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene; **16 PAHs** (total sum PAHs) = sum of LMW, MMW, and HMW PAHs.

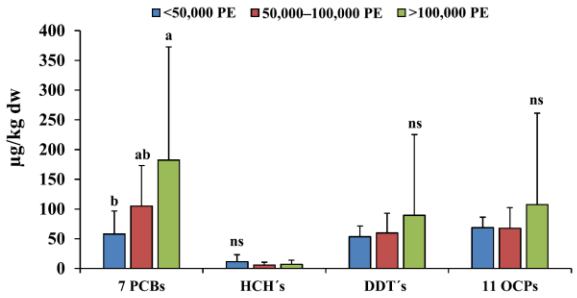


Fig. 2. Average content of 7 PCBs, HCH isomers, DDT congeners, and 11 OCPs in sewage sludge samples in relation to the designed capacity of WWTP expressed by population equivalent (PE).
PE – population equivalent: <50,000 (small WWTP; n = 21), 50,000–100,000 (medium WWTP; n = 11), and >100,000 (large WWTP; n = 8). Different letters indicate significant differences ($P < 0.05$) between tested groups of WWTPs for each respective PAH. Lowercase letters indicate difference by K–W test. **ns** denotes no significant difference between WWTP groups at $P < 0.05$. Abbreviations: **7 PCBs** = sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180; **HCHs** = sum of alpha, beta, gamma, and delta hexachlorocyclohexane isomers (HCH); **DDTs** = sum of 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 2,4′-DDT, 4,4′-DDD, and 4,4′-DDT congeners; **11 OCPs** = sum of HCH isomers, DDT isomers, and hexachlorobenzene (HCB).

significantly different ($P > 0.05$; K–W test) results for the content of the 11 OCPs, HCHs, and DDT congeners in sludges in relation to the designed capacity of WWTPs (Fig. 2), which indicates a similar distribution of these pollutants in the environment of Czech Republic.

3.5. Content of persistent organic pollutants in sludge in relation to the type of sludge stabilisation

Anaerobic and aerobic digestion, which have been widely studied, are the most common processes for treating sewage sludge from WWTPs. However, a comparison of accumulated persistent compounds in sludges

from these treatments still remains ambiguous (Katsoyiannis and Samara, 2005). The content of individual PAHs, PCBs, and OCPs in sludges in relation to the biological stabilisation method of the digested sludge is evaluated in Figs. S4–S6. In many previous studies (Guo et al., 2009; Ning et al., 2014; Olofsson et al., 2013) analysing PAHs in sewage sludges from various WWTPs, the difference between the content of PAHs and PCBs in anaerobically/aerobically treated sludges was not obvious. In the present study, a significantly higher ($P < 0.05$–0.001; PS *t*-test or $P < 0.05$; M-W *U* test) amount of most of the investigated PAHs was found in sewage sludges stabilised under anaerobic conditions compared to the aerobically treated ones. The same was true for the 7 individual PCBs, except for congeners PCB 118 and PCB 180 (Fig. S5) as well as compounds 2,4′-DDE and 4,4′-DDE (Fig. S6).

As shown in Figs. 3 and 4, the use of anaerobic digestion to stabilise the sewage sludge led to the concentration of a significantly higher content ($P < 0.01/0.001$; PS *t*-test) of all PAH groups and the total sum of the 7 PCBs than in aerobically treated sludge. This could be caused by the fact that PAHs are generally formed from the incomplete combustion of fossil fuels and petroleum-based materials in households and plants (Zhang et al., 2012) and as a consequence of increased automobile exhaust and heavy industry (Suman et al., 2016) in very densely populated cities or urban areas where WWTPs with anaerobic stabilisation commonly prevail.

Furthermore, PCBs were used in many voltage converters, capacitors, and many other electrical devices (Iwegbue et al., 2022). Therefore, their residues in anaerobically treated sewage sludge can be expected as these sludges usually come from urban areas where medium or large WWTPs operate using anaerobic stabilisation more frequently for economic reasons. Another explanation was given by Komolafe et al. (2021), who recently published that PAHs are resistant to anaerobic degradation in the stabilisation segment of a WWTP with an up-flow anaerobic sludge blanket reactor, while the biotransformation of aromatics under aerobic conditions is possible. But in our study, this hypothesis could be rejected for our explanation as no relationship between pollutant concentration in sewage sludge and biological treatment represented by BOD was observed. Further, the opposite results were obtained in the case of selected OCPs (Fig. 4) as the content of HCH isomers was significantly increased ($P < 0.01$; M-W *U* test) in aerobically digested sludges. This could be explained by the fact that

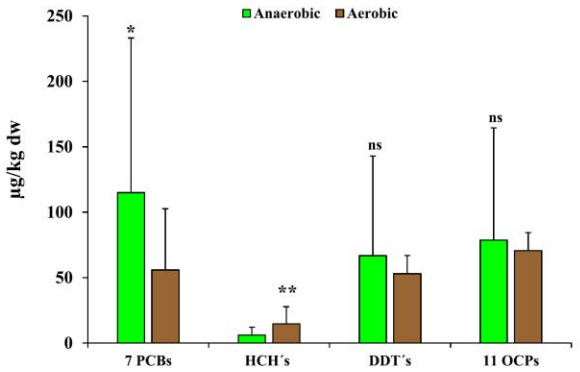


Fig. 4. Average content of 7 PCBs, HCH isomers, DDT congeners, and 11 OCPs in anaerobic digested (n = 27) and aerobic digested (n = 13) sewage sludge.
* indicates significant difference between the technologies ($P < 0.01$; PS *t*-test); ** indicates significant difference between the technologies ($P < 0.01$; M-W *U* test); **ns** denotes no significant difference between WWTP groups at $P = 0.05$. Abbreviations: **7 PCBs** = sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180; **HCHs** = sum of alpha, beta, gamma, and delta hexachlorocyclohexane isomers (HCH); **DDTs** = sum of 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 2,4′-DDT, 4,4′-DDD, and 4,4′-DDT congeners; **11 OCPs** = sum of HCH isomers, DDT isomers, and hexachlorobenzene (HCB).

these sludges usually came from small WWTPs, which mostly operate in agricultural areas. Therefore, we can speculate that these OCPs were used more often there in the past. Based on these findings, their higher abundance in stabilised sludge can be related to higher concentrations of analysed compounds in the wastewater-feeding WWTPs and urban/-rural WWTP environments rather than the type of sludge stabilisation.

Anaerobic digestion of the sludge of mesophilic temperature is used in WWTPs more commonly than the treatment of thermophilic temperature due to its lower process energy demand for reactor heating (Gebreeyessus and Jenicek, 2016). WWTPs with thermophilic anaerobic digestion of sludge are very rare in the Czech Republic; therefore, only three sewage sludge samples were investigated in this study. When we evaluated the mesophilic/thermophilic effect of anaerobic digestion on the concentration of individual PAHs, PCBs, and OCPs (Figs. S7–S9), including the sums of respective pollutants (Figs. 5 and 6), no

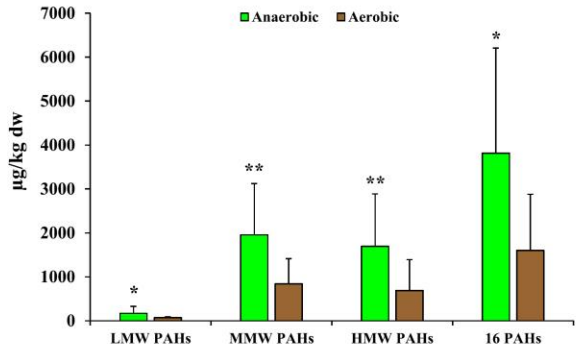


Fig. 3. Average content of LMW PAHs, MMW PAHs, HMW PAHs, and 16 PAHs in anaerobic digested (n = 27) and aerobic digested (n = 13) sewage sludge.
* indicates significant difference between the technologies ($P < 0.01$; PS *t*-test); ** indicates significant difference between the technologies ($P < 0.001$; PS *t*-test). Abbreviations: **LMW PAHs** (low molecular weight PAHs) = sum of naphthalene, acenaphthylene, acenaphthene, and fluorene; **MMW PAHs** (medium molecular weight PAHs) = sum of phenanthrene, anthracene, fluoranthene, and pyrene; **HMW PAHs** (high molecular weight PAHs) = sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene; **16 PAHs** (total sum PAHs) = sum of LMW, MMW, and HMW PAHs.

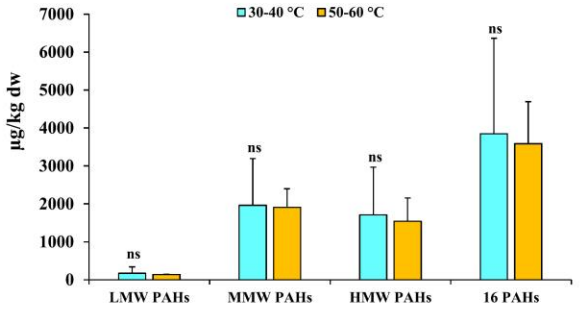


Fig. 5. Average content of LMW PAHs, MMW PAHs, HMW PAHs, and 16 PAHs in anaerobic digested sewage sludge treated by 30–40 °C (mesophilic; n = 24) and 50–60 °C (thermophilic; n = 3) temperatures.
ns denotes no significant difference between WWTP groups at $P = 0.05$. Abbreviations: **LMW PAHs** (low molecular weight PAHs) = sum of naphthalene, acenaphthylene, acenaphthene, and fluorene; **MMW PAHs** (medium molecular weight PAHs) = sum of phenanthrene, anthracene, fluoranthene, and pyrene; **HMW PAHs** (high molecular weight PAHs) = sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene; **16 PAHs** (total sum PAHs) = sum of LMW, MMW, and HMW PAHs.

from these treatments still remains ambiguous (Katsoyiannis and Samara, 2005). The content of individual PAHs, PCBs, and OCPs in sludges in relation to the biological stabilisation method of the digested sludge is evaluated in Figs. S4–S6. In many previous studies (Guo et al., 2009; Ning et al., 2014; Olofsson et al., 2013) analysing PAHs in sewage sludges from various WWTPs, the difference between the content of PAHs and PCBs in anaerobically/aerobically treated sludges was not obvious. In the present study, a significantly higher ($P < 0.05$–0.001; PS *t*-test or $P < 0.05$; M-W *U* test) amount of most of the investigated PAHs was found in sewage sludges stabilised under anaerobic conditions compared to the aerobically treated ones. The same was true for the 7 individual PCBs, except for congeners PCB 118 and PCB 180 (Fig. S5) as well as compounds 2,4′-DDE and 4,4′-DDE (Fig. S6).

As shown in Figs. 3 and 4, the use of anaerobic digestion to stabilise the sewage sludge led to the concentration of a significantly higher content ($P < 0.01/0.001$; PS *t*-test) of all PAH groups and the total sum of the 7 PCBs than in aerobically treated sludge. This could be caused by the fact that PAHs are generally formed from the incomplete combustion of fossil fuels and petroleum-based materials in households and plants (Zhang et al., 2012) and as a consequence of increased automobile exhaust and heavy industry (Suman et al., 2016) in very densely populated cities or urban areas where WWTPs with anaerobic stabilisation commonly prevail.

Furthermore, PCBs were used in many voltage converters, capacitors, and many other electrical devices (Iwegbue et al., 2022). Therefore, their residues in anaerobically treated sewage sludge can be expected as these sludges usually come from urban areas where medium or large WWTPs operate using anaerobic stabilisation more frequently for economic reasons. Another explanation was given by Komolafe et al. (2021), who recently published that PAHs are resistant to anaerobic degradation in the stabilisation segment of a WWTP with an up-flow anaerobic sludge blanket reactor, while the biotransformation of aromatics under aerobic conditions is possible. But in our study, this hypothesis could be rejected for our explanation as no relationship between pollutant concentration in sewage sludge and biological treatment represented by BOD was observed. Further, the opposite results were obtained in the case of selected OCPs (Fig. 4) as the content of HCH isomers was significantly increased ($P < 0.01$; M-W *U* test) in aerobically digested sludges. This could be explained by the fact that

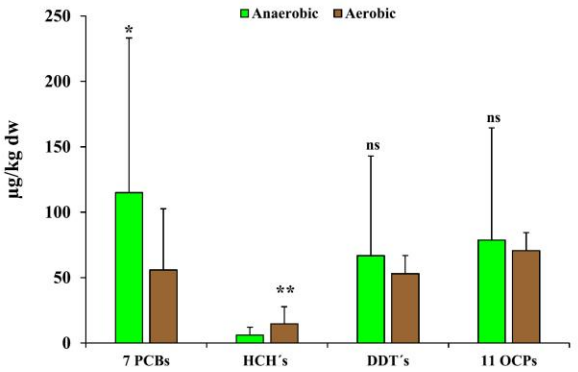


Fig. 4. Average content of 7 PCBs, HCH isomers, DDT congeners, and 11 OCPs in anaerobic digested (n = 27) and aerobic digested (n = 13) sewage sludge.
* indicates significant difference between the technologies ($P < 0.01$; PS *t*-test); ** indicates significant difference between the technologies ($P < 0.01$; M-W *U* test); **ns** denotes no significant difference between WWTP groups at $P = 0.05$. Abbreviations: **7 PCBs** = sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180; **HCHs** = sum of alpha, beta, gamma, and delta hexachlorocyclohexane isomers (HCH); **DDTs** = sum of 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 2,4′-DDT, 4,4′-DDD, and 4,4′-DDT congeners; **11 OCPs** = sum of HCH isomers, DDT isomers, and hexachlorobenzene (HCB).

these sludges usually came from small WWTPs, which mostly operate in agricultural areas. Therefore, we can speculate that these OCPs were used more often there in the past. Based on these findings, their higher abundance in stabilised sludge can be related to higher concentrations of analysed compounds in the wastewater-feeding WWTPs and urban/-rural WWTP environments rather than the type of sludge stabilisation.

Anaerobic digestion of the sludge of mesophilic temperature is used in WWTPs more commonly than the treatment of thermophilic temperature due to its lower process energy demand for reactor heating (Gebreeyessus and Jenicek, 2016). WWTPs with thermophilic anaerobic digestion of sludge are very rare in the Czech Republic; therefore, only three sewage sludge samples were investigated in this study. When we evaluated the mesophilic/thermophilic effect of anaerobic digestion on the concentration of individual PAHs, PCBs, and OCPs (Figs. S7–S9), including the sums of respective pollutants (Figs. 5 and 6), no

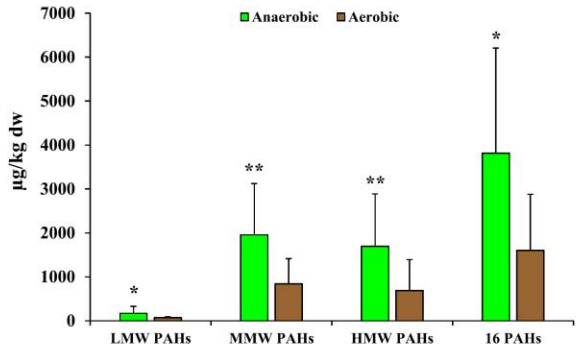


Fig. 3. Average content of LMW PAHs, MMW PAHs, HMW PAHs, and 16 PAHs in anaerobic digested (n = 27) and aerobic digested (n = 13) sewage sludge.
* indicates significant difference between the technologies ($P < 0.01$; PS *t*-test); ** indicates significant difference between the technologies ($P < 0.001$; PS *t*-test). Abbreviations: **LMW PAHs** (low molecular weight PAHs) = sum of naphthalene, acenaphthylene, acenaphthene, and fluorene; **MMW PAHs** (medium molecular weight PAHs) = sum of phenanthrene, anthracene, fluoranthene, and pyrene; **HMW PAHs** (high molecular weight PAHs) = sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene; **16 PAHs** (total sum PAHs) = sum of LMW, MMW, and HMW PAHs.

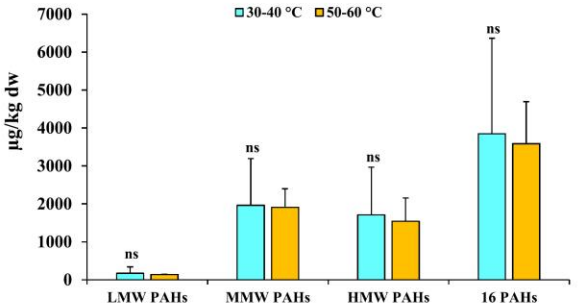


Fig. 5. Average content of LMW PAHs, MMW PAHs, HMW PAHs, and 16 PAHs in anaerobic digested sewage sludge treated by 30–40 °C (mesophilic; n = 24) and 50–60 °C (thermophilic; n = 3) temperatures.
ns denotes no significant difference between WWTP groups at $P = 0.05$. Abbreviations: **LMW PAHs** (low molecular weight PAHs) = sum of naphthalene, acenaphthylene, acenaphthene, and fluorene; **MMW PAHs** (medium molecular weight PAHs) = sum of phenanthrene, anthracene, fluoranthene, and pyrene; **HMW PAHs** (high molecular weight PAHs) = sum of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene; **16 PAHs** (total sum PAHs) = sum of LMW, MMW, and HMW PAHs.

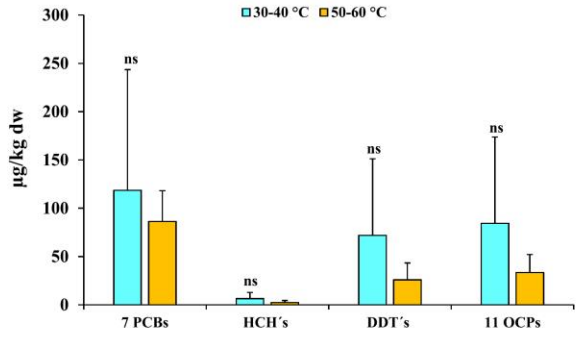


Fig. 6. Average content of 7 PCBs, HCH isomers, DDT congeners, and 11 OCPs in sewage sludge samples according to the temperature of anaerobic digestion technology.
ns denotes no significant difference between WWTP groups at $P = 0.05$. Abbreviations: **7 PCBs** = sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180; **HCHs** = sum of alpha, beta, gamma, and delta hexachlorocyclohexane isomers (HCH); **DDTs** = sum of 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 2,4′-DDT, 4,4′-DDD, and 4,4′-DDT congeners; **11 OCPs** = sum of HCH isomers, DDT isomers, and hexachlorobenzene (HCB).

significantly different ($P > 0.05$) results were obtained. We analysed a small scale of samples in order to have a robust idea if this could imply that, under anaerobic conditions, the temperature variable does not influence the degradation of POPs during the treatment process.

4. Conclusions

The actual contents of 16 PAHs, 7 PCBs, and 11 OCPs in sewage sludge from 40 different WWTPs in the Czech Republic were determined. The average content of OCPs, PCBs and PAHs in sludge was 76.1, 95.7, and 3096 µg/kg dry weight, respectively. Among the tested pollutants, moderate and strong correlations in sludge ($r = 0.40–0.76$) were found, and a linear correlation between WWTP size and pollutant contents in sludge with growing WWTP capacity was evident. The effect of current WWTPs technological parameters and stabilisation technology of sewage sludge (design capacity, wastewater load and hydraulic retention, biochemical and chemical removal efficiencies, sludge retention time and digestion technology) on the content of 34 POPs in sewage sludge was investigated. When evaluating if the contents can be influenced by the common WWTP parameters, no relationship was found between total pollutant contents. Nevertheless, some individual pollutants, such as anthracene and PCB 52, correlated significantly ($P < 0.05$) with biochemical oxygen demand ($r = -0.35$) and chemical oxygen demand removal efficiencies ($r = -0.35$). We observed a statistically higher content of total PAHs and PCBs in anaerobically digested sludges ($P < 0.05$) compared to aerobically digested ones. The effect of mesophilic/thermophilic anaerobic digestion on the content of individual PAHs, PCBs, and OCPs was not obvious, as the thermophilic anaerobic digestion technology of sewage sludge is very rare in the Czech Republic.

Credit author statement

Zdeněk Košnář: Investigation, Methodology, Validation, Formal analysis, Conceptualization, Writing - Original Draft. **Filip Mercl**: Methodology, Validation, Writing - Review & Editing. **Lorenzo Pierdonà**: Formal analysis. **Abraham Demelash Chane**: Formal analysis. **Pavel Míchal**: Methodology, Formal analysis. **Pavel Tlustoš**: Writing - Review & Editing, Supervision, Resources, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by the Ministry of Agriculture of the Czech Republic (QK1710379 and QK21010207) and European Regional Development Fund (project NUTRISK no. CZ.02.1.01/0.0/0.0/16_019/0000845).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122060.

References

Aemig, Q., Chéron, C., Delgenès, N., Jimenez, J., Houot, S., Steyer, J.P., Patureau, D., 2006. Distribution of polycyclic aromatic hydrocarbons (PAHs) in sludge organic matter pools as a driving force of their fate during anaerobic digestion. Waste Manage. (Tucson, Ariz.) 48, 389–396. https://doi.org/10.1016/j.wasman.2015.11.045.

Aparicio, I., Santos, J.L., Alonso, E., 2009. Limitation of the concentration of organic pollutants in sewage sludge for agricultural purposes: a case study in south Spain. Waste Manag. 29 (5), 1747–1753. https://doi.org/10.1016/j.wasman.2008.11.003.

Blanchard, M., Teil, M.J., Ollivon, D., Legenti, L., Chevreuil, M., 2004. Polycyclic aromatic hydrocarbons and polychlorobiphenyls in waste waters and sewage sludges from the Paris area (France). Environ. Res. 95, 184–197. https://doi.org/10.1016/j.envres.2003.07.003.

Bonett, D.G., Wright, T.A., 2000. Sample size requirements for estimating Pearson, Kendall and Spearman correlations. Psychometrika 65 (1), 23–28. https://doi.org/10.1007/BF02294183.

Bright, D.A., Healey, N., 2003. Contaminant risks from biosolids land application: contemporary organic contaminant levels in digested sewage sludge from five treatment plants in Greater Vancouver, British Columbia. Environ. Pollut. 126 (1), 39–49. https://doi.org/10.1016/S0269-7491(03)00148-9.

Cai, Q.Y., Mo, C.H., Wu, Q.T., Zeng, Q.Y., Katsoyiannis, A., 2007. Occurrence of organic contaminants in sewage sludges from eleven wastewater treatment plants, China. Chemosphere 68 (9), 1751–1762. https://doi.org/10.1016/j.chemosphere.2007.03.041.

Dai, J., Xu, M., Chen, J., Yang, X., Ke, Z., 2007. PCDD/F, PAH and heavy metals in the sewage sludge from six wastewater treatment plants in Beijing, China. Chemosphere 66 (2), 353–361. https://doi.org/10.1016/j.chemosphere.2006.04.072.

Derco, J., Dudáš, J., Valičková, M., Šimovičová, K., Kecskés, J., 2015. Removal of micropollutants by ozone based processes. Chem. Eng. Process 94, 78–84. https://doi.org/10.1016/j.cep.2015.03.014.

Dijk, K.C.V., Lesschen, J.P., Oenema, O., 2016. Phosphorus flows and balances of the European union member states. Sci. Total Environ. 542, 1078–1093. https://doi.org/10.1016/j.scitotenv.2015.08.048.

Dubey, M., Mohapatra, S., Tyagi, V.T., Suthar, S., Kazmi, A.A., 2021. Occurrence, fate, and persistence of emerging micropollutants in sewage sludge treatment. Environ. Pollut. 273, 116515 https://doi.org/10.1016/j.envpol.2021.116515.

Evans, J.D., 1996. Straightforward Statistics for the Behavioral Sciences. Brooks/Cole Publishing Co, United States, p. 634. Pacific Grove, -13: 978-0534231002.

Feng, S., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Cheng, D., Varjani, S., Lei, Z., Liu, Y., 2021. Roles and applications of enzymes for resistant pollutants removal in wastewater treatment. Bioresour. Technol. 335, 125278 https://doi.org/10.1016/j.biortech.2021.125278.

Fijalkowski, K., Rorat, A., Grobelak, A., Kacprzak, M.J., 2017. The presence of contaminations in sewage sludge – the current situation. J. Environ. Manag. 203, 1126–1136. https://doi.org/10.1016/j.jenvman.2017.05.068.

Gebreeyessus, G.D., Jenicek, P., 2016. Thermophilic versus mesophilic anaerobic digestion of sewage sludge: a comparative review. Bioengineering 3 (2), 15. https://doi.org/10.3390/bioengineering3020015.

Goberna, M., Simón, P., Hernández, M.T., García, C., 2018. Prokaryotic communities and potential pathogens in sewage sludge: response to wastewaster origin, loading rate and treatment technology. Sci. Total Environ. 615, 360–368. https://doi.org/10.1016/j.scitotenv.2017.09.240.

Guo, L., Zhang, B., Xiao, K., Zhang, Q., Zheng, M., 2009. Levels and distributions of polychlorinated biphenyls in sewage sludge of urban wastewater treatment plants. J. Environ. Sci. 21 (4), 468–773. https://doi.org/10.1016/s1001-0742(08)62293-7.

Harrison, E.Z., Oakes, S.R., Hysell, M., Hay, A., 2006. Organic chemicals in sewage sludges. Sci. Total Environ. 367 (2–3), 481–497. https://doi.org/10.1016/j.scitotenv.2006.04.002.

Hua, L., Wu, W.X., Liu, Y.X., Tientchen, C.M., Chen, Y.X., 2008. Heavy metals and PAHs in sewage sludge from twelve wastewater treatment plants in Zhejiang province. Biomed. Environ. Sci. 21 (4), 345–352. https://doi.org/10.1016/S0895-3988(08)60053-7.

Ighalo, J.O., Yap, P.S., Iwuozor, K.O., Aniagor, C.O., Liu, T., Dulta, K., Iwuchukwu, F.U., Rangabhashiyam, S., 2022. Adsorption of persistent organic pollutants (POPs) from the aqueous environment by nano-adsorbents: a review. Environ. Res. 212, 113123 https://doi.org/10.1016/j.envres.2022.113123.

Iwegbue, C.M.A., Oshenyen, V.E., Tesi, G.O., Olisah, C., Nwajei, G.E., Martincigh, B.S., 2022. Occurrence and spatial characteristics of polychlorinated biphenyls (PCBs) in sediments from rivers in the western Niger delta of Nigeria impacted by urban and industrial activities. Chemosphere, 132671. https://doi.org/10.1016/j.chemosphere.2021.132671.

Jaspers, V., Megson, D., O'Sullivand, G., 2014. Chapter 7 - POPs in the terrestrial environment. In: O'Sullivand, G., Sandau, S. (Eds.), Environmental Forensics for Persistent Organic Pollutants. Elsevier, p. 66. https://doi.org/10.1016/C2011-0-04340-5.

Jones, V., Gardner, M., Ellor, B., 2014. Concentrations of trace substances in sewage sludge from 28 wastewater treatment works in the UK. Chemosphere 111, 478–484. https://doi.org/10.1016/j.chemosphere.2014.04.025.

Katsoyiannis, A., Samara, C., 2005. Persistent organic pollutants (POPs) in the conventional activated sludge treatment process: fate and mass balance. Environ. Res. 97 (3), 245–257. https://doi.org/10.1016/j.envres.2004.09.001.

Komolafe, O., Mrozik, W., Dolfing, J., Acharya, K., Vassalle, L., Mota, C.R., Davenport, R., 2021. Occurrence and removal of micropollutants in full-scale aerobic, anaerobic and facultative wastewater treatment plants in Brazil. J. Environ. Manag. 287, 112286 https://doi.org/10.1016/j.jenvman.2021.112286.

Košnář, Z., Mercl, F., Chane, A.D., Pierdonà, L., Míchal, P., Tlustoš, P., 2021. Occurrence of synthetic polycyclic and nitro musk compounds in sewage sludge from municipal wastewater treatment plants. Sci. Total Environ. 801, 149777 https://doi.org/10.1016/j.scitotenv.2021.149777.

Mailler, R., Gasperi, J., Chebbo, G., Rocher, V., 2014. Priority and emerging pollutants in sewage sludge and fate during sludge treatment. Waste Manage. (Tucson, Ariz.) 34 (7), 1217–1226. https://doi.org/10.1016/j.wasman.2014.03.028.

Man, Y.B., Chow, K.L., Cheng, Z., Mo, W.Y., Chan, Y.H., Lam, J.C.W., Lau, F.T.K., Fung, W.C., Wong, M.H., 2017. Profiles and removal efficiency of polycyclic aromatic hydrocarbons by two different types of sewage treatment plants in Hong Kong. J. Environ. Sci. 53, 196–206. https://doi.org/10.1016/j.jes.2016.04.020.

Nas, B., Argun, M.E., Dolu, T., Ates, H., Yel, E., Koyuncu, S., Dinç, S., Kara, M., 2020. Occurrence, loadings and removal of EU-priority polycyclic aromatic hydrocarbons (PAHs) in wastewater and sludge by advanced biological treatment, stabilization pond and constructed wetland. J. Environ. Manag. 268, 110580 https://doi.org/10.1016/j.jenvman.2020.110580.

Ning, X.A., Lin, M.Q., Shen, L.Z., Zhang, J.H., Wang, J.Y., Wang, Y.J., Yang, Z.Y., Liu, J. Y., 2014. Levels, composition profiles and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in sludge from ten textile dyeing plants. Environ. Res. 132, 112–118. https://doi.org/10.1016/j.envres.2014.03.041.

Olofsson, U., Brorström-Lundén, E., Kylin, H., Haglund, P., 2013. Comprehensive mass flow analysis of Swedish sludge contaminants. Chemosphere 90, 28–35. https://doi.org/10.1016/j.chemosphere.2012.07.002.

Passuello, A., Montse, M., Nadal, M., Schuhmacher, M., Domingo, J.L., 2010. POP accumulation in the food chain: integrated risk model for sewage sludge application in agricultural soils. Environ. Int. 36 (6), 577–583. https://doi.org/10.1016/j.envint.2010.04.015.

Peréz, S., Farré, M., García, M.J., Barceló, D., 2001. Occurrence of polycyclic aromatic hydrocarbons in sewage sludge and their contribution to its toxicity in the ToxAlert® 100 bioassay. Chemosphere 45 (6–7), 705–712. https://doi.org/10.1016/S0045-6535(01)00152-7.

Public Notice No. 437/2016, 2016. Quality requirements of biosolids for the use on agricultural land of 2016. Publ. Law 437, 2016. https://eagri.cz/public/web/mze/legislativa/ostatni/Legislativa-ostatni_uplna-zneni_vyhlaska-2016-437.html.

Roig, N., Jordi Sierra, J., Nadal, M., Martí, E., Navalón-Madrigal, P., Schuhmacher, M., Domingo, J.L., 2012. Relationship between pollutant content and ecotoxicity of sewage sludges from Spanish wastewater treatment plants. Sci. Total Environ. 425, 99–109. https://doi.org/10.1016/j.scitotenv.2012.03.018.

Samuel, O., Othman, M.D.H., Kamaludin, R., Kurniawan, T.A., Li, T., Dzinun, H., Imtiaz, A., 2022. Treatment of oily wastewater using photocatalytic membrane reactors: a critical review. J. Environ. Chem. Eng. 10, 108539 https://doi.org/10.1016/j.jece.2022.108539.

Scheringer, M., Strempel, S., Hukari, S., Ng, C.A., Blepp, M., Hungerbuhler, K., 2012. How many persistent organic pollutants should we expect? Atmos. Pollut. Res. 3, 383–391. https://doi.org/10.5094/APR.2012.044.

Shen, R.J., Luo, Y.M., Hang, G.Y., Teng, Y., Li, Z.G., Wu, L.H., 2007. Contamination of PAHs in sludge samples from the Yangtze river delta area. Pedosphere 17 (3), 373–382. https://doi.org/10.1016/S1002-0160(07)60045-9.

Singh, R.P., Agrawal, M., 2008. Potential benefits and risks of land application of sewage sludge. Waste Manag. 28 (2), 347–358. https://doi.org/10.1016/j.wasman.2006.12.010.

Stasinakis, S.S., 2012. Review on the fate of emerging contaminants during sludge anaerobic digestion. Bioresour. Technol. 121, 432–440. https://doi.org/10.1016/j.biortech.2012.06.074.

Stevens, J.L., Northcott, G.L., Stern, G.A., Tomy, G.T., Jones, K.C., 2003. PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks, and polychlorinated n-alkanes in U.K. sewage sludge: survey results and implications. Environ. Sci. Technol. 37, 462–467. https://doi.org/10.1021/es020161y.

Suciu, N.A., Lamastra, L., Trevisan, M., 2015. PAHs content of sewage sludge in Europe and its use as soil fertilizer. Waste Manage. (Tucson, Ariz.) 41, 119–127. https://doi.org/10.1016/j.wasman.2015.03.018.

Suman, S., Sinha, A., Tarafdar, A., 2016. Polycyclic aromatic hydrocarbons (PAHs) concentration levels, pattern, source identification and soil toxicity assessment in urban traffic soil of Dhanbad, India. Sci. Total Environ. 545–546, 353–360. https://doi.org/10.1016/j.scitotenv.2015.12.061.

Sun, S.J., Zhao, Z.B., Li, B., Ma, L.X., Fu, D.L., Sun, X.Z., Thapa, S., Shen, J.M., Qi, H., Wu, Y.N., 2019. Occurrence, composition profiles and risk assessment of polycyclic aromatic hydrocarbons in municipal sewage sludge in China. Environ. Pollut. 245, 764–770. https://doi.org/10.1016/j.envpol.2018.11.067.

United States Environmental Protection Agency (US EPA). Learn about polychlorinated biphenyls (PCBs). Last updated on June 5, 2022. (Accessed 18 July 2022). https://www.epa.gov/pcbs/learn-about-polychlorinated-biphenyls-pcbs.

Vácha, R., Horváthová, V., Vysloužilová, M., 2005. The application of sludge on agriculturally used soils and the problem of persistent organic pollutants. Plant Soil Environ. 51 (1), 12–18. https://doi.org/10.17221/3550-PSE.

Vácha, R., Vysloužilová, M., Horváthová, V., Čechmánková, J., 2006. Recommended maximum contents of persistent organic pollutants in sewage sludge for application on agricultural soils. Plant Soil Environ. 52 (8), 362–367. https://doi.org/10.17221/3453-PSE.

Vácha, R., Horváthová, V., Vysloužilová, M., Čechmánková, J., 2007. Problem of persistent organic pollutants in sludge for application in agricultural soil. Chem. Listy 101 (10), 811–815. http://www.chemicke-listy.cz/ojs3/index.php/chemicke-listy/article/view/1738/1738.

Wang, X., Xi, B., Huo, S., Sun, W., Pan, H., Zhang, J., Ren, Y., Liu, H., 2013. Characterization, treatment and releases of PBDEs and PAHs in a typical municipal sewage treatment plant situated beside an urban river, East China. J. Environ. Sci. 25 (7), 1281–1290. https://doi.org/10.1016/S1001-0742(12)60201-0.

Wiśniowska, E., Grobelak, A., Kokot, P., Kacprzak, M., 2019. Sludge legislation-comparison between different countries. In: Prasad, et al. (Eds.), Industrial and Municipal Sludge. Butterworth-Heinemann, p. 24. https://doi.org/10.1016/B978-0-12-815907-1.00010-6.

Włodarczyk-Makuła, M., 2012. Half-life of carcinogenic polycyclic aromatic hydrocarbons in stored sewage sludge. Arch. Environ. Protect. 38, 33–44. https://doi.org/10.2478/v10265-012-0016-6.

Wluka, A.K., Huang, Y., Coenen, L., Dsikowitzky, L., Schwarzbauer, J., 2021. Structural diversity of organic contaminants in sewage sludge: a comparison of sewage fingerprints from Germany and China. Discover Water 1, 4. https://doi.org/10.1007/s43832-021-00004-4.

Xu, W., Wang, X., Caia, Z., 2013. Analytical chemistry of the persistent organic pollutants identified in the Stockholm Convention: a review. Anal. Chim. Acta 790, 1–13. https://doi.org/10.1016/j.aca.2013.04.026.

Zhang, L.F., Dong, L., Ren, L.J., Shi, S.X., Zhou, L., Zhang, T., Huang, Y., 2012. Concentration and source identification of polycyclic aromatic hydrocarbons and phthalic acid esters in the surface water of the Yangtze River Delta, China. J. Environ. Sci. 24 (2), 335–342. https://doi.org/10.1016/s1001-0742(11)60782-1.

4.2. Košnář et al. (2021). Occurrence of synthetic polycyclic and nitro musk compounds in sewage sludge from municipal wastewater treatment plants

Authors: Zdeněk Košnář, Filip Mercl, **Abraham Demelash Chane**, Lorenzo Pierdonà, Pavel Míchal, Pavel Tlustoš

Published in the journal: Science of The Total Environment, 801, 149777
Information about the magazine: IF = 10.75 (2021)

Science of the Total Environment 801 (2021) 149777

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Occurrence of synthetic polycyclic and nitro musk compounds in sewage sludge from municipal wastewater treatment plants

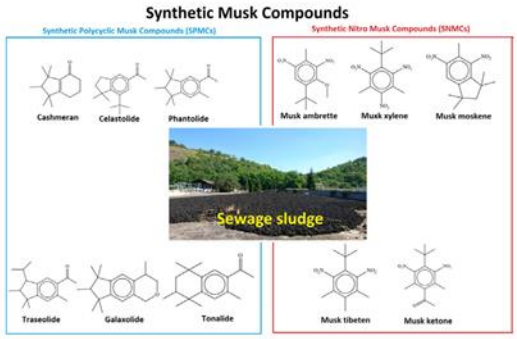
Zdeněk Košnář *, Filip Mercl, Abraham Demelash Chane, Lorenzo Pierdonà, Pavel Míchal, Pavel Tlustoš

Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 165 00, Praha, Suchdol, Czech Republic

HIGHLIGHTS

- Average content of total musk compounds in sewage sludge was 5518 μg/kg dw.
- Six polycyclic musks accounted for 99.5%, remaining five nitro musks were negligible.
- Tonalide correlated with WWTP design capacity significantly ($r = 0.32$, $P < 0.05$).
- Musk contents in sewage sludge did not change over time, with a gap of two years.
- Current WWTP technology has limited effect on musk accumulation in sewage sludge.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 May 2021
Received in revised form 3 August 2021
Accepted 16 August 2021
Available online 20 August 2021

Editor: Dimitra A Lambropoulou

Keywords:
Musks
Organic pollutants
Personal care products
Screening
Biosolids

ABSTRACT

Synthetic musk compounds (SMCs) are widely used as fragrances that can be released from different sources and through the sewer system, finally reaching wastewater treatment plants (WWTPs). In this study, 6 synthetic polycyclic and 5 nitro musk compounds were screened in 55 sewage sludge (SS) samples from 43 different WWTPs in the Czech Republic, and the effect of WWTP technology parameters on SMC content in SS was assessed. Galaxolide and Tonalide were predominant synthetic polycyclic musk compounds (SPMCs) detected in all SS tested and accounted for 99.5% of the average content of sludge SMCs (5518 μg/kg dw). The amount of synthetic nitro musk compounds (SNMCs) in SS samples was negligible. The Tonalide content in SS correlated significantly with the WWTP design capacity ($r = 0.32$, $P < 0.05$). The significant correlation between chemical oxygen demand (COD) removal efficiency and SMCs ($r = -0.37$, $P < 0.05$) partly suggests the recalcitrance of SMCs, mainly of Celestolide, Galaxolide and Tonalide, to biodegradation in WWTPs. A statistically lower SNMC content was found in anaerobically digested sludges than in aerobic ones. There was no significant difference ($P > 0.05$) between the digestion technology as well as the temperature of anaerobic digestion on the SPMC content in sewage sludge. The wastewater (WW) load percentage or WW hydraulic retention time had no influence on the SMC content in the resulting SS. Musk compounds did not change over time when the SS samples were analysed with a gap of two years, suggesting that sewage sludge for soil applications only needs to be analysed for musk compounds once a year. Our study indicates that the currently common WWTP technologies have only very limited potential to affect the accumulation of musk compounds in sewage sludge.

* Corresponding author.
E-mail address: kosnarz@af.czu.cz (Z. Košnář).

https://doi.org/10.1016/j.scitotenv.2021.149777
0048-9697/

1. Introduction

Synthetic musk compounds (SMCs) are semi-volatile cyclic organic chemicals, which are widely used as inexpensive fragrances in washing and cleaning agents, air fresheners, and fixatives in personal care products, including deodorants, cosmetics, and shampoos (Lee et al., 2014; Wong et al., 2019).

Musk compounds can be mainly divided into synthetic polycyclic musk compounds (SPMCs) and synthetic nitro musk compounds (SNMCs) according to their chemical structures. Polycyclic musks comprise acetylated and highly methylated pyran, tetralin, and indane compounds, while nitro musks are alkylated nitro benzenes with a typical musk odour (Liu et al., 2014; Marchal and Beltran, 2016). Galaxolide and Tonalide are the most frequent individual musk compounds among all polycyclic musks, whereas Musk Ketone and Musk Xylene are the most abundant nitro musks (Lignell et al., 2008). It is assumed that SPMCs represent over 90% of the total SMCs consumed in the European and US markets (Lee et al., 2014).

Synthetic musks are highly persistent compounds that may bioaccumulate in water, soil, and the food chain and, therefore, may pose a risk to the environment. The lipophilicity of SMCs indicates a high bioaccumulation potential that concerns not only the environment, but also impacts food safety and, consequently, public health and other species (Cavalheiro et al., 2013; Zeng et al., 2005). Musk compounds are abundant, persistent, poorly biodegradable, and some of them are toxicologically active compounds (Llompart et al., 2003; Shek et al., 2008). Recent studies have shown that SMCs may cause harmful health effects, such as endocrine disruption through anti-estrogenic activity (Wong et al., 2019).

In general, human use and application is the main source of SMCs in the environment. After use, about 77% of SMCs are drained into the sewer system and reach wastewater treatment plants (WWTPs) (Reiner et al., 2007). They enter the wastewater stream by direct release from private households and industry or by aerial deposition on impervious surfaces and runoff into the sewer system. Due to their widespread use, persistence, and low polarity, SMCs have been detected in most environmental matrices in relatively high amounts, including wastewater (Lee et al., 2003), soil (Biel-Maeso et al., 2019; Yang and Metcalfe, 2006), sewage sludge (Sun et al., 2014; Vallecillos et al., 2012), air (Balci et al., 2020; Fontal et al., 2016), fresh water and coastal sediments (Lee et al., 2014; Zhang et al., 2020), and even in crops (Ramos et al., 2019) or human breast milk (Yin et al., 2016).

Due to a low polarity and high octanol-water distribution coefficients (log K_{ow}), SMCs preferentially adsorb onto sewage sludge. The accumulation in sludge is a result of the sorption potential of individual polycyclic musk compounds, as their log K_{ow} is high (4.9–5.4), which means a lipophilic and strong sorption potential (Balk and Ford, 1999; Artola-Garicano et al., 2003; Ternes et al., 2004). The same is true for nitro musks, as they have a comparable log K_{ow} with values ranging from 4.3–5.8 (Paasivirta et al., 2002; Wong et al., 2019). Values of log K_d (solid-liquid distribution coefficient) for Galaxolide and Tonalide range from 3.5–4.3 and 3.7–4.4, respectively, suggesting that musk fragrances are highly sorbed on sewage sludge particles and dissolved only in low amounts (Yang and Metcalfe, 2006). Sewage sludge containing SMCs has, then, a potential to harm biota, including human beings (Hanssen et al., 2013; Liu et al., 2021; Zeng et al., 2005). A study by Balk and Ford (1999) on earthworms (*Eisenia fetida*) and springtails (*Folsomia candida*) predicted no observed effect concentration of the target substances on organisms based on the terrestrial toxicity data with the value of 0.32 mg/kg dw for Galaxolide and Tonalide. According to German proposals, limits of Galaxolide and Tonalide of 10 mg/kg dw and 15 mg/kg dw, respectively, were set for sewage sludge as a soil amendment (Clarke and Smith, 2011). Therefore, sewage sludge should be screened for its SMC content before its use in soil applications.

This study hypothesizes that musk compounds are accumulated in sewage sludge, and their amount can be influenced by the WWTP technology that is used. Therefore, the aim of this study was to investigate the actual contents of synthetic polycyclic and nitro musk compounds in sewage sludge and to elucidate the effect of WWTP technological parameters on the amount of synthetic musk compounds in the resulting sewage sludge.

2. Material and methods

2.1. WWTPs description

In total, 55 samples of sewage sludge were collected from 43 different WWTPs located in the Czech Republic during the years 2017–2019. In order to study potential changes in SMC content over time, samples from 11 WWTPs were collected twice with a gap of two years and one WWTP was screened three times (once a year). The range of WWTP design capacity expressed by population equivalent (PE) was operationally classified into three groups, namely small, medium, and large WWTPs with a WWTP design capacity of <50,000 ($n = 27$), 50,000–100,000 ($n = 17$), and >100,000 ($n = 11$), respectively. Such groups were chosen because anaerobic digestion is usually not used in small WWTPs due to economic reasons, so the current groups better reflect the size of a municipality than technological parameters of a WWTP. In order to elucidate the effect of technological parameters of WWTP on the SMC content in sewage sludge, WWTPs were further divided based on the type of sewage sludge treatment, aerobic digestion and anaerobic digestion. The anaerobic digestion group was further divided into two sub-classes, mesophilic and thermophilic, based on the temperature used for the digestion. For each WWTP, several other technological parameters were obtained from the WWTP operators. Investigated parameters were: average wastewater load expressed as a percentage from the WWTP design capacity, average hydraulic retention time of wastewater in WWTP, efficiency of biochemical oxygen demand (BOD) removal, efficiency of chemical oxygen demand (COD) removal, and average hydraulic retention time of sludge in the stabilization segment of WWTP. A detailed description of individual WWTPs is given in Table S1. Most of the WWTP operators provided the sewage sludge samples and technological parameters only under the condition of anonymity, so the exact locations of individual WWTPs cannot be given.

2.2. Sewage sludge sample collection and analysis

From each WWTP, approximately 10 kg of fresh stabilized sewage sludge was collected as a result of at least 10 random subsamples thoroughly mixed. Raw sludge samples were transported immediately after collection at 4 °C to the laboratory for further analyses. In the laboratory, samples were freeze-dried and milled to a fine powder prior to the analyses. Determination of initial water content in the samples was measured gravimetrically as a weight difference before and after freeze-drying. The pH value of dry sewage sludge was determined in the supernatant after 2 h extraction in 0.01 M CaCl2 (1/5; w/v). Electrical conductivity was measured in the supernatant after 1 h extraction in demineralized water (1/10; w/v). Ash content of the samples was determined gravimetrically after burning the samples at 550 °C for 4 h. Total contents of C, H, N, and S in the samples were determined using an elemental analyser (CHNS Vario MACRO cube, Elementar Analysensysteme V3.1.1, Hanau, Germany) (Table S2). The content of O was calculated according to Wang et al. (2013) as follows: $O\ \% = 100\% - (C + H + N + S + \text{ash}, \%)$. Molar atomic ratios (H/C, O/C, C/N) and ((O + N)/C) were calculated on a moist- and ash-free basis.

2.3. Synthetic musk compounds

In this study, 11 selected synthetic musk compounds were analysed (Table S3). Six were polycyclic musks: Cashmeran® (DPMI; 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)indanone), Celestolide® (ADBI; 4-

acetyl-1,1-dimethyl-6-tert-butyldihydroindene), Phantolide® (AHDI; 6-acetyl-1,1,2,3,3,5-hexamethyl-dihydroindene), Traseolide® (ATII; 5-acetyl-1,1,2,6-tetramethyl-3-isopropyl-dihydroindene), Galaxolide® (HHCB; 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyrane), and Tonalide® (AHTN; 7-acetyl-1,1,3,4,4,6-hexamethyl-tetrahydronaphthalene), while the other 5 were nitro musks: Musk Ambrette (MA; 2,6-dinitro-3-methoxy-4-tert-butyl toluene), Musk Xylene (MX; 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene), Musk Moskene (MM: 4,6-dinitro-1,1,3,3,5-pentamethylindane), Musk Tibeten (MT: 1-tert-butyl-2,6-dinitro-3,4,5-trimethylbenzene), and Musk Ketone (MK; 1-tert-butyl-3,5-dimethyl-2,6-dinitro-4-acetylbenzene).

2.4. Musk compound extraction

Musk compounds were extracted from the sewage sludge according to a modified method previously described by Clara et al. (2011). Briefly, 0.5 g of freeze-dried sludge sample in a 50 mL glass centrifuge tube was spiked with 1 mL of *p*-terphenyl-d_{14} (B/N surrogate mix, Restek, Bellefonte, USA) at a concentration of 500 ng/mL in hexane (95%, for GC/ECD residue analysis, Chromservis, Praha, Czech Republic) as a surrogate standard (Table S4) to get the extraction efficiency. 10 mL of ethanol (98%, p.a., Praha, Chromservis, Czech Republic), 10 mL of sodium acetate (Lachner, Neratovice, Czech Republic) buffer (pH 3.4), and 10 mL of hexane were added one after another. Samples were then put into a rotary shaker (GFL 3017, DKSH, Zürich, Switzerland) for 30 min at 150 rpm and ultrasonicated for 30 min at 35 °C (Bandelin Sonorex Digitec DT510H, Berlin, Germany). Samples were centrifuged (Rotina 420R, Hettich, Tuttlingen, Germany) for 10 min at 2500 rpm (~1328 × g) and 20 °C. The hexane layer was collected at the top and filtered into another glass tube for evaporation under a gentle stream of nitrogen. The samples were then reconstituted in 2 mL of hexane and purified on a solid phase extraction (SPE) cartridge (Strata SI – silica 1 g, Phenomenex, Torrance, California, USA). The silica gel was activated with 10 mL of hexane. After the activation, the dissolved samples were passed through the column and eluted with 10 mL of hexane-ethyl acetate mixture (9:1, v/v) (Chromatopur G, Chromservis, Praha, Czech Republic). Sample extracts were again evaporated to dryness under a gentle stream of nitrogen and reconstituted with 2 mL of hexane. Finally, 900 μL of the extracted sample was mixed with 100 μL of internal standard mix consisting of Tonalide-d_3 (Neochema, Bodenheim, Germany), Musk Xylene-d_{15} (LGC Standards, Middlesex, UK), and PCB 116-d_5 (Neochema, Bodenheim, Germany) in hexane (200 ng/mL of each) in a 2 mL amber vial. Tonalide-d_3 and Musk Xylene-d_{15} (Table S5) were used as the internal standard for SPMCs and SNMCs, respectively. PCB 116-d_5 was used as the internal standard for *p*-terphenyl-d_{14}.

2.5. Instrumental analysis

The chromatographic analysis of individual musk analytes was performed on a gas chromatograph equipped with a mass spectrometer detector with triple quadrupole using an electron-impact ionization source (7000D GC/MS/MS, Agilent Technologies, Santa Clara, USA) and PAL RTC auto sampler system (CTC Analytics AG, Zwingen, Switzerland). The separation of analytes was carried out using a chromatography capillary column (length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm, Restek-XLB, Bellefonte, USA). Injection conditions were set in the splitless mode with an injection volume of 2 μL, helium flow of 1.0 mL/min, pressure of 21.9 psi, and purge flow of 50 mL/min at 1 min. The helium carrier gas (6.0 purity) flowed at a velocity of 17.1 cm/s. The temperature program of the oven was initially held at 60 °C for 1 min, then raised to 220 °C/min at a rate of 40 °C/min, then to 320 °C at a rate of 10 °C/min, and held for 1 min at a final temperature of 320 °C. The mass spectrometer was operated in a dynamic multiple reaction monitoring mode. MS/MS transitions and collision energies of each compound are shown in Table S6. A musk mix standard solution (Neochema, Bodenheim, Germany) in hexane was used for the eight-point (0.5, 1, 5, 10, 50, 100, 500, and 1000 ng/mL) calibration of individual 11 musk compounds, with $R^2 > 0.995$ for all musk compounds. The method limit of detection (MLOD) of individual musks was in the range 0.5–1.0 ng/mL, corresponding to 0.4–2 μg/kg dw (Table S7). Fresh calibration blanks and quality control of surrogates at 500 ng/mL were also prepared alongside with each sample batch. All 11 musk compounds were below MLOD in the blanks. Differences in surrogate standard check were lower than 10%. The precision of the method was verified by the recovery of each compound of the spiked blank and spiked sewage sludge samples (Table S7). Obtained results were corrected for the extraction efficiency for each sample. The average recovery of *p*-terphenyl-d_{14} was 90.2%.

2.6. Statistics and data evaluation

In this study, SPMCs, SNMCs, and SMCs denote the sum of 6 synthetic polycyclic musk compounds, the sum of 5 synthetic nitro musk compounds, and the sum of all 11 synthetic musk compounds, respectively. Data were checked for normal distribution (Shapiro-Wilk test) and were tested on equal variance. *t*-test and one-way analysis of variance (ANOVA) followed by Tukey's HSD test were done to compare if the treatments were significantly different ($P < 0.05$) in cases where the data followed the assumptions. In case of not normally distributed data, Mann-Whitney Rank Sum (MWR) test and Kruscal-Wallis (KW) test were applied to compare two or more groups, respectively. Pearson correlation analysis was performed to identify the influence of selected technological parameters of WWTPs on the content of SMCs found in the samples. Strength of the correlation was evaluated according to Evans (1996) as follows: 0.00–0.19 very weak, 0.20–0.39 weak, 0.40–0.59 moderate, 0.60–0.79 strong, and 0.80–1.00 very strong. For statistical purposes, contents below MLOD were replaced by one-half of the MLOD (Antweiler and Taylor, 2008). Statistical analyses and figures were carried out using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA).

3. Results and discussion

3.1. Occurrence of synthetic musk compounds in sewage sludge

Synthetic polycyclic musk compounds (SPMCs) were found at a higher frequency and content than synthetic nitro musk compounds (SNMCs) in all WWTPs sludges, as shown in Table 1. Individual musk compounds of Galaxolide and Tonalide were found in all 55 sewage sludge samples. Except of two sewage sludge samples, Celastolide, Phantolide, and Traseolide were also detected in all samples. The Cashmeran content was below MLOD (0.4 μg/kg dw) in all investigated sewage sludge samples, which was similar to results of Vallecillos et al. (2012) who reported that Cashmeran was found in only one sewage sample (n = 8), with a content of 90.8 μg/kg dw. Galaxolide and Tonalide were the most predominant individual musk compounds in our samples, which constituted about 78.2% and 20.4% of the average total content of SMCs (5518 μg/kg dw), respectively. Previous reports also showed that these two compounds were the most abundant (Zeng et al., 2005; Shek et al., 2008; Lv et al. (2010); Guo et al., 2010; Hu et al., 2011; Ramos et al., 2019). As presented in Table 1, the content of Galaxolide and Tonalide varied from 718.3 to 8399 μg/kg dw and 245.7 to 1980 μg/kg dw, respectively. The average contents of Galaxolide and Tonalide in our sewage sludge samples were in the range of previously reported values in Beijing, China (260–12,590 μg/kg dw and 10–2560 μg/kg dw, respectively (Hu et al., 2011)) and Kentucky, USA (20–36,000 μg/kg dw and 20–7200 μg/kg dw, respectively (Horii et al., 2007)). However, Sun et al. (2014) reported higher Galaxolide and Tonalide contents in sewage sludge samples (up to 91,000 and 15,000 μg/kg dw, respectively) in Cincinnati, USA. A study by Ramos et al. (2019) also reported higher mean values of both compounds in Portugal than the current study.

Other polycyclic musk compounds measured in this study were found in the range from < MLOD to 402.6 μg/kg dw. Celastolide and

Table 1
Evaluation of 11 SMCs content present in 55 sewage sludge samples from different WWTPs.

Compounds	n > MLOD	Average (μg/kg dw)	SD	Median (μg/kg dw)	Min (μg/kg dw)	Max (μg/kg dw)	CV (%)
Cashmeran	0	–	–	–	<MLOD	–	–
Celestolide	53	12.6	6.6	11.8	<MLOD	41.4	52
Phantolide	53	7.9	5.5	6.7	<MLOD	29.9	69
Traesolide	53	25.0	52.5	18.5	<MLOD	402.6	210
Musk Ambrette	34	4.5	20.5	1.2	<MLOD	153.5	456
Galaxolide	55	4320	1573	4514	718.3	8399	36
Tonalide	55	1126	421.2	1155	245.7	1980	37
Musk Xylene	18	3.5	7.8	1.2	<MLOD	51.2	225
Musk Moskene	4	4.3	21.7	1.2	<MLOD	162.0	510
Musk Tibeten	36	9.2	18.6	1.3	<MLOD	68.9	202
Musk Ketone	47	4.9	8.7	1.3	<MLOD	56.5	179
SPMCs	55	5492	1943	5680	981.1	9809	35
SNMCs	35	26.3	40.4	9.7	<MLOD	208.8	154
SMCs	55	5518	1950	5686	996.1	9819	35

n > MLOD = number of samples in which the compound was detected, MLOD = method limit of detection, SD = standard deviation, CV = coefficient of variation, - = not determined, as the compound was < MLOD (0.4–2 μg/kg dw) in all samples tested, SPMCs = sum of synthetic polycyclic musk compounds, SNMCs = sum of synthetic nitro musk compounds, SMCs = sum of synthetic musk compounds.

Phantolide contents in our sewage samples were comparable to values reported in Portugal (Ramos et al., 2019). Conversely, higher amounts of these compounds were found in Switzerland (Kupper et al., 2004) and China (Shek et al., 2008).

The Traseolide content in this study (<MLOD – 402.6 μg/kg dw) was comparable to the reported values in China, from 15 to 300 μg/kg dw (Hu et al., 2011). Similarly, Müller and Böhmer (2006) also reported that the Traseolide content ranged from 30 to 460 μg/kg dw.

Individual synthetic nitro musk compounds (SNMCs) were detected in selected sewage sludge samples at lower amounts for the majority of the investigated WWTPs, with a mean of 26.3 μg/kg dw. In the present study, individual musk compounds of the SNMCs group were in the range of <MLOD to 162.0 μg/kg dw. Musks Ketone, Tibeten, and Ambrette were found in 85, 65, and 62% of the 55 investigated WWTPs sludge samples, respectively. On the other hand, Musk Xylene and Musk Moskene were only detected in 33 and 7% of the 55 investigated sewage samples, respectively. Musk Xylene was not detected by many previous studies (Lv et al., 2010; Hu et al., 2011), and most of the other individual SNMCs were also not detected (Berset et al., 2000; Ramos et al., 2019). Lv et al. (2010) reported a Musk Ketone content in the range of <MLOD – 24.4 μg/kg dw, which was similar to the current study, but Hu et al. (2011) found a higher amount of Musk Ketone of up to 530 μg/kg dw. Likewise, Guo et al. (2010) reported similar values.

The total content of SMCs ranged from 996.1 to 9819 μg/kg dw in our sewage sludge samples, with an average of 5518 μg/kg dw, and SPMCs constituted about 99.5% of that amount. The reason for the lower average content of SNMCs (26.3 μg/kg dw) than SPMCs (5492 μg/kg dw) in the current study might be due to the restrictions in producing these compounds in Europe because of concerns about their toxicity; therefore, their use in personal care products has decreased after 2008 (Taylor et al., 2014; Ramos et al., 2019). However, nitro musks are still added in many cheap consumer products, leading to their presence in environmental samples and biota (Smyth et al., 2008; Yin et al., 2016).

3.2. Correlation analyses among individual musk compounds

We observed a statistically significant correlation between the content of selected synthetic musk compounds in 55 sewage sludge samples, as shown in Table 2. Individual polycyclic musk compounds have a statistically significant positive correlation between each other, e.g., very strong correlation between Galaxolide/Tonalide ($r = 0.80$, $P < 0.001$). The strong positive significant correlation was also found among Galaxolide, Tonalide and total SMCs content, with the values of $r = 0.99$ ($P < 0.001$) and $r = 0.87$ ($P < 0.001$), respectively. This was expected, as these compounds constituted about 78.2% and 20.4% of the average total content of SMCs, as shown in Table 1. Furthermore, significant moderate correlations between Tonalide/Phantolide ($r = 0.54$, $P < 0.001$), Phantolide/Celestolide ($r = 0.52$, $P < 0.001$), and many others were observed (Table 2).

Similar to the present study, Yang and Metcalfe (2006) found a positive significant correlation ($r = 0.973$) between Galaxolide/Tonalide in sewage sludge from Peterborough, Canada. Many other studies also reported significant correlations between Galaxolide/Tonalide (Shek et al., 2008; Hu et al., 2011; Sun et al., 2014; Ramos et al., 2019), Tonalide/Phantolide (Shek et al., 2008; Guo et al., 2010), and Tonalide/Celastolide (Shek et al., 2008; Ramos et al., 2019), indicating that these polycyclic musks in sewage sludge may come from the same source. Due to their wide use in personal care products and detergents, the major sources of these synthetic musk compounds are households (domestic sources), whereas industrial sources seem to be of minor importance (Clara et al., 2011).

Some synthetic nitro musks also had a strong correlation between each other, e.g., Musk Ambrette with Musk Moskene ($r = 0.77$, $P < 0.001$). On the other hand, a weak positive correlation ($r = 0.34$, $P < 0.001$) was found between nitro musk (Musk Moskene) and polycyclic musk (Galaxolide) as well as for Musk Moskene and Tonalide. A significant negative correlation was observed between Musk Moskene and Celastolide ($r = -0.27$, $P < 0.01$). A study by Lee et al. (2010) only observed a relationship among Galaxolide, Tonalide, and Musk Ketone in sewage sludge. Overall, the current study found a significantly weak correlation between SPMCs and SNMCs ($r = 0.21$, $P < 0.05$).

3.3. Influence of WWTP technology on the content of synthetic musk compounds in sewage sludge

Pearson correlation analysis was also used to understand the influence of selected technological properties of sampled WWTPs (Table S1) on the basic physico-chemical characteristics of 55 sewage sludge sample tested (Table S2). Pearson correlation coefficients (r) derived from the correlation analysis showed a weak correlation among variables tested, as shown in Table S8.

Furthermore, Pearson correlation coefficients (r) for individual musk compounds, overall SMCs, and selected technological parameters of WWTPs are shown in Table 3. For all the individual musk compounds, only Tonalide correlated significantly with the WWTP design capacity ($r = 0.32$, $P < 0.05$).

Wastewater load percentage and wastewater hydraulic retention time in WWTP have no influence on the content of musk compounds in sewage sludge, as these WWTP technological parameters showed no significant correlation with sewage sludge musk contents ($P > 0.05$).

A study by Sun et al. (2014) also found no correlation between the content of selected musk compounds (Galaxolide and Tonalide) and biological treatment of WWTP, in which activated sludge types were used

Table 2
Pearson correlation coefficients (r) for 11 individual musks, SPMCs, SNMCs, and SMCs derived from correlation analysis of 55 sludge samples.

Variables	DPMI	ABDI	AHDI	ATII	MA	HHCB	AHTN	MX	MM	MT	MK	SPMCs	SNMCs	SMCs
DPMI	1	–	–	–	–	–	–	–	–	–	–	–	–	–
ABDI		1	0.52***	ns	ns	0.42***	0.45***	ns	-0.27**	0.25**	ns	0.44***	ns	0.44***
AHDI			1	ns	ns	0.28**	0.54***	ns	ns	ns	ns	0.35***	ns	0.34***
ATII				1	ns	ns	0.25**	ns	ns	ns	ns	0.19*	ns	0.19*
MA					1	ns	ns	0.77***	ns	ns	ns	ns	0.51***	0.02*
HHCB						1	0.80***	ns	0.34***	ns	ns	0.99***	0.25**	0.99***
AHTN							1	ns	0.23*	ns	ns	0.87***	ns	0.87***
MX								1	ns	ns	ns	ns	0.52***	ns
MM									1	ns	ns	ns	0.57***	0.33***
MT										1	0.50***	ns	0.53***	ns
MK											1	ns	0.38***	ns
∑SPMCs												1	0.21*	1.00***
∑SNMCs													1	ns
∑SMCs														1

The significance of observed Pearson correlation coefficients (r): * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant at $\alpha = 95\%$, - not estimated (<MLOD), Abbreviations DPMI = Cashmaran, ABDI = Celestolide, AHDI = Phantolide, ATII = Traseolide, MA = Musk Ambrette, HHCB = Galaxolide, AHTN = Tonalide, MX = Musk Xylene; MM = Musk Moskene, MT = Musk Tibeten, MK = Musk Ketone, SPMCs = sum of synthetic polycyclic musk compounds, SNMCs = sum of synthetic nitro musk compounds, SMCs = sum of synthetic musk compounds.

for the analyses. When Liu et al. (2014) used only anaerobically digested sewage sludge samples collected from different WWTPs in 23 Chinese cities, they found a strong positive correlation between SMCs/Total organic carbon content (TOC) in sewage sludge and the serving population of the WWTPs. Yang and Metcalfe (2006) indicated that BOD removal efficiency and TOC were two significant independent variables correlated with the contents of both Galaxolide and Tonalide. In our study, a significant ($P < 0.05$) moderate correlation was found only between Musk Ambrette as well as Musk Xylene and BOD removal efficiency ($r = -0.56$ and $r = -0.48$, respectively). When BOD removal efficiency represents the WWTP efficiency in removal of biologically degradable organic compounds (Sholz, 2006), it may indicate that Musk Ambrette and Musk Xylene concentrations in sewage sludge can be at least partly affected by autochthonous microorganisms. That may also partially explain the relatively low content of these compounds in sewage sludge samples. In case of other individual musk compounds, no relationship was found which is consistent with Gonzalez-Gil et al. (2016) who reported that musk compounds (Galaxolide and Tonalide) were not biotransformed during the wastewater treatment. Katsoyiannis and Samara (2004) suggested that compounds with a strong hydrophobic character are principally removed through sorption to sludge particles and transferred to the sludge processing systems.

A negative correlation was observed among the COD removal efficiency and Celastolide, Galaxolide, Tonalide, and Musk Ambrette. COD is defined as the amount of oxygen equivalents consumed in the chemical oxidation of organic matter by a strong oxidant. The COD test uses strong oxidants to oxidize organic matter that microorganisms may oxidize only partially or not at all (Hu and Grasso, 2005). As there were no significant correlations of Celastolide, Galaxolide, Tonalide with BOD removal efficiency but contents of these compounds as well as the sums significantly correlated with COD removal efficiency (SPMCs ($r = -0.36$, $P < 0.05$), SNMCs ($r = -0.33$, $P < 0.01$), and SMCs ($r = -0.37$, $P < 0.05$)), it indicates the recalcitrance of these compounds to the degradation in WWTPs. We hypothesize that the negative correlation coefficients are due to the dilution of these compounds in sludge as there is a higher rate of conversion of organic pollution to sludge with increasing COD removal efficiency.

In comparison to our study, when Simonich et al. (2002) investigated relationship between BOD removal efficiency and concentration of selected musk compounds in final effluent, no significant correlation was found. Yang and Metcalfe (2006) observed that the overall removal efficiency of synthetic musks from the aqueous sewage in the WWTP could be more than 43.3%, but removal occurred mainly by partitioning into the biosolids due to their high log K_{ow}.

In our study, the retention time of sewage sludge in stabilization significantly correlated only with Musk Tibeten ($r = -0.33$, $P < 0.01$). Therefore, it can be assumed that most of the musk compound content will not decrease with prolonged retention time of sludge in the stabilization.

3.4. Occurrence of synthetic musk compounds in sewage sludge according to WWTP design capacity

The assessment of average synthetic musk compound content based on the design capacity of WWTPs was performed. As indicated in Fig. 1, the most dominant individual synthetic polycyclic musk compound, Galaxolide, showed the highest content for large WWTPs, with a value of 5065.1 μg/kg dw. The Galaxolide content increased with increasing

Table 3
Pearson correlation coefficients (r) for individual musks, SPMCs, SNMCs, SMCs and main technological parameters of WWTPs derived from correlation analysis of 55 sludge samples.

Variables	Celestolide	Phantolide	Traseolide	Musk Ambrette	Galaxolide	Tonalide	Musk xylene	Musk moskene	Musk tibeten	Musk ketone	∑SPMCs	∑SNMCs	∑SMCs
WWTP design capacity	ns	ns	ns	ns	ns	0.32*	ns	ns	ns	ns	ns	ns	ns
Wastewater load percentage	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Wastewater hydraulic retention time in WWTP	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
BOD removal efficiency	ns	ns	ns	−0.56***	ns	ns	−0.48***	ns	ns	ns	ns	ns	ns
COD removal efficiency	−0.32*	ns	ns	−0.59***	−0.35**	−0.34*	ns	ns	ns	ns	−0.36**	−0.33*	−0.37**
Retention time of sewage sludge in the stabilization	ns	ns	ns	ns	ns	ns	ns	ns	−0.33*	ns	ns	ns	ns

Cashmeran is not considered, as it was < MLOD (0.4–2 μg/kg dw) in all samples tested, The significance of observed Pearson correlation coefficients (r): * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant at $\alpha = 95\%$, BOD = biochemical oxygen demand, COD = chemical oxygen demand, SPMCs = sum of synthetic polycyclic musk compounds, SNMCs = sum of synthetic nitro musk compounds, SMCs = sum of synthetic musk compounds.

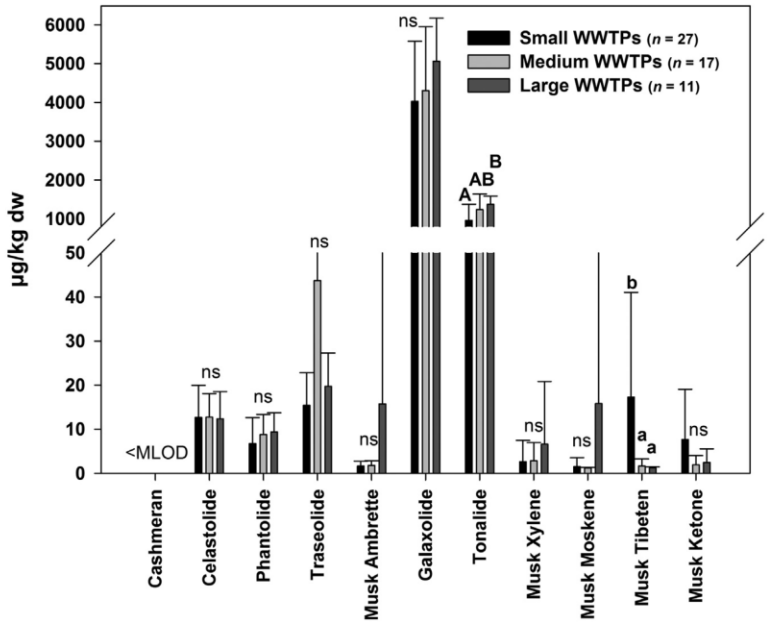


Fig. 1. Average content of 11 individual synthetic musk compounds in sewage sludge according to WWTPs design capacity.
Different letters indicate significant differences ($P < 0.05$) between tested groups of WWTPs for each respective musk compound. Uppercase letters indicate difference determined by HSD test, while lowercase letters indicate difference by KW test. ns denotes no significant difference between WWTP groups at $P < 0.05$. PE (population equivalent) values for Small WWTPs, Medium WWTPs, and Large WWTPs are <50,000; 50,000–100,000, and >100,000, respectively.

WWTP design capacity, but the increments were not significantly different ($P > 0.05$). Tonalide, the second dominant musk from the SPMCs group, also showed a constant increase in the average content as the design capacity of WWTPs increased, with the value ranging from 958.2 μg/kg dw (small WWTPs) to 1368.6 μg/kg dw (large WWTPs). This increase in the content was significantly different ($P < 0.05$, HSD test) and could be supported by a significant correlation found in Table 3.

Other individual SPMCs, Celastolide, Phantolide, and Traseolide showed no significant difference ($P > 0.05$) in their contents when the WWTP design capacity increased. Cashmeran was not evaluated, as the content was below MLOD (0.4 μg/kg dw) in all investigated sewage sludge samples. However, Musk Tibeten from the nitro musk compounds group showed a different trend, as the highest ($P < 0.05$, KW test) content (17.3 μg/kg dw) was found in small WWTPs. In the case of other nitro musks, the content was not statistically different ($P > 0.05$) with the increasing WWTP design capacity.

The average content of SPMCs in Fig. 2 gradually increased from small to large WWTPs, and the same was true for SMCs. However, the increments were not statistically different ($P > 0.05$). Conversely, the

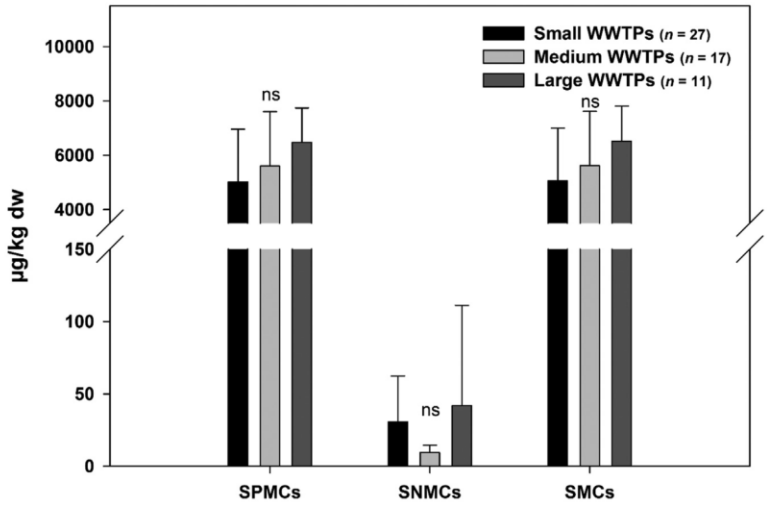


Fig. 2. Average content of synthetic polycyclic musk compounds (SPMCs), synthetic nitromusk compounds (SNMCs), and sum of synthetic musk compounds (SMCs) in sewage sludge according to WWTPs design capacity.
ns denotes no significant difference between WWTP groups at $P < 0.05$.

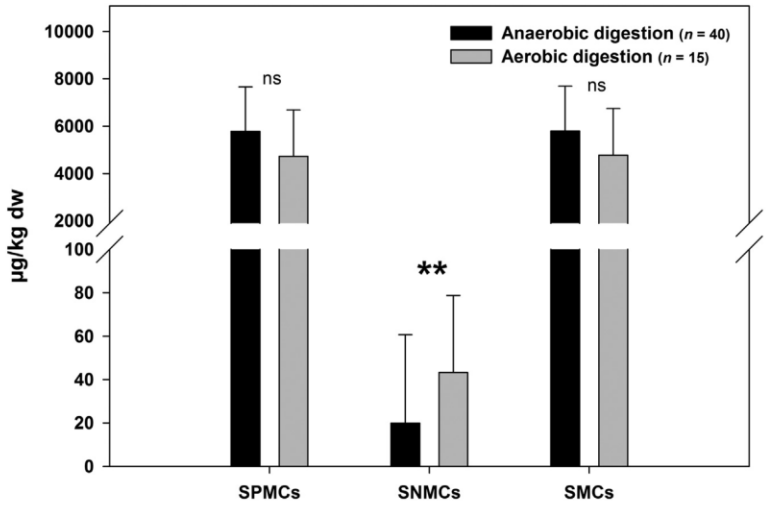


Fig. 3. Average content of synthetic polycyclic musk compounds (SPMCs), synthetic nitromusk compounds (SNMCs), and sum of synthetic musk compounds (SMCs) in sewage sludge samples according to digestion technology used for the stabilization of the sewage sludge.
** indicates significant difference between the technologies ($P < 0.01$; MWR test); ns denotes no significant difference between WWTP groups at $P = 0.05$.

lowest average content of SNMCs was found in medium WWTPs, but the change was not statistically different ($P > 0.05$). Fig. 2 also shows that the average sum of SPMCs and overall SMCs content showed comparable results for all ranges of WWTP capacities. This was expected, as more than 90% of SPMCs of the total SMCs are used in the production of personal care products sold in European markets (Lee et al., 2014).

As shown in Fig. 2, a higher average content of SPMCs than SNMCs was observed in sewage sludge samples of each WWTP design capacity group. This could be linked with the suggestion that the market of personal care products in Czech Republic is dominated by polycyclic musks (Hajšlová and Šetková, 2004). One could expect huge changes of SMCs in sewage sludges from WWTPs with different capacities, as Liu et al. (2014) found a strong significant relationship between the serving population of WWTPs and SMCs content. Liu et al. (2014) suggested that larger WWTPs are commonly found in larger cities where people could use more fragrances, but in the current study, we did not find significant differences ($P > 0.05$) of SMCs when the WWTPs design capacity was increased. We hypothesize that this phenomenon may be due to different cultural habits of the citizens.

3.5. Occurrence of synthetic musk compounds in sewage sludge according to digestion technology, temperature of anaerobic digestion, and over time

The content of SMCs according to the digestion technology used for the stabilization of sewage sludge is shown in Fig. 3. The influence of aerobic and anaerobic digestion on the content of SMCs in sewage sludge was not obvious, as no significant difference ($P > 0.05$) was found between the SMC content in sewage sludge collected from WWTPs where anaerobic and aerobic digestion was used. The same was true for the content of polycyclic musk compounds (SPMCs). A statistically lower content ($P < 0.01$; MWR test) of nitro musk compounds

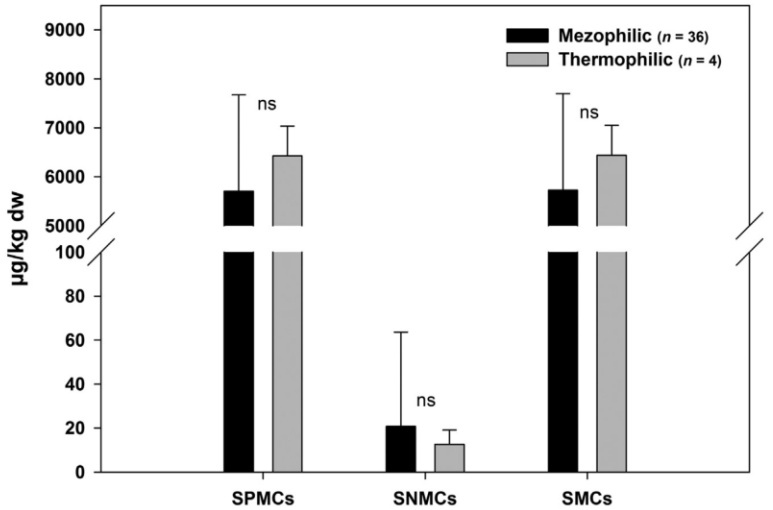


Fig. 4. Average content of synthetic polycyclic musk compounds (SPMCs), synthetic nitromusk compounds (SNMCs), and sum of synthetic musk compounds (SMCs) in sewage sludge samples according to the temperature of anaerobic digestion technology.
ns denotes no significant difference between WWTP groups at $P < 0.05$.

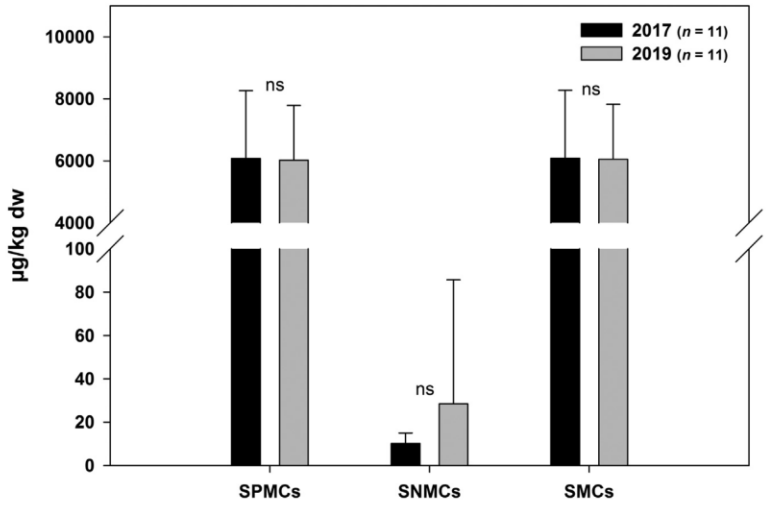


Fig. 5. Changes in average content of synthetic polycyclic musk compounds (SPMCs), synthetic nitromusk compounds (SNMCs), and sum of synthetic musk compounds (SMCs) in sewage sludge samples over time.
ns denotes no significant difference between WWTP groups at $P < 0.05$.

(SNMCs) was found in anaerobically treated sewage sludge than in aerobically treated ones.

This may lead to the suggestion that, in the WWTPs where anaerobic digestion is used, autochthonous microorganisms could be involved in the degradation of nitro musks, so the content in the resulting sewage sludge is lowered. To elucidate the mechanism of nitro musk compound reduction by sewage sludge treatment under anaerobic conditions, further research needs to be conducted. Ghattas et al. (2017) reviewed that the reduction of nitro groups under anaerobic conditions occurs, in general, when microorganisms create a reducing environment by which electrons are transferred to reducible substrates, such as nitro–aromatics. In comparison to nitro musks, Gonzalez-Gil et al. (2016) described that polycyclic musk fragrances (Galaxolide and Tonalide) were not subjected to biotransformation processes during the anaerobic digestion of sewage sludge. Tasselli and Guzzella (2020) reported that even in activated sludge treatment, which is a process for treating sewage or industrial wastewaters using aeration and a biological floc composed of bacteria and protozoa, it was not possible to remove and degrade polycyclic musks.

Unfortunately, as the content of nitro musks in sewage sludge was about 0.5% of the total musk content in this current study, a recommendation to reduce nitro musk compounds by anaerobic digestion will have a negligible effect on the total musk compounds in the resulting sewage sludge. Moreover, the influence of anaerobic digestion temperature on musk sewage sludge content shown in Fig. 4 was not observed, as the content of polycyclic, nitro, and total musks in sewage sludge treated anaerobically under mesophilic and thermophilic conditions was not significantly different ($P < 0.05$). This can be supported by the study of Gonzalez-Gil et al. (2016), who found a comparable content of polycyclic musk – Galaxolide in a laboratory treated sewage sludge under mesophilic and thermophilic conditions.

The study of potential changes of SMC content over the time 2017–2019 was performed, and the results are shown in Fig. 5. The content of polycyclic, nitro, and total content of musk compounds in sewage sludge was not statistically different ($P > 0.05$) when the sewage sludge sample was collected from the respective WWTP with a gap of two years. This indicates that the presence of musk compounds in sewage sludge is not prone to fluctuate with time. Thus, screening of the sewage sludge for musk compounds once a year should be enough, as in the case of other persistent organic pollutants (PAHs and PCBs) in sewage sludge according to the Czech legislative limit for soil application (Public Notice No. 437/2016, 2016).

4. Conclusions

Sewage sludge samples were collected from 55 WWTPs in different locations of the Czech Republic and screened for 11 selected synthetic musk compounds (SMCs). Predominant synthetic polycyclic musk compounds (SPMCs) were Galaxolide and Tonalide, while Musk Ketone and Musk Xylene were the most abundant synthetic nitro musk compounds (SNMCs). Total content of SMCs ranged from 996.1 to 9819 µg/kg dw in sewage sludge, with an average of 5518 µg/kg dw, and SPMCs constituted about 99.5% of that amount.

Individual polycyclic musks contents correlated between each other, indicating a single source. Tonalide content correlated significantly with the WWTP design capacity ($r = 0.32$, $P < 0.05$) and showed a significant increment ($P < 0.05$, HSD test) when WWTPs design capacity increased. Wastewater load percentage and wastewater hydraulic retention time in WWTPs had no influence on the musks in sewage sludge. A weak significant correlation between COD removal efficiency and SMCs ($r = -0.37$, $P < 0.05$) but no correlation with BOD removal efficiency was found suggesting the recalcitrance of the SMCs to biodegradation. The retention time of sewage sludge stabilization in WWTPs only significantly correlated with Musk Tibeten ($r = -0.33$, $P < 0.05$). Only in the case of total sum of 5 synthetic nitro musk compounds in sludge (~0.5%), a statistically ($P < 0.01$, MWR test) lower content was observed in sewage sludge collected from WWTPs with anaerobic digestion than with aerobic digestion. There was no significant difference ($P > 0.05$) between mesophilic and thermophilic conditions of anaerobic digestion technology on SMCs content in sewage sludge.

The current study suggests that the accumulation of the major musk compounds in sewage sludge is not affected by the current WWTPs technologies. Innovative sewage sludge treatment processes should be involved prior to its use in agriculture.

CRediT authorship contribution statement

Zdeněk Košnář: Conceptualization, Methodology, Investigation, Data curation, Visualization Writing - original draft, review & editing. **Filip Mercl:** Project administration, Conceptualization, Methodology,

Validation, Formal analysis, Data analysis, Writing - review & editing, **Abraham Demelash Chane**: Investigation, Writing - original draft, **Lorenzo Pierdonà**: Investigation, **Pavel Míchal**: Sample and data collection, **Pavel Tlustoš**: Supervision, Editing, Project administration, Funding acquisition.

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.

Acknowledgements

This study was supported by the Czech Ministry of Agriculture (QK1710379) and by the European Regional Development Fund (project NUTRISK no. CZ.02.1.01/0.0/0.0/16_019/0000845).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149777.

References

Antweiler, R.C., Taylor, H.E., 2008. Evaluation of statistical treatments of left-censored environmental data using coincident uncensored data sets: I. Summary statistics. Environ. Sci. Technol. 42, 3732–3738. https://doi.org/10.1021/es071301c.

Artola-Garicano, E., Borkent, I., Hermens, J.L.M., Vaes, W.H.J., 2003. Removal of two polycyclic musks in sewage treatment plants: freely dissolved and total concentrations. Environ. Sci. Technol. 37, 3111–3116. https://doi.org/10.1021/es020226x.

Balci, E., Genisoglu, M., Sofuoglu, S.C., Sofuoglu, A., 2020. Indoor air partitioning of synthetic musk compounds: gas, particulate matter, house dust, and window film. Sci. Total Environ. 729, 138798. https://doi.org/10.1016/j.scitotenv.2020.138798.

Balk, F., Ford, R.A., 1999. Environmental risk assessment for the polycyclic musks, AHTN and HHCB: II. Effect assessment and risk characterisation. Toxicol. Lett. 111, 81–94. https://doi.org/10.1016/S0378-4274 (99)00170-8.

Berset, J.D., Bigler, P., Herren, D., 2000. Analysis of nitro musk compounds and their amino metabolites in liquid sewage sludges using NMR and mass spectrometry. Anal. Chem. 72, 2124–2131. https://doi.org/10.1021/ac991006d.

Biel-Maeso, M., Corada-Fernández, C., Lara-Martín, P.A., 2019. Removal of personal care products (PCPs) in wastewater and sludge treatment and their occurrence in receiving soils. Water Res. 150, 129–139. https://doi.org/10.1016/j.watres.2018.11.045.

Cavalheiro, J., Prieto, A., Monperrus, M., Etxebarria, N., Zuloaga, O., 2013. Determination of polycyclic and nitro musks in environmental water samples by means of microextraction by packed sorbents coupled to large volume injection-gas chromatography–mass spectrometry analysis. Anal. Chim. Acta 773, 68–75. https://doi.org/10.1016/j.aca.2013.02.036.

Clara, M., Gans, O., Windhofer, G., Krenn, U., Hartl, W., Braun, K., Scharf, S., Scheffknecht, C., 2011. Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. Chemosphere 82, 1116–1123. https://doi.org/10.1016/j.chemosphere.2010.11.041.

Clarke, B.O., Smith, S.R., 2011. Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environ. Int. 37, 226–247. https://doi.org/10.1016/j.envint.2010.06.004.

Evans, J.D., 1996. Straightforward Statistics for the Behavioral Sciences. Pacific Grove: Brooks/Cole Publishing Co, United States, p. 634 ISBN-13: 978-0534231002.

Fontal, M., van Drooge, B.L., Grimalt, J.O., 2016. A rapid method for the analysis of methyl dihydrojasmonate and galaxolide in indoor and outdoor air particulate matter. J. Chromatogr. A 1447, 135–140. https://doi.org/10.1016/j.chroma.2016.04.028.

Ghattas, A.K., Fischer, F., Wick, A., Ternes, T.A., 2017. Anaerobic biodegradation of (emerging) organic contaminants in the aquatic environment. Water Res. 116, 268–295. https://doi.org/10.1016/j.watres.2017.02.001.

Gonzalez-Gil, L., Papa, M., Feretti, D., Ceretti, E., Mazzoleni, G., Steimberg, N., Pedrazzani, R., Bertanza, G., Lema, J.M., Carballa, M., 2016. Is anaerobic digestion effective for the removal of organic micropollutants and biological activities from sewage sludge? Water Res. 102, 211–220. https://doi.org/10.1016/j.watres.2016.06.025.

Guo, R., Lee, I.S., Kim, U.J., Oh, J.E., 2010. Occurrence of synthetic musks in korean sewage sludges. Sci. Total Environ. 408, 1634–1639. https://doi.org/10.1016/j.scitotenv.2009.12.009.

Hajšlová, J., Šetková, L., 2004. Synthetic musks in bioindicators: monitoring data of fish and human milk samples from the Czech Republic. In: Rimkus, G.G. (Ed.), Series Anthropogenic Compounds. The Handbook of Environmental Chemistry. vol 3X. Springer, Berlin, Heidelberg. https://doi.org/10.1007/b14120.

Hanssen, L., Warner, N.A., Braathen, T., Odland, J.Ø., Lund, E., Nieboer, E., Sandanger, T.M., 2013. Plasma concentrations of cyclic volatile methylsiloxanes (cVMS) in pregnant and postmenopausal norwegian women and self-reported use of personal care products (PCPs). Environ. Int. 51, 82–87. https://doi.org/10.1016/j.envint.2012.10.008.

Horii, Y., Reiner, J.L., Loganathan, B.G., Senthil Kumar, K., Sajwan, K., Kannan, K., 2007. Occurrence and fate of polycyclic musks in wastewater treatment plants in Kentucky and Georgia, USA. Chemosphere 68, 2011–2020. https://doi.org/10.1016/j.chemosphere.2007.04.054.

Hu, Z., Grasso, D., 2005. Chemical oxygen demand. In: Paul, W., Alan, T., Colin, P. (Eds.), Encyclopedia of Analytical Science, second edition Elsevier, pp. 325–330 https://doi.org/10.1016/j.jwpe.2020.101850.

Hu, Z., Shi, Y., Zhang, S., Niu, H., Cai, Y., 2011. Assessment of synthetic musk fragrances in seven wastewater treatment plants of Beijing, China. Bull. Environ. Contam. Toxicol. 86, 302–306. https://doi.org/10.1007/s00128-011-0215-1.

Katsoyiannis, A., Samara, C., 2004. Persistent organic pollutants (POPs) in the sewage treatment plant of Thessaloniki, northern Greece: occurrence and removal. Water Res. 38, 2685–2698. https://doi.org/10.1016/j.watres.2004.03.027.

Kupper, T., Berset, J.D., Etter-Holzer, R., Furrer, R., Tarradellas, J., 2004. Concentrations and specific loads of polycyclic musks in sewage sludge originating from a monitoring network in Switzerland. Chemosphere 54, 1111–1120. https://doi.org/10.1016/j.chemosphere.2003.09.023.

Lee, H.B., Peart, T.E., Sarafin, K., 2003. Occurrence of polycyclic and nitro musk compounds in Canadian sludge and wastewater samples. Water Qual. Res. J. 38, 683–702. https://doi.org/10.2166/wqrj.2003.043.

Lee, I.S., Lee, S.H., Oh, J.E., 2010. Occurrence and fate of synthetic musk compounds in water environment. Water Res. 44, 214–222. https://doi.org/10.1016/j.watres.2009.08.049.

Lee, I.S., Kim, U.J., Oh, J.E., Choi, M., Hwang, D.W., 2014. Comprehensive monitoring of synthetic musk compounds from freshwater to coastal environments in Korea: with consideration of ecological concerns and bioaccumulation. Sci. Total Environ. 470–471, 1502–1508. https://doi.org/10.1016/j.scitotenv.2013.07.070.

Lignell, S., Darnerud, P.O., Aune, M., Cnattingius, S., Hajslova, J., Setkova, L., Glynn, A., 2008. Temporal trends of synthetic musk compounds in Mother's milk andassociations with personal use of perfumed products. Environ. Sci. Technol. 42, 6743e6748. https://doi.org/10.1021/es800626n.

Liu, N., Shi, Y., Li, W., Xu, L., Cai, Y., 2014. Concentrations and distribution of synthetic musks and siloxanes in sewage sludge of wastewater treatment plants in China. Sci. Total Environ. 476–477, 65–72. https://doi.org/10.1016/j.scitotenv.2013.12.124.

Liu, J., Zhang, W., Zhou, Q., Zhou, Q., Zhang, Y., Zhu, L., 2021. Polycyclic musks in the environment: a review of their concentrations and distribution, ecological effects and behavior, current concerns and future prospects. Crit. Rev. Environ. Sci. Technol. 51, 323–377. https://doi.org/10.1080/10643389.2020.1724748.

Llompart, M., Garcí´a-Jares, C., Salgado, C., Polo, M., Cela, R., 2003. Determination of musk compounds in sewage treatment plant sludge samples by solid-phase microextraction. J. Chromatogr. A 999, 185–193. https://doi.org/10.1016/S0021-9673(03)00449-7.

Lv, Y., Yuan, T., Hu, J., Wang, W., 2010. Seasonal occurrence and behavior of synthetic musks (SMs) during wastewater treatment process in Shanghai, China. Sci. Total Environ. 408, 4170–4176. https://doi.org/10.1016/j.scitotenv.2010.05.003.

Marchal, M., Beltran, J., 2016. Determination of synthetic musk fragrances. Int. J. Environ. Anal. Chem. 96, 1213–1246. https://doi.org/10.1080/03067319.2016.1249479.

Müller, J., Böhmer, W., 2006. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants. part 1: behaviour of polycyclic musks in sewage sludge of different treatment plants in summer and winter (5 pp). J. Soils Sediments 6, 231–235. https://doi.org/10.1065/jss2006.10.187.1.

Paasivirta, J., Sinkkonen, S., Rantalainen, A.L., Broman, D., Zebühr, Y., 2002. Temperature dependent properties of environmentally important synthetic musks. Environ. Sci. Pollut. Res. 9, 345–355. https://doi.org/10.1007/BF02987579.

Public Notice No. 437/2016, 2016. Quality Requirements of Biosolids for the Use on Agricultural Land, Legal Code of Ministry of the Environment of the Czech Republic.

Ramos, S., Homem, V., Santos, L., 2019. Development and optimization of a QuEChERS-GC–MS/MS methodology to analyse ultraviolet-filters and synthetic musks in sewage sludge. Sci. Total Environ. 651, 2606–2614. https://doi.org/10.1016/j.scitotenv.2018.10.143.

Reiner, J.L., Berset, J.D., Kannan, K., 2007. Mass flow of polycyclic musks in two wastewater treatment plants. Arch. Environ. Contam. Toxicol. 52, 451–457. https://doi.org/10.1007/s00244-006-0203-3.

Shek, W.M., Murphy, M.B., Lam, J.C.W., Lam, P.K.S., 2008. Synthetic polycyclic musks in Hong Kong sewage sludge. Chemosphere 71, 1241–1250. https://doi.org/10.1016/j.chemosphere.2007.11.069.

Sholz, M., 2006. Chapter 16 - constructed wetlands. Wetland Systems to Control Urban Runoff. Elsevier, pp. 91–110 https://doi.org/10.1016/B978-044452734-9/50019-0.

Simonich, S.L., Federle, T.W., Eckhoff, W.S., Rottiers, A., Webb, S., Sabaliunas, D., de Wolf, W., 2002. Removal of fragrance materials during U.S. and european wastewater treatment. Environ. Sci. Technol. 36, 2839–2847. https://doi.org/10.1021/es025503e.

Smyth, S.A., Lishman, L.A., McBean, E.A., Kleywegt, S., Yang, J.J., Svoboda, M.L., Lee, H.B., Seto, P., 2008. Seasonal occurrence and removal of polycyclic and nitro musks from wastewater treatment plants in Ontario, Canada. J. Environ. Eng. Sci. 7, 299–317. https://doi.org/10.1139/S08-002.

Sun, P., Casteel, K., Dai, H., Wehmeyer, K.R., Kiel, B., Federle, T., 2014. Distributions of polycyclic musk fragrance in wastewater treatment plant (WWTP) effluents and sludges in the United States. Sci. Total Environ. 493, 1073–1078. https://doi.org/10.1016/j.scitotenv.2014.04.038.

Tasselli, S., Guzzella, L., 2020. Polycyclic musk fragrances (PMFs) in wastewater and activated sludge: analytical protocol and application to a real case study. Environ. Sci. Pollut. Res. 27, 30977–30986. https://doi.org/10.1007/s11356-019-06767-7.

Taylor, K.M., Weisskopf, M., Shine, J., 2014. Human exposure to nitro musks and the evaluation of their potential toxicity: an overview. Environ. Health 13, 14. https://doi.org/10.1186/1476-069X-13-14.

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38, 4075–4084. https://doi.org/10.1016/j.watres.2004.07.015.

Vallecillos, L., Borrull, F., Pocurull, E., 2012. Determination of musk fragrances in sewage sludge by pressurized liquid extraction coupled to automated ionic liquid-based headspace single-drop microextraction followed by GC-MS/MS. J. Sep. Sci. 35, 2735–2742. https://doi.org/10.1002/jssc.201200326.

Wang, M., Peng, C., Chen, W., Markert, B., 2013. Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicol. Environ. Saf. 97, 242–247. https://doi.org/10.1016/j.ecoenv.2013.07.032.

Wong, F., Robson, M., Melymuk, L., Shunthirasingham, C., Alexandrou, N., Shoeib, M., Luk, E., Helm, P., Diamond, M.L., Hung, H., 2019. Urban sources of synthetic musk compounds to the environment. Environ. Sci. Process. Impacts 21, 74–88. https://doi.org/10.1039/C8EM00341F.

Yang, J.J., Metcalfe, C.D., 2006. Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. Sci. Total Environ. 363, 149–165. https://doi.org/10.1016/j.scitotenv.2005.06.022.

Yin, J., Wang, H., Li, J., Wu, Y., Shao, B., 2016. Occurrence of synthetic musks in human breast milk samples from 12 provinces in China. Food Addit. Contam., Part A 33, 1219–1227. https://doi.org/10.1080/19440049.2016.1201219.

Zeng, X., Sheng, G., Xiong, Y., Fu, J., 2005. Determination of polycyclic musks in sewage sludge from Guangdong, China using GC–EI-MS. Chemosphere 60, 817–823. https://doi.org/10.1016/j.chemosphere.2005.04.026.

Zhang, H., Bu, Q., Wu, D., Yu, G., 2020. Polycyclic musks in surface water and sediments from an urban catchment in the megacity Beijing, China. Environ. Pollut. 263, 114548. https://doi.org/10.1016/j.envpol.2020.114548.

4.3. Chane et al. (2024). Persistent polycyclic aromatic hydrocarbons removal from sewage sludge-amended soil through phytoremediation combined with solid-state ligninolytic fungal cultures

Authors: **Abraham Demelash Chane**, Zdeněk Košnář, Tereza Hřebečková, Miroslav Jozífek, Petr Doležal, Pavel Tlustoš

Published in the journal: Fungal Biology, 128, 1675-1683
Information about the magazine: IF = 2.5 (2023)

Fungal Biology 128 (2024) 1675–1683

Contents lists available at ScienceDirect

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

Persistent polycyclic aromatic hydrocarbons removal from sewage sludge-amended soil through phytoremediation combined with solid-state ligninolytic fungal cultures

Abraham Demelash Chane [a], Zdeněk Košnář [a,*], Tereza Hřebečková [a], Miroslav Jozífek [b], Petr Doležal [a], Pavel Tlustoš [a]

[a] Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 165 00, Praha-Suchdol, Czech Republic
[b] Department of Horticulture, Czech University of Life Sciences Prague, Kamýcká 129, 165 00, Praha-Suchdol, Czech Republic

ARTICLE INFO

Handling Editor: Yuyi Yang

Keywords:
PAH removal
Anaerobically stabilized sludge
Phytoremediation
Mycoremediation
Plant uptake

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are widely present in the environment, causing increasing concern because of their impact on soil health, food safety and potential health risks. Four bioremediation strategies were examined to assess the dissipation of PAHs in agricultural soil amended with sewage sludge over a period of 120 days: soil-sludge natural attenuation (SS); phytoremediation using maize (*Zea mays* L.) (PSS); mycoremediation (MR) separately using three white-rot fungi (*Pleurotus ostreatus*, *Phanerochaete chrysosporium* and *Irpex lacteus)*; and plant-assisted mycoremediation (PMR) using a combination of maize and fungi. In the time frame of the experiment, mycoremediation using *P. chrysosporium* (MR-PH) exhibited a significantly higher ($P < 0.05$) degradation of total PAHs compared to the SS and PSS treatments, achieving a degradation rate of 52 %. Both the SS and PSS treatments demonstrated a lower degradation rate of total PAHs, with removal rates of 18 % and 32 %, respectively. The PMR treatments showed the highest removal rates of total PAHs at the end of the study, with degradation rates of 48–60 %. In the shoots of maize, only low- and medium-molecular-weight PAHs were found in both the PSS and PMR treatments. The calculated translocation and bioconversion factors always showed values < 1. The analysed enzymatic activities were higher in the PMR treatments compared to other treatments, which can be positively related to the higher degradation of PAHs in the soil.

1. Introduction

Sewage sludge is essentially an organic residue, containing high microbial biomass as well as high organic matter, N, P, and Zn contents, among others. If properly treated and applied to land, it can improve soil's quality thus improving the productivity of agricultural crops and revegetation of disturbed ecosystems, such as mining tailing areas. However, Sewage sludge also has high amounts of potentially toxic elements in its composition, heavy metals, organic pollutants, and emerging contaminants (Cieślik et al., 2015; Tyagi and Lo, 2013).

Incorporating sewage sludge into the soil can also have implications for soil microbes, plants and animals due to the accumulation of pollutants (Liu et al., 2017). It also contributes to the sustainable crop and food production and helps meeting sustainable development goals (SDGs) especially in developing countries (Kumar et al., 2022). Additionally, it is economically viable approach due to its beneficial impact on the agrochemical and biological properties of the soil (Wu et al., 2017; Wołejko et al., 2018). According to Chojnacka et al. (2023), sludge production in the Czech Republic for the year 2017 reached 223,000 tonnes dw, of which approximately 33 % was used for composting while the majority (up to 45 %) was utilized for agricultural purposes. However, the average application of wastewater sewage sludge in 13 countries in the European Union (EU) was recorded at 22.6 % and 22.1 % of the respective production amounts in 2013 and 2014.

As previously indicated, the utilization of sewage sludge in soil can impact soil microbes, plants, and animals by potentially accumulating pollutants (Liu et al., 2017). Polycyclic aromatic hydrocarbons (PAHs) are among the most environmentally hazardous compounds due to their persistence and toxicity. Studies have identified their presence in various environmental compartments including soil, water, and air (Gupte et al., 2016; Kadri et al., 2017a; Patel et al., 2020). It is important to consider the presence of PAHs within the sludge they can be taken up by plants and ultimately find their way into the human body through the

* Corresponding author.
E-mail address: kosnarz@af.czu.cz (Z. Košnář).

https://doi.org/10.1016/j.funbio.2024.01.007
Received 21 November 2023; Received in revised form 26 January 2024; Accepted 29 January 2024
Available online 30 January 2024

Abbreviations

ACE	Acenaphthylene
ACEN	Acenaphthene
ANT	Anthracene
BaA	Benzo[a]anthracene
BbF	Benzo[b]fluoranthene
BCF	bioconcentration factor
BkF	Benzo[k]fluoranthene
BghiP	Benzo[g,h,i]perylene
BaP	Benzo[a]pyrene
CHR	Chrysene
DBA	Dibenz[a,h]anthracene
FLU	Fluorene
FLUO	Fluoranthene
HMW	high molecular weight
IYP	Indeno[l,2,3-c,d] pyrene
LAC	laccase
LMW	low molecular weight
MMW	medium molecular weight
MR	mycoremediation of PAHs in sludge-amended soil
MR-PL	soil-sludge with *P. ostreatus* substrate
MR-PH	soil-sludge with *P. chrysosporium*
MR-IR	soil-sludge with *I. lacteus* substrate
MnP	manganese peroxide
PAHs	polycyclic aromatic hydrocarbons
PSS	phytoremediation of PAHs in planted-contaminated soil-sludge
PMR	plant-assisted microbial bioremediation
PMR-PL	soil-sludge with *P. ostreatus* substrate
PMR-PH	soil-sludge with *P. chrysosporium* substrate
PMR-IR	soil-sludge with *I. lacteus* substrate
SS	soil-sludge
TF	transfer factor

food chain (Włóka et al., 2013; Wołejko et al., 2018; Zhang et al., 2018). Their accumulation in the soil environment is of increasing concern because of their impact on soil health, food safety and the potential carcinogenic and mutagenic health risks (Barbosa et al., 2023; Moubarz et al., 2023). According to Suciu et al. (2015), the overall PAH content in sewage sludge among EU countries ranged from 10 to 3910 μg/kg dry weight (dw). Meanwhile, Feng et al. (2019) reported PAH concentrations in soils amended with sludge to be within the range of 149–307 μg/kg dw. Furthermore, Ma et al. (2015) observed variations in the concentrations of 16 PAHs in soils, ranging from 10 to 5911 μg/kg dw. PAHs present challenges for degradation in natural settings due to their lipophilic nature. Furthermore, their persistence increases with higher molecular weights in the environment, as highlighted by (Haritash and Kaushik, 2009). Understanding the degradation of PAHs in soil is complex due to adsorption phenomena and the limited bioavailability of these compounds. Factors such as environmental conditions, microbial communities, and the chemical nature of the contaminants contribute to the intricacies of bioremediation (Kadri et al., 2017b).

Bioremediation is an attractive approach for the removal of PAHs from the environment and specific microbes are used to break down the organic pollutants (Abatenh et al., 2017). Many microorganisms have been reported to efficiently degrade PAHs, for instance, Gao et al. (2010) mentioned that the white-rot fungi of *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Irpex lacteus* and *Bjerkandera adusta* were suitable degraders of PAHs in the soil. The ability to generate ligninolytic extracellular oxidative enzymes has rendered these fungi highly suitable for the decomposition and mineralization of the organic polymer lignin, and these enzymes have the capability of cleaving the benzene ring of PAHs (Kijpornyongpan et al., 2022). PAHs derived from soil or sludge compost-amended soil could be degraded by plants together with microorganisms in a soil–plant system (Gworek et al., 2016; Zhu et al., 2017). Phytoremediation could be implemented with various plants to degrade, extract and immobilize PAHs from the soil (Abdel-Shafy and Mansour, 2018). Guo et al. (2017a) studied the phytoremediation of PAHs using maize (*Zea mays* L.) in the soil and found that their removal ranged from 69.2 % to 78.4 %. The current study hypothesizes that the combined use of white-rot fungi and plants could significantly enhance the removal of PAHs from the soil compared to other bioremediation strategies.

The study aims were to evaluate four different strategies for PAH degradation: natural attenuation in biosolid-amended soil; mycoremediation utilizing three strains of solid-state white-rot fungi cultures, namely *P. ostreatus*, *P. chrysosporium* and *I. lacteus*; phytoremediation using maize (*Zea mays* L.); and microbial-assisted phytoremediation, which involves a combination of maize (*Zea mays* L.) and three different strains of solid-state white-rot fungi cultures (*P. ostreatus*, *P. chrysosporium* and *I. lacteus*). Additionally, the study aim is to evaluate the effect of these treatments on the uptake of PAHs by plants.

2. Materials and methods

2.1. Solvents, analytical standards, and materials

GC/MS–MS grade *n*-hexane (Chromasolv™), acetone (Pestinorm®) and ethanol (p.a., 99.8 %) were used to prepare an extraction solution of acetone–hexane (1:1 *v/v*). All solvents were purchased from Altium International (Czech Republic). PAH-Mix 16 (EPA 550, EPA 610) containing 16 individual PAHs prioritized by the USEPA in cyclohexane at 100 μg/ml was used as a standard stock mixture (Table S1). Deuterated compounds (naphthalene-d_8, acenaphthene-d_8, phenanthrene-d_{10}, chrysene-d_{12}, perylene-d_{12} and PCB 116-d_5; 100 μg/ml of each) were used as the internal standard stock mixture solution (Table S2) and a surrogate standard solution of deuterated *p*-terphenyl-d_{14} in dichloromethane at 1000 μg/ml (Table S3) was used for the method recovery (Chromservis, Czech Republic). Strata SI-1 SPE cartridges manufactured by Phenomenex (USA) were purchased from Chromservis (Czech Republic) and Whatman® Grade 40 filters were purchased from Supelco (USA). The PAH abbreviations are listed in Table S1 and the basic physical and chemical properties of the 16 individual PAHs are summarized in Table S4.

2.2. Soil and sludge description

The experimental soil, a Cambisol characterized by an acidic pH_{CaCl2} 5.4, was obtained from Humpolec, Czech Republic (49°33′16″N, 15°21′2″E). Prior to the start of the experiment, the soil underwent trace PAH analysis, revealing levels that remained below the quantification limit, ranging between 1.7 and 5.4 μg/kg dw. The anaerobically stabilized sewage sludge, collected from a wastewater treatment plant accommodating to a population equivalent of 198,000, exhibited concentrations of low, medium, and high molecular weight PAHs, as well as Σ16 PAHs, at 291.3, 3497.4, 3193, and 6981.6 μg/kg dw, respectively.

2.3. Solid-state fungal substrate description

Three ligninolytic fungal species – *Pleurotus ostreatus* (Jacq.) P. Kumm., strain HK35 (*P. ostreatus*), *P. chrysosporium* (Burds.) (*P. chrysosporium*) and *I. lacteus* (Fr.), strain 931 (*I. lacteus*) – were obtained from the deposit of the Department of Horticulture, Czech

University of Life Sciences, Prague. Solid-state ligninolytic fungi cultivation, the conditions and a description of the colonized wheat straw carrier substrates were specified by Košnář et al. (2019) and Chane et al. (2023a).

2.4. Experimental setup

The experimental study was carried out for 120 days in the outdoor and roofed vegetation hall that controls atmospheric precipitation under natural daylight to resemble the actual field conditions. Polyethylene pots with a total volume of 6 l were used, with holes in the bottom. To catch possible leachate, another similar plastic pot without holes was used under the perforated one. Each pot was filled with 4 kg dw of experimental soil and 1 kg of fresh sewage sludge thoroughly mixed. Four replicates were subjected to each treatment as follows: (A) soil-sludge (SS) represented only the intrinsic clean-up ability of PAH-contaminated sludge-amended soil; (B) mycoremediation of PAHs in sludge-amended soil inoculated by different solid-state cultivated white-rot fungi substrates (MR): (i) soil-sludge with *P. ostreatus* substrate (MR-PL), (ii) soil-sludge with *P. chrysosporium* (MR-PL) and (iii) soil-sludge with *I. lacteus* substrate (MR-PL); (C) phytoremediation of PAHs in maize-contaminated soil-sludge (PSS); and (D) plant-assisted microbial bioremediation through the different solid-state white-rot fungi strains (PMR): (i) soil-sludge with *P. ostreatus* substrate (PMR-PL), (ii) soil-sludge with *P. chrysosporium* substrate (PMR-PH) and (iii) soil-sludge with *I. lacteus* substrate (PMR-IR). For the phytoremediation treatments, the pots were planted with maize (*Zea mays* L. var. Colisee) seeds obtained from KWS Saat in Germany. Initially, eight maize seeds were sown in each pot. After a period of two weeks following germination, only four plants were left to grow and the pots were randomized every week. The soil was irrigated with distilled water to maintain the water content of the soil at 60 % water-holding capacity, while pots were regularly weighed to add water when needed throughout the experimental period. At the end of the experiment, the plants were harvested and divided into shoots and roots. The roots underwent a gentle wash with demi-water to remove any rhizospheric soil adhering to them. Subsequently, the shoots and roots were separately cut into smaller pieces and subjected to lyophilization, milling and storage at 4 °C in preparation for the PAH analyses. Three samples from each pot were collected at 0 and 120 days of the experiment by using a stainless-steel tool and then mixed thoroughly to obtain a representative sample. Soil samples were lyophilized, ground, sieved at 2 mm mesh and stored at −20 °C until PAH extraction and other analyses.

2.5. PAH extraction, analysis of PAHs and enzymatic activities

The PAHs from the soil were extracted and analysed based on the protocol of Chane et al. (2023a). Briefly, 1 g dw of soil was mixed with 10 ml of ethanol, sodium acetate buffer (pH 3.4) and *n*-hexane and surrogate standard solution at 500 ng/ml, shaken for 1 h in an orbital shaker (GFL 3017, Germany) and then extracted with the ultrasonic extraction system for 30 min at a bath temperature of 35 °C. The upper hexane layer was filtered through a Whatman® Grade 40 filter and rinsed with 5 ml of hexane. Re-extraction was done following the same procedure by adding 5 ml of a hexane–acetone mixture (1:1, *v/v*). The extract was mixed with the previous one, concentrated to 1.0 ml and evaporated to near dryness (0.2 ml) using nitrogen. The sample extract was cleaned through SPE cartridges then concentrated again to 0.2 ml by using nitrogen. Before PAH analysis, 100 μl of the internal standard solution was added to the extract to a final volume of 2 ml. Samples of plant biomass were extracted using the same procedure described above.

Separation, identification and determination of individual PAHs were performed using an Agilent 8890 gas chromatograph coupled with an Agilent 7000D triple quadrupole detector (Santa Clara, USA) and PAL RTC automatic sampler system (CTC Analytics, Switzerland). More detailed chromatographic conditions were described by Chane et al. (2023b). Detection was performed using a mass spectrometer in dynamic multiple reaction monitoring mode (Table S5). The mean recovery of the *p*-terphenyl-d_{14} surrogate standard obtained from each sample extraction was 94 %. The limit of detection of the individual PAHs was in the range of 0.5–5.0 ng/ml. In this study, the total concentration of PAHs was determined by summing the 16 individual USEPA PAHs. These PAHs were categorized into three groups based on their molecular weight and the number of rings: (1) low-molecular-weight (LMW) PAHs – the sum of NAP, ACY, ACE, FLU, PHE and ANT; (2) medium-molecular-weight (MMW) PAHs – the sum of FLUO, PYR, BaA and CHR; and (3) high-molecular-weight (HMW) PAHs – the sum of BbF, BkF, BaP, IPY, DBA and BghiP.

Soil activities of hydrolytic and ligninolytic enzymes were analysed according to Hřebečková et al. (2020) and measured from lyophilized soil samples using a Tecan 96-well plate spectrophotometer (Infinite M200, Tecan Trading, Switzerland) in four replicates.

2.6. Statistical analysis and data processing

Statistical analysis was conducted to evaluate the differences between treatments using one-way analysis of variance followed by Tukey's test at a significance level of $P < 0.05$ through the Statistica 7 software package (StatSoft, USA); *t*-tests were employed to assess the statistical significance of the datasets between two sampling times, specifically at 0 and 120 days. Standard deviations (SD) were also performed using Microsoft Excel 2010 (Microsoft Corporation, USA). Mean ± SD values were used to express the data, with distinct letters shown above the bars in the figures and within the tables representing significant differences ($P < 0.05$) among the different concentrations. The PAH concentrations in the soil and plant tissues were calculated based on their dry weight. The bioconcentration factor (BCF) was determined by calculating the ratio of the PAH concentration in maize roots (C_{roots}) to the PAH concentration in the soil (C_{soil}), whereas the transfer factor (TF) was calculated by dividing the PAH concentration in the shoots (C_{shoots}) by the PAH concentration in the roots (C_{roots}). The calculations for both the BCF and the TF were performed using Microsoft Excel 2010 (Microsoft Corporation, USA).

3. Results and discussion

3.1. PAHs in non-treated sludge-soil (SS)

Over the course of the experiment, the soil-sludge treatment showed a slight reduction in the concentration of investigated individual PAHs compounds (Figs. 1–3). During the entire experimental period of 120 days, the initial total PAH concentration in the SS treatment (Table S6), which was 1165 μg/kg dw, only decreased by 18 % (Fig. 4), which was not significantly different ($P < 0.05$) compared to its respective initial content at the start of the experiment. The result was in accordance with Houshani et al. (2019), who reported a total PAHs maximum degradation rate of 31 % from bare (non-planted) soil after 120 days of experimentation. In contrast to the findings of the current study, Feng et al. (2019) reported a higher degradation of PAHs in non-planted soil amended with sewage sludge (a value of 53 %), with total PAHs concentrations of 149.3–306.7 μg/kg dw. Similarly, Tomczyk et al. (2020) also reported better degradation of Σ16 PAHs in sewage sludge-amended soil (in the range of 48–57 %) compared to the current study. However, the residual PAHs concentration was decreased negligibly (Tables S6 and S7). The degradation of PAHs can be enhanced by photodegradation and leaching processes. However, the removal of PAHs from soil can be limited by factors such as the oxygen supply provided by the sewage sludge and its specific surface area (Kottler and Alexander, 2001).

In terms of individual PAHs, 2–3-ring PAHs exhibited a higher removal percentage. Specifically, ACY, NAP ACY, and ACE had removal values of 33.6 %, 56.7 % and 78.6 %, respectively (Fig. 1). A higher

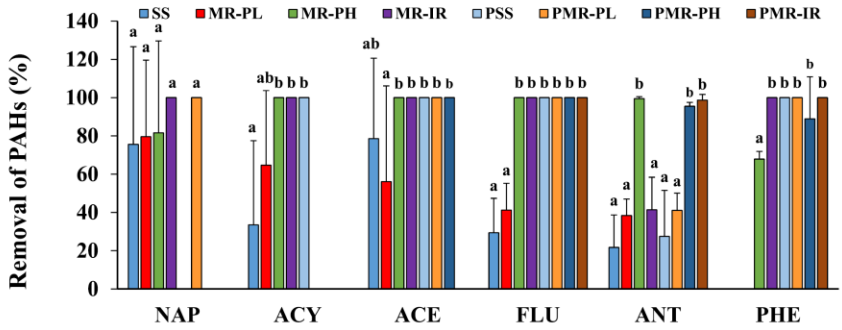


Fig. 1. The percentage removal (%) of individual low molecular weight PAHs from soil-sludge at the end of the experiment
The data represent means (±SD) of four replicate measurements. The same lowercase letters above bars indicate that differences among treatments were not significant at $P < 0.05$ as determined by Turkey's test. Abbreviations: SS = natural attenuation of soil-sludge; MR-PL = mycoremediation of soil-sludge with fungal substrates of *P. ostreatus*; MR-PH = mycoremediation of soil-sludge with fungal substrates of *P. chrysosporium*; MR-IR = mycoremediation of soil-sludge with fungal substrates of *I. lacteus*; PSS = Phytoremediation of soil-sludge using maize; PMR-PL = plant assisted mycoremediation of soil-sludge with fungal strains of *P. ostreatus*; PMR-PH = plant assisted mycoremediation of soil-sludge with fungal strains of *P. chrysosporium*; PMR-IR = plant assisted mycoremediation of soil-sludge with fungal strains of *I. lacteus*.

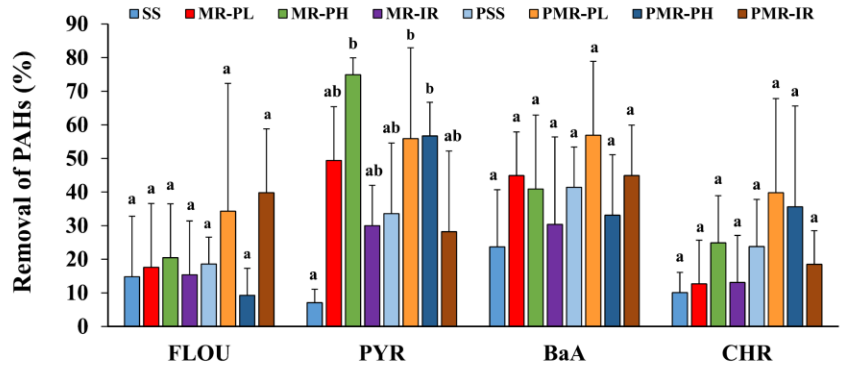


Fig. 2. The percentage removal (%) of individual medium molecular weight PAHs from soil-sludge at the end of the experiment
The data represent means (±SD) of four replicate measurements. The same lowercase letters above bars indicate that differences among treatments were not significant at $P < 0.05$ as determined by Turkey's test. Abbreviations: SS = natural attenuation of soil-sludge; MR-PL = mycoremediation of soil-sludge with fungal substrates of *P. ostreatus*; MR-PH = mycoremediation of soil-sludge with fungal substrates of *P. chrysosporium*; MR-IR = mycoremediation of soil-sludge with fungal substrates of *I. lacteus*; PSS = Phytoremediation of soil-sludge using maize; PMR-PL = plant assisted mycoremediation of soil-sludge with fungal strains of *P. ostreatus*; PMR-PH = plant assisted mycoremediation of soil-sludge with fungal strains of *P. chrysosporium*; PMR-IR = plant assisted mycoremediation of soil-sludge with fungal strains of *I. lacteus*.

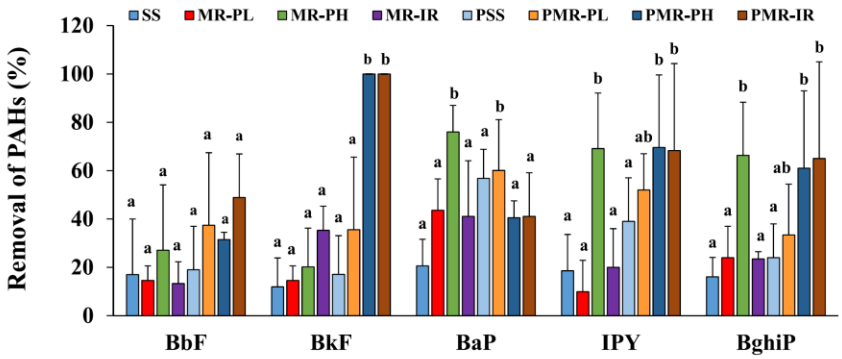


Fig. 3. The percentage removal (%) of individual high molecular weight PAHs from soil-sludge at the end of the experiment
The data represent means (±SD) of four replicate measurements. The same lowercase letters above bars indicate that differences among treatments were not significant at $P < 0.05$ as determined by Turkey's test. Abbreviations: SS = natural attenuation of soil-sludge; MR-PL = mycoremediation of soil-sludge with fungal substrates of *P. ostreatus*; MR-PH = mycoremediation of soil-sludge with fungal substrates of *P. chrysosporium*; MR-IR = mycoremediation of soil-sludge with fungal substrates of *I. lacteus*; PSS = Phytoremediation of soil-sludge using maize; PMR-PL = plant assisted mycoremediation of soil-sludge with fungal strains of *P. ostreatus*; PMR-PH = plant assisted mycoremediation of soil-sludge with fungal strains of *P. chrysosporium*; PMR-IR = plant assisted mycoremediation of soil-sludge with fungal strains of *I. lacteus*.

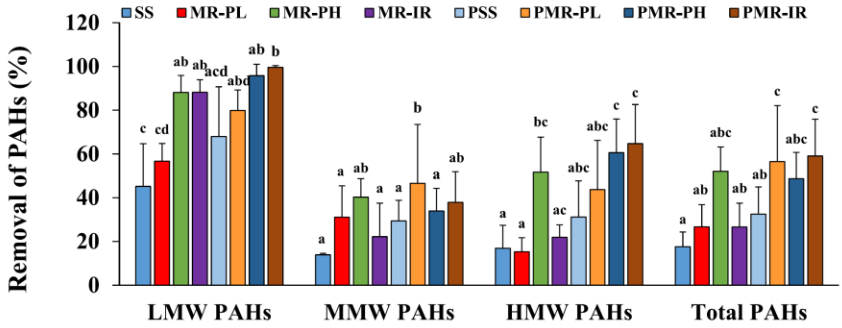


Fig. 4. The percentage removal (%) of LMW PAHs, MMW PAHs, HMW PAHs, and Total sum of PAHs from soil-sludge at the end of the experiment
The data represent means (±SD) of four replicate measurements. The same lowercase letters above bars indicate that differences among treatments were not significant at $P < 0.05$ as determined by Turkey's test. Abbreviations: SS = natural attenuation of soil-sludge; MR-PL = mycoremediation of soil-sludge with fungal substrates of *P. ostreatus*; MR-PH = mycoremediation of soil-sludge with fungal substrates of *P. chrysosporium*; MR-IR = mycoremediation of soil-sludge with fungal substrates of *I. lacteus*; PSS = Phytoremediation of soil-sludge using maize; PMR-PL = plant assisted mycoremediation of soil-sludge with fungal strains of *P. ostreatus*; PMR-PH = plant assisted mycoremediation of soil-sludge with fungal strains of *P. chrysosporium*; PMR-IR = plant assisted mycoremediation of soil-sludge with fungal strains of *I. lacteus*; LMW, MMW, and HMW PAHs: low, medium and high molecular weight polycyclic aromatic hydro carbons, respectively.

degradation of low molecular weight PAHs can be attributed to their chemical structure, which makes them more easily degradable and prone to volatilization (Tomczyk et al., 2020). In contrast, degradation of 4–6-ring PAHs was relatively lower, as PYR, CHR and BkF were negligibly removed with values of 7.1 %, 10.1 % and 11.9 %, respectively (Figs. 2 and 3). Similar findings were reported by García-Sánchez et al. (2018), where removal of PAHs from the soil decreased as the molecular weights of the PAHs increased. The limited PAH decrease can be attributed to the higher hydrophobicity and lower bioavailability of high molecular weight PAHs, as they exhibit a stronger affinity for adsorption onto the soil matrix. According to a study by Baldantoni et al. (2017), after 140 days there was a significant reduction of over 60 % in the concentration of ANT, while the concentration of BaP decreased by < 40 %. The degradation rate of ANT was higher, with only 5 % of its initial concentration remaining after 5 months. On the other hand, BaP showed a slower degradation rate in soil, as it retained approximately 50 % of its original concentration even after 9 months. This is likely to be because BaP has lower water solubility than ANT and tends to adhere to the soil organic matter, making it less available to microbial communities and therefore less biodegradable. Enzymatic activities were examined to evaluate their contribution to the degradation of PAHs (Table 1). In the SS treatment, no significant differences ($P < 0.05$) were observed between the initial time and the values recorded at the end of the experiment, which indicates no disturbances of the sludge-soil microorganisms. Similarly, in the study by Košnář et al. (2019), no significant differences were observed in the investigated ligninolytic enzymes (LAC and MnP) or other extracellular enzymes at the end of the experiment when compared to their respective initial values.

Table 1
The activities of selected extracellular enzymes in the sewage sludge amended soil at the end of an experiment.

	Lipase (μmol MUFY/g dw)	Laccase (mmol ABTS/g dw)	Mn-peroxidase (mmol DMAB/g dw)
SS	856 ± 160a	13.6 ± 17a	2.9 ± 5.7 ab
MR-PL	743 ± 435a	30.3 ± 28b	n.d.
MR-PH	499 ± 82a	21.6 ± 14 ab	n.d.
MR-IR	491 ± 118a	14.5 ± 12a	3.2 ± 6.5 ab
PSS	571 ± 157a	17.9 ± 9 ab	2.6 ± 4.1 ab
PMR-PL	542 ± 97a	32.7 ± 38b	5.3 ± 7.2c
PMR-PH	527 ± 77a	25.2 ± 11b	2.5 ± 3.5 ab
PMR-IR	723 ± 195a	29.1 ± 25b	3.7 ± 4.1b

The data represent means (±SD) of four replicate measurements. The same lowercase letters within the same column indicate lack of statistical differences ($P < 0.05$) among treatments as determined by Turkey's test. Abbreviations: n.d. = not detected; SS = natural attenuation of soil-sludge; MR-PL, MR-PH, MR-IR = mycoremediation of soil-sludge with fungal substrates of *P. ostreatus, P. chrysosporium, I. lacteus*, respectively; PSS = Phytoremediation of soil-sludge using maize; PMR-PL, PMR-PH, PMR-IR: plant assisted mycoremediation of soil-sludge with fungal strains of *P. ostreatus, P. chrysosporium, I. lacteus*, respectively.

3.2. Mycoremediation of PAHs in sludge-soil (MR)

The mycoremediation (MR) treatments appeared to be more promising for removing PAHs from the sludge-soil compared to the soil-sludge (SS) treatment. The Σ16 PAHs in mycoremediation by *P. chrysosporium* (MR-PH) treatment showed a reduction of 832 μg/kg dw within the experimental time frame (Tables S8 and S9), which accounted for a removal percentage of 52 % (Fig. 4). The decrease was significantly higher ($P < 0.05$) than the soil-sludge and other mycoremediation treatments. The pattern of degradation of LMW PAHs was higher than HMW PAHs, with the percentage removal by mycoremediation treatments in the following decreasing order: LMW > MMW > HMW. Ligninolytic fungi of *P. chrysosporium* showed the best degradation potential of LMW PAHs, with a value of 84.4 %. The other fungi species, *P. ostreatus* and *I. lacteus*, removed 48 % and 60 % of LMW PAHs, respectively (Fig. 4). Byss et al. (2008) found a similar degradation rate of low molecular weight PAHs compared to the current study using *I. lacteus* and higher degradation using *P. ostreatus*, in the range of 33–59 % and 48–72 %, respectively. However, in the current study, 51.7 %, 22.1 % and 15.3 % of high molecular weight PAHs were removed by *P. chrysosporium*, *I. lacteus* and *P. ostreatus*, respectively (Fig. 3). Previous studies have provided evidence that white-rot fungi, specifically *P. chrysosporium*, exhibit a remarkable ability to efficiently oxidize and mineralize various aromatic compounds found in polluted soils. These compounds encompass a wide range of substances, including HMW BaP (Hadibarata et al., 2022; Abo-State et al., 2021). Oleszczuk (2006) also mentioned that the degradation of high molecular weight PAHs (5–6 rings) can be achieved through co-metabolic processes.

Based on the individual PAH removal, both *P. chrysosporium* and *I. lacteus* degraded ACY, ACE and FLU completely (Fig. 1), while *P. ostreatus* removed 38–79 % of LMW PAHs (Fig. 4). However, significantly lower removal rates of individual HMW PAHs from soil, such as BkF, BbF, BaP, IPY and BghiP, were observed in sludge-soil with *P. ostreatus* (MR-PL) and *I. lacteus* (MR-IR), ranging from 8.1 to 33.9 μg/kg dw (Table S8). Similarly, Covino et al. (2010) reported a lower degradation of medium molecular weight and high molecular weight PAHs by *P. ostreatus*, including CHRY, BbF and BkF. The reported

degradation rates were 29.7 %, 39.7 % and 32.8 %, respectively. On the other hand, *P. chrysosporium* (MR-PH) treatment showed better significant removal of total high molecular weight PAHs ($P < 0.05$) than other MR treatments and the SS treatment, with a 416 μg/kg dw decrease from the initial concentration after 120 days of the experiment (Table S9). Soil-sludge with *P. ostreatus* substrate (MR-PL) treatment had significantly higher ($P < 0.05$) laccase activities compared to the other MR and SS treatments conducted. Moreover, soil-sludge with *P. chrysosporium* (MR-PH) treatment also showed comparable laccase activity after 120 days of the experiment (a value of 21.5 mmol ABTS/g dw) but registered significantly higher ($P < 0.05$) degradation of total PAHs compared to the soil-sludge (SS) and other mycoremediation (MR) treatments (Fig. 4). Extracellular manganese peroxidase activity was not found with either the *P. ostreatus* substrate (MR-PL) or *P. chrysosporium* (MR-PH) treatment. Surprisingly, *P. chrysosporium* showed better degradation performance of PAHs in the MR treatments but the enzyme concentrations were not satisfactory compared to other MR treatments. In fact, *P. chrysosporium* can adsorb PAHs in its mycelial pellets owing to its abundant conjugated structures (C=C and aromatic components) and high carbon content (Gu et al., 2015). Previous studies have indicated that the process of biosorption, specifically the adsorption of PAHs, by the fungus of *P. chrysosporium* may be a crucial factor in the removal of PAHs. This is due to several characteristics of the fungal mycelium, such as its significant carbon content, its aliphatic and aromatic components and its various chemical groups (Li et al., 2010; Chen and Ding, 2012).

3.3. Phytoremediation of PAHs in sludge-soil (PSS)

Dissipation of the total PAHs from phytoremediated sludge-soil was significantly higher ($P < 0.05$) compared to the soil-sludge (SS) treatment but lower than for the mycoremediation (MR) treatments. The detected degradation rate during the time frame of the study was 32 %, accounting for a PAH concentration of 301 μg/kg dw (Fig. 4). García-Sánchez et al. (2018) also reported the removal of Σ16 PAHs by using maize after 120 days of experiment, with a higher degradation rate of 37.1 %. A study by Feng et al. (2019) reported higher degradation of total PAHs in the sewage sludge-amended soils after 126 days of experiment, with a removal rate of 60.1 % by using the tall fescue plant. Guo et al. (2017b) also reported improved degradation of total PAHs with the presence of maize root exudates during their incubation experiment on aged agricultural soil, varying from 69.2 % to 78.4 %.

Degradation of the sum of low molecular weight PAHs was higher than that of high molecular weight PAHs, with a removal rate of 68 % (Fig. 1). Among the individual low molecular weight PAHs, ACE, FLU and PHE were completely removed on phytoremediation soil-sludge (PSS) treatment (Fig. 1). These higher dissipation rates could be attributed to the active participation of maize plants in the rhizospheric breakdown of PAHs (Segura and Ramos, 2013; Sun et al., 2013). LMW-PAHs might also exhibit a faster process of adsorption and transfer within plant cells compared to HMW-PAHs (Ramezanzadeh Arvanaghi et al., 2017). BaP and IPY had the highest removal rates among the high molecular weight PAHs, with values of 56.8 % and 39.0 %, respectively (Fig. 3). The enzymatic activities of lipase, laccase and manganese peroxidase in the PSS treatment did not show significant differences compared to the soilsludge (SS) treatments ($P < 0.05$) (Table 1). However, laccase activity was significantly lower in the PSS treatment compared to the plant-assisted mycoremediation (PMR) treatments at the end of the experiment. Nevertheless, the dissipation of PAHs from planted soil-sludge was significantly ($P < 0.05$) higher than in bare sludge-soil. This could potentially be attributed to the action of plant enzymes released through root exudates, which enhance the accessibility of PAHs and provide additional materials for co-metabolic degradation. Additionally, the soil environment is modified in a manner that creates a more favourable condition for the indigenous microbial degradation of PAHs (Hamdi et al., 2012; Feng et al., 2014; Grifoni et al., 2020).

3.4. Plant-assisted mycoremediation of PAHs in sludge-soil (PMR)

During the experimental period, the highest reduction in total PAHs concentrations was observed in plant-assisted mycoremediation treatments utilizing *I. lacteus* substrate (PMR-IR) and *P. ostreatus* substrate (PMR-PL). These treatments achieved average reductions of 60 % and 57 %, respectively. These values were significantly greater ($P < 0.05$) compared to the degradations achieved in other experimental treatments (Fig. 4). Furthermore, when using *P. ostreatus* in plant-assisted mycoremediation, that treatment also demonstrated a more satisfactory degradation potential of PAHs than the SS and PSS treatments, with a removal rate of 57 % at the end of the experiment. For plant-assisted mycoremediation (PMR) treatments, the concentration of total PAHs in the soil at the end of the experiment showed a significant decrease compared to their respective initial concentrations (Table S7). The plant assisted mycoremediation treatments with *P. ostreatus* substrate (PMR-PL) and *with P. chrysosporium* substrate (PMR-PH) were able to remove ACE and FLU completely (100 %), and with *I. lacteus* substrate (PMR-IR) treatment showed a removal rate higher than 99 % for FLU, ANT and PHE compared to their initial concentrations (Fig. 1). Similarly, Deng and Zeng (2017) have studied the degradation of PHE by *P. chrysosporium* combined with alfalfa, resulting in a degradation rate of 85 % compared to its initial concentration after 60 days. BkF, IPY and BghiP were dissipated in the soil of both the *with P. chrysosporium* substrate (PMR-PH) and with *I. lacteus* substrate (PMR-IR) treatments, with a removal rate of 61–100 % in the time frame of study, while other medium molecular weight and high molecular weight PAHs compounds exhibited a lower dissipation rate of 9–44 %. The removal of IPY and BghiP in PMR treatments was significantly higher ($P < 0.05$) compared to SS treatment at the end of the experiment. During the experimental time frame, the enzymatic activities of laccase and manganese peroxidase in the PMR treatments were significantly higher in comparison with both the SS and PSS treatments (Table 1). Indeed, root exudates serve as a carbon source and nutrient supply that enhance the proliferation of soil fungi (Liu et al., 2013). As also noted by Muratova et al. (2009), root exudates play a role in stimulating the growth and vitality of soil microorganisms, potentially resulting in improved enzyme secretion. This finding suggests that the increased enzymatic activity could be a contributing factor to the higher removal rates of total PAHs observed in the PMR treatments compared to the other studied treatments. The potential for large-scale application of plant-assisted mycoremediation seems promising, given its heightened efficiency in PAH removal by enhancing enzymatic activity. Choosing treatments exhibiting notably higher degradation rates allows for the strategic selection of specific fungal strains, optimizing the degradation of PAHs.

3.5. Bioconcentration of PAHs in maize

Fig. 5 shows the bioconcentration of PAHs in the roots and shoots of maize in the experimental period. The yield of dried roots and above ground biomass at the end of study is shown in Table S12. The sum of low molecular weight PAH concentrations in the root ranged from 21.4 to 42.8 μg/kg dw. In all the PSS and PMR treatments, the root samples showed a higher concentration of ANT compared to other compounds, with values ranging from 21.4 to 36.6 μg/kg dw (Table S10). This could be attributed to the compound's higher solubility (76 μg/l) (Table S4) compared to other low molecular weight PAHs. Additionally, according to Wild et al. (2005), the uptake of ANT by maize was directly associated with water uptake, in which the compound's solubility is a contributing factor. Another reason is most likely the degree and nature of its interactions with the cell walls and other structures. Following ANT, FLUO was observed in the concentration range 14.7–23.1 μg/kg dw in the root samples (Table S10). The concentrations of medium molecular weight and high molecular weight PAHs in the root samples of maize ranged from 22.0 to 47.6 μg/kg dw (Fig. 5). The concentration of ∑16 PAHs in the phytoremediation soil-sludge (PSS) treatment reached a

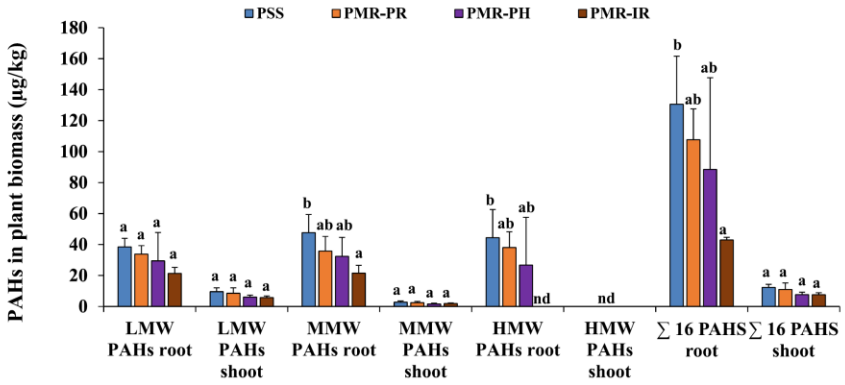


Fig. 5. The concentration of the total sum of LMW, MMW, and HMW PAHs and total PAHs in plant biomass (µg/kg dw) at the end of the 120–day experiment
The data represent means (±SD) of four replicate measurements. The same lowercase letters above bars indicate that differences among treatments were not significant at $P < 0.05$ as determined by Turkey's test. Abbreviations: nd = not detected; PSS = Phytoremediation of soil-sludge using maize; PMR-PL = plant assisted mycoremediation of soil-sludge with fungal strains of *P. ostreatus*; PMR-PH = plant assisted mycoremediation of soil-sludge with fungal strains of *P. chrysosporium*; PMR-IR = plant assisted mycoremediation of soil-sludge with fungal strains of *I. lacteus*; LMW, MMW, and HMW PAHs: low, medium and high molecular weight polyaromatic hydro carbons, respectively.

significantly higher ($P < 0.05$) value of 131 µg/kg dw than the other plant-assisted mycoremediation (PMR) treatments, with values in the range of 43–108 µg/kg dw (Fig. 5). This could be due to the increased microbial activity due to the addition of fungal substrate influencing the availability of PAHs and its uptake by plants (Houshani et al., 2019).

In the maize shoots, only ACE, ANT, FLUO and PYR were detected, belonging to the group of low molecular weight and medium molecular weight PAHs (Table S11); it was observed that the concentration of LMW PAHs was higher than that of the medium molecular weight PAHs. Among the low molecular weight PAHs, a higher concentration was found in the case of ANT, with the value varying from 5.7 to 8.9 µg/kg dw for both the PSS and plant-assisted mycoremediation (PMR) treatments. On the other hand, FLUO and PYR were found at concentrations of 0.8–1.8 and 0.6–1.1 µg/kg dw, respectively (Table S11). In the PM-IR treatment, while ANT was found in the roots, other compounds within the group of low molecular weight and high molecular weight PAHs were not detected in either the root or shoot of maize. The absence of high molecular weight PAHs in maize shoots suggests a possible reason why PAHs with more aromatic rings are more hydrophobic and physically less able to traverse cell membranes (Gao and Zhu, 2004).

A significant ($P < 0.05$) positive correlation (Table S13) was found between the maize root yield and the concentrations of medium molecular weight and high molecular weight PAHs in the roots. BghiP, IPY, CHR and BaP showed a positive correlation, with coefficients of 0.57, 0.64, 0.72 and 0.78, respectively. Among the compounds detected in the shoots of maize, only FLUO had a significantly positive correlation with the above-ground biomass yield, with a coefficient of 0.50.

Tables 2 and 3 present the BCF and TF results for the PAHs in maize; BCF and TF calculations were employed to assess the plant's capacity for accumulating PAHs in its tissues from the soil, thus providing an insight into the bioaccumulation and transfer of PAHs from roots to shoots, respectively. The findings revealed that the plant biomass exhibited higher BCF values of 0.53–0.99 for ANT, indicating significant accumulation of this compound, facilitated by both fungal strains and ANT removal from the soil. During PMR-PH treatment, PYR, IPY and BghiP showed notably higher ($P < 0.05$) BCF values compared to the other treatments.

Specifically, ACE demonstrated a significantly higher ($P < 0.05$) TF value of 0.92 in the PSS treatment than with the PMR treatments. The other detected compounds, namely ANT, FLUO and PYR, exhibited TF values of 0.26–0.07. In all investigated treatments, the TF and BCF values were <1, indicating safe production of maize on sludge-amended soil. These values imply that maize had limited capacity to extract

Table 2
Bioconcentration factor of detected PAHs in the roots of maize.

	PSS	PMR-PL	PMR-PH	PMR-IR
ANT	0.68 ± 0.09a	0.53 ± 0.06a	0.99 ± 0.7b	0.95 ± 0.07b
FLOU	0.18 ± 0.05b	0.11 ± 0.02 ab	0.09 ± 0.02a	0.09 ± 0.01 ab
PYR	0.17 ± 0.04 ab	0.09 ± 0.02a	0.20 ± 0.07b	0.06 ± 0.01a
BaA	0.09 ± 0.05a	0.08 ± 0.09a	0.04 ± 0.02a	0.02 ± 0.001a
CHR	0.11 ± 0.04a	0.06 ± 0.02a	0.15 ± 0.09a	–
BbF	0.20 ± 0.09a	0.11 ± 0.04a	0.07 ± 0.08a	–
BaP	0.18 ± 0.04a	0.11 ± 0.04a	0.16 ± 0.02a	–
IPY	0.25 ± 0.09a	0.15 ± 0.05a	0.54 ± 0.06b	–
BghiP	0.19 ± 0.7a	0.13 ± 0.01a	0.38 ± 0.04b	–

The data represent means (±SD) of four replicate measurements. The same lowercase letters within the same row indicate lack of statistical differences ($P < 0.05$) among treatments as determined by Turkey's test. Abbreviations: - = not estimated; PSS = Phytoremediation of soil-sludge using maize; PMR-PL, PMR-PH, PMR-IR: plant assisted mycoremediation of soil-sludge with fungal strains of *P. ostreatus, P. chrysosporium, I. lacteus*, respectively.

Table 3
Transfer factor of detected PAHs from roots into the shoots of maize.

	PSS	PMR-PL	PMR-PH	PMR-IR
ACE	0.9 ± 0.4a	0.95 ± 0.8a	0.31 ± 0.09a	n.d.
ANT	0.24 ± 0.05a	0.25 ± 0.07a	0.21 ± 0.11a	0.27 ± 0.08a
FLUO	0.08 ± 0.03a	0.07 ± 0.03a	0.05 ± 0.03a	0.09 ± 0.02a
PYR	0.07 ± 0.02a	0.11 ± 0.03a	0.07 ± 0.05a	0.09 ± 0.02a

The data represent means (±SD) of four replicate measurements. The same lowercase letters within the same row indicate lack of statistical differences ($P < 0.05$) among treatments as determined by Turkey's test. Abbreviations: n.d. = not detected; PSS = Phytoremediation of soil-sludge using maize; PMR-PL, PMR-PH, PMR-IR: plant assisted mycoremediation of soil-sludge with fungal strains of *P. ostreatus, P. chrysosporium, I. lacteus*, respectively.

considerable quantities of PAHs from the soil and transport them to the shoots (Agnello et al., 2016). The lipophilic and hydrophobic nature of MMW and HMW PAHs has been identified as the main reason for the restricted translocation from roots to shoots (Gao et al., 2010; Houshani et al., 2019).

4. Conclusion

The plant-assisted mycoremediation treatments achieved the most effective removal of PAHs from the soil in this study. The degradation

rates of total PAHs of these treatments using different ligninolytic fungi species ranged between 48 % and 60 %. Among the different groups of PAHs, the LMW PAHs showed a greater tendency to be removed by all tested treatments compared to the other PAH groups. Mycoremediation treatment using the fungus *P. chrysosporium* showed a significantly higher degradation rate of total PAHs compared to the natural attenuation sludge-soil and the other fungal strains used in the treated sludge-soil, achieving removal rates of 60 %. While most of the analysed PAHs were found in the roots of maize, only four PAHs were detected in the maize shoots and their concentrations were relatively low. Moreover, the BCF and TF were <1, indicating that the PAHs were not significantly accumulated or transported within the plant. Notably, the plant-assisted mycoremediation approach displayed higher enzymatic activities compared to the other studied treatments, ultimately resulting in better degradation of the PAHs by the end of the experiment. Future investigations could explore the complex interactions among diverse plant and fungi species, soil components, and PAHs by delving into varied enzymatic activities and the associated biochemical pathways in the degradation process. Moreover, conducting extensive, long-term studies to assess the sustained effectiveness and viability of plant-assisted mycoremediation would offer insights into treatment stability and its prolonged impact on soil health.

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.

Acknowledgements

The authors gratefully acknowledge the support given by the Czech Ministry of Agriculture (Projects QK1710379 and QK1910235) and by the European Regional Development Fund (Project NUTRISK: No. CZ.02.1.01/0.0/0.0/16_019/0000845).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2024.01.007.

References

Abatenh, E., Gizaw, B., Tsegaye, Z., Wassie, M., Abatenh, E., Gizaw, B., Tsegaye, Z., Wassie, M., 2017. The role of microorganisms in bioremediation- A review. Open Journal of Environmental Biology 2, 38–46. https://doi.org/10.17352/ojeb.000007.

Abdel-Shafy, H.I., Mansour, M.S.M., 2018. Phytoremediation for the Elimination of metals, Pesticides, PAHs, and other pollutants from wastewater and soil. In: Kumar, V., Kumar, M., Prasad, R. (Eds.), Phytobiont and Ecosystem Restitution. Springer, Singapore, pp. 101–136. https://doi.org/10.1007/978-981-13-1187-1_5.

Abo-State, M.A.M., Osman, M.E., Khattab, O.H., El-Kelani, T.A., Abdel-Rahman, Z.M., 2021. Degradative pathways of polycyclic aromatic hydrocarbons (PAHs) by Phanerochaete chrysosporium under optimum conditions. Journal of Radiation Research and Applied Sciences 14, 507–520. https://doi.org/10.1080/16878507.2021.2001247.

Agnello, A.C., Bagard, M., van Hullebusch, E.D., Esposito, G., Huguenot, D., 2016. Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. Sci. Total Environ. 563 (564), 693–703. https://doi.org/10.1016/j.scitotenv.2015.10.061.

Baldantoni, D., Morelli, R., Bellino, A., Prati, M.V., Alfani, A., De Nicola, F., 2017. Anthracene and benzo(a)pyrene degradation in soil is favoured by compost amendment: Perspectives for a bioremediation approach. J. Hazard Mater. 339, 395–400. https://doi.org/10.1016/j.jhazmat.2017.06.043.

Barbosa, F., Rocha, B.A., Souza, M.C.O., Bocato, M.Z., Azevedo, L.F., Adeyemi, J.A., Santana, A., Campiglia, A.D., 2023. Polycyclic aromatic hydrocarbons (PAHs): updated aspects of their determination, kinetics in the human body, and toxicity. J. Toxicol. Environ. Health, Part A B 26, 28–65. https://doi.org/10.1080/10937404.2022.2164390.

Byss, M., Elhottová, D., Tříska, J., Baldrian, P., 2008. Fungal bioremediation of the creosote-contaminated soil: influence of Pleurotus ostreatus and Irpex lacteus on polycyclic aromatic hydrocarbons removal and soil microbial community composition in the laboratory-scale study. Chemosphere 73, 1518–1523. https://doi.org/10.1016/j.chemosphere.2008.07.030.

Chane, A.D., Košnář, Z., Hřebečková, T., Wiesnerová, L., Jozífek, M., Doležal, P., Praus, L., Tlustoš, P., 2023a. Bioremediation of the synthetic musk compounds Galaxolide and Tonalide by white rot fungal strain-assisted phytoremediation in biosolid-amended soil. Chemosphere 328, 138605. https://doi.org/10.1016/j.chemosphere.2023.138605.

Chane, A.D., Košnář, Z., Hřebečková, T., Wiesnerová, L., Jozífek, M., Doležal, P., Praus, L., Tlustoš, P., 2023b. Bioremediation of the synthetic musk compounds Galaxolide and Tonalide by white rot fungal strain-assisted phytoremediation in biosolid-amended soil. Chemosphere 328, 138605. https://doi.org/10.1016/j.chemosphere.2023.138605.

Chen, B., Ding, J., 2012. Biosorption and biodegradation of phenanthrene and pyrene in sterilized and unsterilized soil slurry systems stimulated by Phanerochaete chrysosporium. J. Hazard Mater. 229 (230), 159–169. https://doi.org/10.1016/j.jhazmat.2012.05.090.

Chojnacka, K., Skrzypczak, D., Szopa, D., Izydorczyk, G., Moustakas, K., Witek-Krowiak, A., 2023. Management of biological sewage sludge: fertilizer nitrogen recovery as the solution to fertilizer crisis. J. Environ. Manag. 326, 116602 https://doi.org/10.1016/j.jenvman.2022.116602.

Cieślik, B.M., Namieśnik, J., Konieczka, P., 2015. Review of sewage sludge management: standards, regulations and analytical methods. J. Clean. Prod. 90, 1–15. https://doi.org/10.1016/j.jclepro.2014.11.031.

Covino, S., Čvančarová, M., Muzikář, M., Svobodová, K., D'annibale, A., Petruccioli, M., Federici, F., Křesinová, Z., Cajthaml, T., 2010. An efficient PAH-degrading Lentinus (Panus) tigrinus strain: effect of inoculum formulation and pollutant bioavailability in solid matrices. J. Hazard Mater. 183, 669–676. https://doi.org/10.1016/j.jhazmat.2010.07.078.

Deng, S., Zeng, D., 2017. Removal of phenanthrene in contaminated soil by combination of alfalfa, white-rot fungus, and earthworms. Environ. Sci. Pollut. Res. 24, 7565–7571. https://doi.org/10.1007/s11356-017-8466-y.

Feng, Lijuan, Zhang, L., Feng, Li, 2014. Dissipation of polycyclic aromatic hydrocarbons in soil amended with sewage sludge compost. International Biodeterioration & Biodegradation. Challenges in Environmental Science and Engineering. https://doi.org/10.1016/j.ibiod.2014.04.012. CESE-2013 95, 200–207.

Feng, L.-J., Zhang, L.-Q., Feng, L., Li, J.-L., 2019. Dissipation of polycyclic aromatic hydrocarbons (PAHs) in soil amended with sewage sludge and sludge compost. Environ. Sci. Pollut. Res. 26, 34127–34136. https://doi.org/10.1007/s11356-018-3383-2.

Gao, D., Du, L., Yang, J., Wu, W.-M., Liang, H., 2010. A critical review of the application of white rot fungus to environmental pollution control. Crit. Rev. Biotechnol. 30, 70–77. https://doi.org/10.3109/07388550903427272.

Gao, Y., Zhu, L., 2004. Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 55, 1169–1178. https://doi.org/10.1016/j.chemosphere.2004.01.037.

García-Sánchez, M., Košnář, Z., Mercl, F., Aranda, E., Tlustoš, P., 2018. A comparative study to evaluate natural attenuation, mycoaugmentation, phytoremediation, and microbial-assisted phytoremediation strategies for the bioremediation of an aged PAH-polluted soil. Ecotoxicol. Environ. Saf. 147, 165–174. https://doi.org/10.1016/j.ecoenv.2017.08.012.

Grifoni, M., Rosellini, I., Angelini, P., Petruzzelli, G., Pezzarossa, B., 2020. The effect of residual hydrocarbons in soil following oil spillages on the growth of *Zea mays* plants. Environ. Pollut. 265, 114950 https://doi.org/10.1016/j.envpol.2020.114950.

Gu, H., Luo, X., Wang, H., Wu, L., Wu, J., Xu, J., 2015. The characteristics of phenanthrene biosorption by chemically modified biomass of Phanerochaete chrysosporium. Environ. Sci. Pollut. Res. 22, 11850–11861. https://doi.org/10.1007/s11356-015-4451-5.

Guo, M., Gong, Z., Miao, R., Su, D., Li, X., Jia, C., Zhuang, J., 2017a. The influence of root exudates of maize and soybean on polycyclic aromatic hydrocarbons degradation and soil bacterial community structure. Ecol. Eng. 99, 22–30. https://doi.org/10.1016/j.ecoleng.2016.11.018.

Guo, M., Gong, Z., Miao, R., Su, D., Li, X., Jia, C., Zhuang, J., 2017b. The influence of root exudates of maize and soybean on polycyclic aromatic hydrocarbons degradation and soil bacterial community structure. Ecol. Eng. 99, 22–30. https://doi.org/10.1016/j.ecoleng.2016.11.018.

Gupte, A., Tripathi, A., Patel, H., Rudakiya, D., Gupte, S., 2016. Bioremediation of Polycyclic Aromatic Hydrocarbon (PAHs): A Perspective. The Open Biotechnology Journal 10. https://doi.org/10.2174/1874070701610010363.

Gworek, B., Klimczak, K., Kijeńska, M., Gozdowski, D., 2016. Comparison of PAHs uptake by selected Monocotyledones and Dicotyledones from municipal and industrial sewage sludge. Environ. Sci. Pollut. Res. 23, 19461–19470. https://doi.org/10.1007/s11356-016-7130-2.

Hadibarata, T., Kristanti, R.A., Bilal, M., Al-Mohaimeed, A.M., Chen, T.-W., Lam, M.K., 2022. Microbial degradation and transformation of benzo[a]pyrene by using a white-rot fungus Pleurotus eryngii F032. Chemosphere 307, 136014. https://doi.org/10.1016/j.chemosphere.2022.136014.

Hamdi, H., Benzarti, S., Aoyama, I., Jedidi, N., 2012. Rehabilitation of degraded soils containing aged PAHs based on phytoremediation with alfalfa (Medicago sativa L.). Int. Biodeterior. Biodegrad. 67, 40–47. https://doi.org/10.1016/j.ibiod.2011.10.009.

Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J. Hazard Mater. 169, 1–15. https://doi.org/10.1016/j.jhazmat.2009.03.137.

Houshani, M., Salehi-Lisar, S.Y., Motafakkerazad, R., Movafeghi, A., 2019. Uptake and distribution of phenanthrene and pyrene in roots and shoots of maize (*Zea mays* L.).

Environ. Sci. Pollut. Res. 26, 9938–9944. https://doi.org/10.1007/s11356-019-04371-3.
Hřebečková, T., Wiesnerová, L., Hanč, A., 2020. Changes in layers of laboratory vermicomposting using spent mushroom substrate of Agaricus subrufescens P. J. Environ. Manag. 276, 111340 https://doi.org/10.1016/j.jenvman.2020.111340.
Kadri, T., Rouissi, T., Kaur Brar, S., Cledon, M., Sarma, S., Verma, M., 2017a. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: a review. J. Environ. Sci. 51, 52–74. https://doi.org/10.1016/j.jes.2016.08.023.
Kadri, T., Rouissi, T., Kaur Brar, S., Cledon, M., Sarma, S., Verma, M., 2017b. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: a review. J. Environ. Sci. 51, 52–74. https://doi.org/10.1016/j.jes.2016.08.023.
Kijpornyongpan, T., Schwartz, A., Yaguchi, A., Salvachúa, D., 2022. Systems biology-guided understanding of white-rot fungi for biotechnological applications: a review. iScience 25, 104640. https://doi.org/10.1016/j.isci.2022.104640.
Košnář, Z., Částková, T., Wiesnerová, L., Praus, L., Jablonský, I., Koudela, M., Tlustoš, P., 2019. Comparing the removal of polycyclic aromatic hydrocarbons in soil after different bioremediation approaches in relationto the extracellular enzyme activities. J. Environ. Sci. 76, 249–258. https://doi.org/10.1016/j.jes.2018.05.007.
Kottler, B.D., Alexander, M., 2001. Relationship of properties of polycyclic aromatic hydrocarbons to sequestration in soil. Environ. Pollut. 113, 293–298. https://doi.org/10.1016/S0269-7491(00)00189-5.
Kumar, P., Kumar, V., Adelodun, B., Bedeković, D., Kos, I., Širić, I., Alamri, S.A.M., Alrumman, S.A., Eid, E.M., Abou Fayssal, S., Goala, M., Arya, A.K., Bachheti, A., Choi, K.S., Ajibade, F.O., Silva, L.F.O., 2022. Sustainable use of sewage sludge as a casing material for button mushroom (agaricus bisporus) cultivation: experimental and prediction modeling studies for uptake of metal elements. J Fungi (Basel) 8, 112. https://doi.org/10.3390/jof8020112.
Liu, S., Luo, Y., Cao, Z., Wu, L., Wong, M., 2013. Effect of ryegrass (Lolium multiflorum L.) growth on degradation of benzo [a] pyrene and enzyme activity in soil. J. Food Agric. Environ. 11, 1006–1011.
Li, Y., Chen, B., Zhu, L., 2010. Enhanced sorption of polycyclic aromatic hydrocarbons from aqueous solution by modified pine bark. Bioresource Technology 101, 7307–7313. https://doi.org/10.1016/j.biortech.2010.04.088.
Liu, X., Liu, W., Wang, Q., Wu, L., Luo, Y., Christie, P., 2017. Soil properties and microbial ecology of a paddy field after repeated applications of domestic and industrial sewage sludges. Environ. Sci. Pollut. Res. 24, 8619–8628. https://doi.org/10.1007/s11356-017-8543-2.
Ma, W.-L., Liu, L.-Y., Tian, C.-G., Qi, H., Jia, H.-L., Song, W.-W., Li, Y.-F., 2015. Polycyclic aromatic hydrocarbons in Chinese surface soil: occurrence and distribution. Environ. Sci. Pollut. Res. 22, 4190–4200. https://doi.org/10.1007/s11356-014-3648-3.
Moubarz, G., Saad-Hussein, A., Shahy, E.M., Mahdy-Abdallah, H., Mohammed, A.M.F., Saleh, I.A., Abo-Zeid, M.A.M., Abo-Elfadl, M.T., 2023. Lung cancer risk in workers occupationally exposed to polycyclic aromatic hydrocarbons with emphasis on the role of DNA repair gene. Int. Arch. Occup. Environ. Health 96, 313–329. https://doi.org/10.1007/s00420-022-01926-9.
Muratova, A., Pozdnyakova, N., Golubev, S., Wittenmayer, L., Makarov, O., Merbach, W., Turkovskaya, O., 2009. Oxidoreductase activity of sorghum root exudates in a phenanthrene-contaminated environment. Chemosphere 74, 1031–1036. https://doi.org/10.1016/j.chemosphere.2008.11.011.
Oleszczuk, P., 2006. Persistence of polycyclic aromatic hydrocarbons (PAHs) in sewage sludge-amended soil. Chemosphere 65, 1616–1626. https://doi.org/10.1016/j.chemosphere.2006.03.007.
Patel, A.B., Shaikh, S., Jain, K.R., Desai, C., Madamwar, D., 2020. Polycyclic aromatic hydrocarbons: sources, toxicity, and remediation approaches. Front. Microbiol. 11.
Ramezanzadeh Arvanaghi, H., Aminfar, Y., Amiri Sadeghan, A., Dolatabadi, N., 2017. Phytoremediation of polycyclic aromatic hydrocarbons (PAHs): air-plant-soil interactions. J. Biodivers. Environ. Sci. (JBES) 10, 178–184.
Segura, A., Ramos, J.L., 2013. Plant–bacteria interactions in the removal of pollutants. Current Opinion in Biotechnology, Energy biotechnology • Environmental biotechnology 24, 467–473. https://doi.org/10.1016/j.copbio.2012.09.011.
Suciu, N.A., Lamastra, L., Trevisan, M., 2015. PAHs content of sewage sludge in Europe and its use as soil fertilizer. Waste Manag. 41, 119–127. https://doi.org/10.1016/j.wasman.2015.03.018.
Sun, B., Ling, W., Wang, Y., 2013. Can root exudate components influence the availability of pyrene in soil? Journal of Soils and Sediments 13, 1161–1169.
Tomczyk, B., Siatecka, A., Jędruchniewicz, K., Sochacka, A., Bogusz, A., Oleszczuk, P., 2020. Polycyclic aromatic hydrocarbons (PAHs) persistence, bioavailability and toxicity in sewage sludge- or sewage sludge-derived biochar-amended soil. Sci. Total Environ. 747, 141123 https://doi.org/10.1016/j.scitotenv.2020.141123.
Tyagi, V.K., Lo, S.-L., 2013. Sludge: a waste or renewable source for energy and resources recovery? Renew. Sustain. Energy Rev. 25, 708–728. https://doi.org/10.1016/j.rser.2013.05.029.
Wild, E., Dent, J., Thomas, G.O., Jones, K.C., 2005. Direct observation of organic contaminant uptake, storage, and metabolism within plant roots. Environ. Sci. Technol. 39, 3695–3702. https://doi.org/10.1021/es048136a.
Włóka, D., Kacprzak, M., Rosikoń, K., Fijałkowski, K., 2013. A study of migration of polycyclic aromatic hydrocarbons in a sewage sludge-soil system. Environ. Protect. Eng. 115–124.
Wołejko, E., Wydro, U., Jabłońska-Trypuć, A., Butarewicz, A., Łoboda, T., 2018. The effect of sewage sludge fertilization on the concentration of PAHs in urban soils. Environ. Pollut. 232, 347–357. https://doi.org/10.1016/j.envpol.2017.08.120.
Wu, D., Chu, S., Lai, C., Mo, Q., Jacobs, D.F., Chen, X., Zeng, S., 2017. Application rate and plant species affect the ecological safety of sewage sludge as a landscape soil amendment. Urban For. Urban Green. 27, 138–147. https://doi.org/10.1016/j.ufug.2017.07.003.
Zhang, C., Xu, Y., Zhao, M., Rong, H., Zhang, K., 2018. Influence of inoculating white-rot fungi on organic matter transformations and mobility of heavy metals in sewage sludge based composting. J. Hazard Mater. 344, 163–168. https://doi.org/10.1016/j.jhazmat.2017.10.017.
Zhu, F., Storey, S., Ashaari, M.M., Clipson, N., Doyle, E., 2017. Benzo(a)pyrene degradation and microbial community responses in composted soil. Environ. Sci. Pollut. Res. 24, 5404–5414. https://doi.org/10.1007/s11356-016-8251-3.

4.4. Chane et al. (2023). Bioremediation of the synthetic musk compounds Galaxolide and Tonalide by white rot fungal strain-assisted phytoremediation in biosolid-amended soil.

Authors: **Abraham Demelash Chane**, Zdeněk Košnář, Tereza Hřebečková, Lucie Wiesnerová Miroslav Jozífek, Petr Doležal, Lukáš Praus, Pavel Tlustoš

Published in the journal: Chemosphere, 328, 138605
Information about the magazine: IF = 8.1 (2023)

Chemosphere 328 (2023) 138605

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Bioremediation of the synthetic musk compounds Galaxolide and Tonalide by white rot fungal strain-assisted phytoremediation in biosolid-amended soil

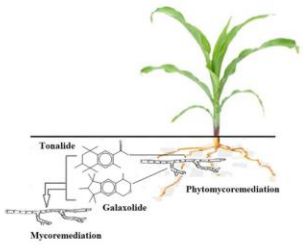
Abraham Demelash Chane [a], Zdeněk Košnář [a,*], Tereza Hřebečková [a], Lucie Wiesnerová [b,c], Miroslav Jozífek [b], Petr Doležal [a], Lukáš Praus [d], Pavel Tlustoš [a]

[a] Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 165 00, Praha, Suchdol, Czech Republic
[b] Department of Horticulture, Czech University of Life Sciences Prague, Kamýcká 129, 165 00, Praha, Suchdol, Czech Republic
[c] Faculty of Medicine in Pilsen, Department of Medical Chemistry and Biochemistry, Charles University in Prague, Husova 3, 301 00, Plzeň, Czech Republic
[d] Laboratory of Environmental Chemistry, Czech University of Life Sciences Prague, Kamýcká 129, 165 00, Praha, Suchdol, Czech Republic

HIGHLIGHTS

- Removal of Galaxolide and Tonalide by natural attenuation was negligible.
- Solely phytoremediation of musks was more effective than natural attenuation.
- *Pleurotus ostreatus* was the most efficient fungi in mycoremediation of musks.
- Highest Tonalide removal occurred by *Phanerochaete*-assisted phytoremediation.
- Bioaccumulation factors of Galaxolide and Tonalide were lower than 1.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Shaohua Chen

Keywords:
Polycyclic musks
Ligninolytic fungi
Dissipation
Sewage sludge
Enzymatic activities

ABSTRACT

The study was aimed to conduct the bioremediation of synthetic musks by four species of white rot fungi combined with phytoremediation (*Zea mays*) in biosolid-amended soils where only Galaxolide (HHCB) and Tonalide (AHTN) were found as other musks were below the detection limit (0.5–2 μg/kg dw). The HHCB and AHTN concentration in natural attenuation treated soil was decreased by not more than 9%. In solely mycoremediation, *Pleurotus ostreatus* was found to be the most efficient fungal strain, with the higher ($P < 0.05$) HHCB and AHTN removal (51.3% and 46.4%). Phytoremediation-only of biosolid-amended soil was also able to remove HHCB and AHTN from soil significantly ($P < 0.05$) in comparison to the control treatment without plants which resulted in the final concentration for both compounds of 56.2 and 15.3 μg/kg dw, respectively. Using white rot fungus-assisted phytoremediation, only *P. ostreatus* decreased the HHCB content in soil significantly ($P < 0.05$) by 44.7%, when compared to the initial concentration. While using *Phanerochaete chrysosporium*, the AHTN concentration was decreased by 34.5%, which was a significantly lower concentration at the end of experiment compared to the initial value. Via fungus-assisted phytoremediation, the enzymatic activity and fungal biomass were increased, probably due to the presence of roots in association with the soil microbiome, in the process increasing the degradation of fragrances accordingly. This could lead to a higher ($P < 0.05$) AHTN removal in

* Corresponding author. CZU Prague, Kamýcká 129, 165 00, Praha, Czech Republic.
E-mail address: kosnarz@af.czu.cz (Z. Košnář).

https://doi.org/10.1016/j.chemosphere.2023.138605
Received 22 November 2022; Received in revised form 23 February 2023; Accepted 3 April 2023
Available online 5 April 2023

P. chrysosporium assisted phytoremediation. Estimated HHCB and AHTN bioaccumulation factors in maize were lower than 1, therefore no environmental risk would be posed.

1. Introduction

Synthetic musk compounds are represented by polycyclic and nitro musks; however, the production of nitro musks was banned due to their bioaccumulative tendency (Lange et al., 2015). The most used synthetic musk compounds, Galaxolide (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran) and Tonalide (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene), since their development in the 1950s, are commonly used as additives in perfumes, powders and gel detergent, liquid and solid body or hair soaps, detergents and others, which are added to give a good odour (Li et al., 2016). The extensive production and usage of synthetic musk compounds lead to their occurrence in sewage sludge (Košnář et al., 2021; Sun et al., 2014), cosmetics (Nakata et al., 2015), soil (Biel-Maeso et al., 2019; DiFrancesco et al., 2004, sediment (Lou et al., 2016; Zhang et al., 2020), crops (Böhmer, 2007; Macherius et al., 2012) and air (Balci et al., 2020; Xie et al., 2007). The majority of Galaxolide (AHTN) and Tonalide (HHCB), up to 90%, reaching sewage sludges produced by wastewater treatment plants (WWTPs) is sorbed into sludge particles (Lou et al., 2016). Previous reports have shown that in Europe and the United States, of the produced biosolids about 50% is being applied to land, which means several million tons of dry biosolids annually (Kinney and Heuvel, 2020).

Amending the soil by using biosolids can supply essential nutrients to plants by substituting inorganic fertilisers (Seleiman et al., 2020). However, the application of sludge contributes through increasing toxic contaminants in the receiving soil to their presence in the food web. Synthetic musk compounds have been found in sludge directly from WWTPs as well as in composted sludge (Chen et al., 2014; Košnář et al., 2021; Ramos et al., 2021). Synthetic musks' relatively high *n*-octanol/water partition coefficient (log K_{OW}) lead to increased lipophilicity. Therefore, they can be with ease adsorbed by particulate organic content towards their accumulation in soils and sediments and are resistant to many biological and chemical degradation methods due to a low bioavailability (Chen et al., 2014; Hu et al., 2011; Wang et al., 2013a). Previous studies have indicated that these contaminants will last long in the soil after application and could transfer into the food chain, and their bioaccumulative tendency has been reported (Homem et al., 2017; Rivier et al., 2019). Böhmer (2007) estimated the half-life of HHCB and AHTN dissipation in biosolid-amended soil to achieve 1–1.5 and 2–24 years, respectively. DiFrancesco et al. (2004) reported the dissipation of fragrance compounds in the soil; however, some synthetic musk compounds including Tonalide were found after 1 year in the biosolid-amended soil.

Phytoremediation can be seen as the usage of plants to facilitate the degradation of pollutants in various ways, including compound scatter, breakdown and transformation. Through phytoremediation, fragrances and many other organic contaminants are taken up by plants, but for some long-lasting compounds such as persistent organic pollutants phytoremediation alone can be slow and inefficient (Macherius et al., 2012; Zhang et al., 2017). For instance, the phytoremediation of polycyclic aromatic hydrocarbons (PAHs) in the soil is only around 9–24% efficient due to a low bioavailability (Parrish et al., 2004). To improve the efficiency of phytoremediation, microbe-assisted phytoremediation is a low-cost, reliable and environmentally friendly approach (Yang et al., 2020).

White rot fungi are known to produce of many ligninolytic enzymes for the degradation of complex organic structures, such as lignin. Ligninolytic enzymes also enables them to decompose a variety of persistent organic pollutants, which could be a promising option to degrade synthetic musk compounds due to their capability to break a benzene ring. White rot fungi can produce many extracellular enzymes, namely lignin peroxidases, manganese-dependent peroxidases and laccases (Cajthaml et al., 2009; Gao et al., 2010). Most commonly used white rot fungi are *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Bjerkandera adusta*, *Trametes versicolor*, *Irpex lacteus* and many other fungi (Mir-Tutusaus et al., 2018). Previously published studies showed the ability of different plants to uptake musks from the soil, but there is a lack of investigation of the behaviour of synthetic musk compounds in plant-soil systems amended with different white rot fungi species related to the soil enzymatic activities.

The objectives of this research were: (a) to compare the degradation of synthetic musk compounds in biosolid-amended soil in planted (via maize *Zea mays*) and non-planted treatments, and (b) to assess the effectiveness of three different white rot fungi species, namely *Pleurotus ostreatus*, *Phanerochaete chrysosporium* and *Irpex lacteus* in the removal of synthetic musks planted with *Zea mays* compared to non-planted treatments, related to the soil microbial and enzymatic activity.

2. Material and methods

2.1. Extraction solvents and analytical standards

Solvents used in the extraction process were *n*-hexane (Chromsolv for pesticide residue analysis), acetone (Pestinorm), and ethanol (p.a.) all obtained from Chromservis (Czech Republic). Internal standards of Tonalide-d_3, musk xylene-d_{15} and PCB 116-d_5 were provided by Neochema (Germany) and LGC Standards (UK). The stock solution of 11 synthetic musk compounds was obtained from Neochema (Germany). *p*-terphenyl-d_{14} purchased from Chromservis, Czech Republic was used as a spiking surrogate.

2.2. Soil and sludge description

The representative soil used in the experiment was obtained from a long-term trial site (Humpolec, Czech Republic at 49.5540547 N, 15.3499731 E). The air-dried, homogenised soil, sieved (<2 mm) for the removal of stones, large roots and branches, showed a pH ($CaCl_2$), EC (μS/cm) and CEC ($mmol^{(+)}/kg$) of 5.4, 329 and 76.8, respectively. Texture composition was as follows: sandy loam (clay, 5.8%; silt, 43.6%; sand, 50.6% *w/w*). The total nitrogen and total carbon contents were 1.9 and 19.3 g/kg dry weight (dw), respectively. The total element content was determined for S (0.40 g/kg dw), K (3.8 g/kg dw), P (1.1 g/kg dw), Ca (2.2 g/kg dw) and Mg (2.8 g/kg dw). More detailed measurements of the physiochemical properties of sludge and soil are shown in the supplementary material. The background contamination of experimental soil by 11 screened synthetic musk compounds (Table S1) was checked and no synthetic musk compounds (SMCs) were detected because the concentration of screened musks was lower than the detection limit (0.4–2.0 μg/kg dw) in Table S5.

The anaerobically stabilised sewage sludge was collected from a WWTP serving around 200,000 inhabitants and with a wastewater load of 38%. The wastewater hydraulic retention time in WWTP was 35 days, with biochemical and chemical oxygen demand removal efficiencies of 96.8% and 95.4%, respectively. The representative sludge sample of 10 kg fresh weight consisted of four randomly taken subsamples of anaerobically treated sludge. Thoroughly mixed sludge was then transported at 4 °C to the laboratory for further homogenisation. Prior to analysis, the homogenised sludge was freeze-dried and kept in a fridge in amber glass bottles. The remaining sludge used for soil amendment was kept at 4 °C. The physiochemical properties of the sludge were determined: a dry matter content of 23.3% and a pH ($CaCl_2$) of 6.50. The elemental

composition of C, H, N and S was 272.1, 45.1, 41.1 and 11.7 g/kg dw, respectively, and the C/N ratio was 7.8. The sewage sludge was also analysed for SMCs before amendment. The contents of individual musks were Cashmeran 8.9 μg/kg dw, Celastolide 5.7 μg/kg dw, Phantolide 20.9 μg/kg dw, Galaxolide 4161 μg/kg dw, Tonalide 1053 μg/kg dw, other musks (Traseolide, musk ambrette, musk xylene, musk moskene, musk tibeten, musk moskene) were not detected and the resulting ∑SMCs was 5256 μg/kg dw.

2.3. Preparation and characterisation of fungal substrates

Wheat straw pellets were used as a lignocellulosic substrate for growing three ligninolytic fungal species. *Pleurotus ostreatus* (Jacq.) P. Kumm., strain HK35 (*P. ostreatus*), *Phanerochaete chrysosporium* (Burds.) (*P. chrysosporium*) and *Irpex lacteus* (Fr.), strain 931 (*I. lacteus*) were obtained from the Department of Horticulture, CZU Prague and collection of basidiomycetes owned by Institute of Microbiology, Academy of Sciences, Czech Republic. The inoculum of each fungus was prepared following the methodology described by Košnář et al. (2019). Briefly, each fungus was cultured on 2% (*w/v*) malt extract-glucose agar to get the mycelium of the respective fungi. The resulting mycelium was applied as fungal spawn on wheat grain. That substrate, in 1-L glass bottles containing gypsum 5% (*w/w*), was autoclaved for 2 h at 120 °C, inoculated with agar pieces containing mycelia and cultivated at room temperature for two weeks. Sterilised wheat straw pellets, in a 2.5-L plastic container with a moisture content of 60% (*w/w*), were inoculated with previously made substrate with each culture. Mycoremediation substrates with white rot fungi were cultured for 4 weeks until the whole layer of straw pellets was overgrown by fungal mycelium. The substrate grown with fungi for the bioremediation experiment was characterized by dryness and total organic matter of 33.7 and 96.9%, respectively. Other properties determined were C, H, N and S contents, with values of 457.1, 69.5, 3.4 and 1.0 g/kg dw, respectively.

2.4. Experimental setup

The bioremediation experiment was carried out in eight treatments, each in four replications as follows: A) biosolids soil (SS) represented natural attenuation of synthetic musks in sludge-amended soil; B) mycoremediation of synthetic musks from soils amended with biosolids using different white rot fungi (M): i) (M-PL) soil-sludge substrate with *P. ostreatus*, ii) (M − PH) soil-sludge substrate with *P. chrysosporium*, iii) (M-IR) soil-sludge substrate with *I. lacteus*; C) phytoremediation of synthetic musks from soil amended with sludge (PSS); D) phytoremediation, together with different white rot fungi, of synthetic musks from sludge-amended soil (PM), i) (PM-PL) planted soil-sludge substrate with *P. ostreatus*, ii) (PM-PH) planted soil-sludge substrate with *P. chrysosporium*, iii) (PM-IR) planted soil-sludge substrate with *I. lacteus*. In our study, maize (*Zea mays* L. var. Colisee) (KWS Saat, Germany) was tested in phytoremediation treatments. For each treatment, 4 kg dw of soil was mixed with 1 kg of fresh sludge and mycoremediation treatments had 0.2 kg of fresh substrate grown with the respective white rot fungus placed in plastic pots (6 L with height, top diameter = 21 cm, bottom diameter = 18.0 cm) with three holes at the bottom. Another plastic pot without holes under the perforated one was used to catch the possible leachate which was returned on top of the soil. Planted pots were sown with eight maize seeds per pot, and then, 2 weeks after germination, four seedlings of a similar size were selected and left in the pot, for the rest of the experiment. The soil of planted and non-planted treatments was irrigated with distilled water to maintain a 60% water-holding capacity during the experiment. All pots were placed randomly in the outdoor and roofed vegetation hall that controls atmospheric precipitation, which in most cases resembles the actual field conditions.

Soil samples consisting of three thoroughly mixed subsamples per pot were collected at days 0 and 120 of the experiment from the whole soil profile. Soil samples for synthetic musk analyses were lyophilised, ground and stored in a freezer before analysis. Soil samples for ergosterol and extracellular enzyme activity analyses were stored at 4 °C. Plants were harvested after 120 days of the experiment and separated into shoots and roots. Roots were separated from the soil carefully and rinsed with demi-water. Roots and shoots were cut into smaller pieces and lyophilised, milled and stored at 4 °C before the analyses of SMCs.

2.5. Synthetic musks extraction and analysis

SMCs in solid samples were extracted based on the method developed by Košnář et al. (2021). In a brief, 1 g of solid sample with replicate (n = 4) from each treatment was placed into 50-mL glass tubes and spiked with 1 mL of surrogates at 500 ng/mL (Table S2). Then, the following extraction solvents were added: 10 mL of ethanol, sodium acetate buffer (pH 3.4) and *n*-hexane. The mixture was shaken on GFL 3017 shaker (DKSH, Switzerland) at 150 revolutions per minute (rpm) for 30 min, followed by immersion in an ultrasonic tank (Bandelin Sonorex, DT510H, Germany) at 35 °C for 30 min. After balancing the weight of extracts with pure *n*-hexane, samples were centrifuged (1328×*g*) on Rotina 420 R (Hettich, Germany) for 10 min at laboratory temperature. The *n*-hexane solvent was collected and filtered using 150 mm filter paper (Whatman®, Grade 40). Re-extraction was done by further ultrasonication after adding 5 mL of *n*-hexane and repeating the procedure described above. The poured *n*-hexane from the extraction was evaporated to near dryness (~0.2 mL) using nitrogen. A clean-up procedure was carried out using solid phase extraction (SPE) with Strata SI-1 silica gel cartridges (Phenomenex, USA) for the extraction of musk from the initial sludge and Strata FL (Florisil™) cartridges (Phenomenex, USA) for the sludge-amended soil and biomass samples to minimise possible matrix effects as was suggested by Vallecillos et al. (2015). The SPE was conditioned with 10 mL of *n*-hexane–ethyl acetate mixture (9: 1 *v/v*). Re-dissolved extracts (2 mL) in *n*-hexane were loaded onto silica gel. Elution was done with 10 mL of *n*-hexane and washing with an *n*-hexane–ethyl acetate mixture (1: 1 *v/v*). Combined eluate and washing fractions were again concentrated to a near dryness, and then *n*-hexane was added to 2 mL. Final extracts were mixed with 100 μL of internal standards (Table S3) in a 2-mL amber vial consisting of 200 ng/mL of each in *n*-hexane.

2.6. Synthetic musks GC/MS/MS analysis

Target compounds were analysed using the Agilent 8890 gas chromatograph with Agilent 7000D triple quadrupole detector (Agilent GC/MS/MS, California, USA) and automatic sampler system PAL RTC (CTC Analytics AG, Switzerland) as was by Košnář et al. (2021). In a brief, the target musk compounds were separated on a 30 m × 0.25 mm × 0.25 μm XLB Restek column (Bellefonte, USA). Pure 6.0 helium with the flow (1.0 mL per minute), pressure (23 psi) and purge (50 mL per minute) was used as a carrier gas in the splitless regime. Starting oven temperature was 60 °C (1 min), then 220 °C (40 °C per minute) and finally 320 °C (10 °C per minute) kept for 1 min. Mass spectrometer transitions of individual musk analytes are summarised in Table S4.

2.7. Quality assurance, control and method precision

To prevent external contamination by target SMCs all glassware were properly rinsed with non-polar solvents of acetone and *n*-hexane three times before each analysis, followed by heating to dryness. The calibration standards (0.5, 1, 5, 10, 50, 100, 500 and 1000 ng/mL) were done from the stock solution (5000 ng/mL) of 11 synthetic musk mixture (Neochema, Germany) with pure *n*-hexane, with $R^2 > 0.995$ for each musk, and directly injected to construct the calibration curve, as was recommended by Ramos et al. (2019). The method limit of detection (MLOD) of individual musks was in the range of 0.5–1.0 ng/mL (Table S5). Procedural and technical blanks were run together with the

samples and fresh calibration every time to find possible background musks concentration. Technical blanks of pure *n*-hexane which were not subjected to the extraction procedure were regularly performed between the samples during the analysis to prevent the possible carry-over of measured analytes. Musk compounds in procedural blanks were below the limit of detection. Together with sample extracts and procedural blanks, fresh calibration standards, technical blanks, and check standards were measured in duplicate. Check standards of *p*-terphenyl-d_{14} in pure hexane at 500 ng/mL were used to determine the actual added concentration and stability of GC/MS/MS and its condition during the analyses as well. Extraction efficiencies of the surrogate standard calculated as extracted concentration/added concentration * 100% were used for the correction of each sample results. The mean *p*-terphenyl-d_{14} recovery from the extraction of solid samples was 94%.

2.8. Total biomass of fungi

The total fungal biomass of soil was estimated by the estimation of ergosterol content in the treated soil, as was described by Djajakirana et al. (1996), employing high-performance liquid chromatography with reversed-phase. Extraction of ergosterol proceeded from 1 g of fresh soil in flat-bottom glass bottles. Then, 25 mL of methanol was added and shaken at 250 rpm for 30 min. The filtered reaction mixture was evaporated at 40 °C using a laboratory rotavapor (BÜCHI Labortechnik AG, Flawil, Switzerland) to near dryness. The soil extract was then re-constituted with 1 mL of methanol and filtered using a 0.45-μm nylon syringe filter into a vial. Quantification of ergosterol content was performed using a calibration curve (10–1000 ng/mL) made of ergosterol standard (Merck Life Science, Czech Republic).

2.9. Soil enzymatic activity

For the determination of ligninolytic (MnP: manganese-dependent peroxidase, Lac: laccase) and hydrolytic lipase (Lip) enzymes, an extraction mixture contained 0.2 ± 0.001 g of a lyophilised soil and 20 mL of 50 mM phosphate buffering solution (pH 7.0). The suspension was homogenised using an IKA T25 miller (IKA®-Werke GmbH & Co. KG, Germany) for 30 s. The suspension was then mixed with 2.50 mM 4-methylumbelliferyl-caprylate to quantify the enzymatic activity of Lip, as a change in fluorescence. To determine MnP and Lac, the suspension had to be filtered and desalted (PD-10 columns). The activity of both MnP and Lac must be measured immediately after substrate application (Baldrian, 2009). Lac activity was measured spectrophotometrically at 420 nm, and MnP activity at a wavelength of 590 nm. Both enzymes were measured as a change in absorbance, for 12 min every 2 min (7 × 2 min) using a Tecan 96-well plate reader (Infinite M200, Tecan Trading AG, Switzerland) according to the procedure developed by Baldrian (2009). All activities were expressed in U/g soil dw. One unit (U) is the amount of enzyme which produces one μmol of the respective reaction product per minute.

2.10. Data evaluation and statistics

Statistical evaluation was performed with the Statistica 7 software package (StatSoft, USA). A paired sample *t*-test and one-way ANOVA. Tukey's test was applied for the statistical significance determination, where $P < 0.05$ data were significant. Significant differences among each treatment as determined by Tukey's test were indicated by different letters in Tables and Figures. Bioconcentration factor (BF) was used to calculate the accumulation ratio of synthetic musk compounds in the roots and shoots of plants. The calculation of bioconcentration factor, and percentage of removal, including graphs, was done using Microsoft Excel 10 (Microsoft Corporation, USA).

3. Results and discussion

3.1. Synthetic musks bioremediation in soil

3.1.1. Natural attenuation of sludge-amended soil

Screening the treated soil for 11 major SMCs showed only Galaxolide (HHCB) and Tonalide (AHTN) (Table S1) detection at any stage of the experiment as other screened musks were below the detection limit (0.4–2.0 μg/kg dw). The After 120 days other musks except for HHCB and AHTN in sludge-amended soil was expected, as very low contents of other musks were found in the initial sludge. This is consistent with previous studies that more than 90% of musks present in sludge were the sum of HHCB and AHTN. Only a portion of fresh sewage sludge was added into the experimental soil (5% *w/w*); due to this, musks of low content in the experimental sludge were not detected in sludge-amended soil because of the dilution effect. Therefore, this study focused on the behaviour of HHCB and AHTN in biosolid amended soil in the presence of fungi, which was compared to the amended plant-soil system. When sludge was applied to the soil without plants and fungal substrate (SS treatment), which represented the natural attenuation of amended soil, the AHTN content dropped only 8.8% from the initial concentration, with a concentration of 22 μg/kg dw at the end of the bioremediation experiment (Table 1). After 120 days of the experiment, the removal of HHCB in the naturally attenuated soil was similar to the decrease of AHTN (Table 1). No statistically significant differences ($P < 0.05$) from the respective initial concentrations (Figs. 1 and 2) were observed in that non-planted sludge-amended soil without fungal substrate. Böhmer (2007) observed that after 259 days of their experiment, AHTN and HHCB showed slow degradation, with 75 and 50% of the content remaining in the biosolid-amended soils, respectively. A similar study estimated that HHCB and AHTN can persist in sludge-amended soil, as the degradation half-lives degradation ranged from 1 to 1.5 and 2–24 years, respectively. The lower dissipation of musk compounds registered in biosolid-amended soil (SS treatment) could be due to their physico-chemical properties, since HHCB and AHTN have a strongly lipophilic character and low water solubility, makes them difficult to easily degrade or leach (Table S1). However, the slow removal of musks from sludge-amended soil (SS treatment) in the current study could be

Table 1
The removal percentage of Galaxolide (HHCB) and Tonalide (AHTN) at each treatment after 120 days of experiment and their residual content in the soil (means ± SD; n = 4).

Treatment	Galaxolide (HHCB)		Tonalide (AHTN)	
	Removal (%)	Residual (μg/kg dw)	Removal (%)	Residual (μg/kg dw)
SS	8.5 ± 1.0 c	66.5 ± 18.8 abc	8.8 ± 4.5 e	22.3 ± 2.9 abc
M-PL	58.9 ± 5.2 a	42.6 ± 5.3 a	49.7 ± 11.6 a	15.6 ± 1.4 a
M-PH	21.6 ± 15.5 bc	54.6 ± 3.7 ab	29.2 ± 2.3 d	18.1 ± 0.7 ab
M-IR	21.1 ± 17.0 bc	57.7 ± 16 ab	23.9 ± 2.7 cd	16.6 ± 2.2 ab
PSS	46.3 ± 0.5 ab	56.2 ± 3.1 ab	44.8 ± 6.3 ab	15.3 ± 1.5 a
PM- PL	44.7 ± 6.3 ab	39.8 ± 9.2 a	37.8 ± 10.3 abc	19.0 ± 5.6 ab
PM- PH	23.9 ± 16.1 bc	65.9 ± 12 abc	34.5 ± 9.2 bcd	17.4 ± 3.1 ab
PM- IR	15.1 ± 10.9 bc	65.9 ± 17 abc	27.1 ± 4.3 cd	20.5 ± 4.8 abc

Different letters within the same column indicate significant differences ($P < 0.05$) among each treatment as determined by Tukey's test. Abbreviations: SS – sewage sludge amended soil (control), M-PL – mycoremediation with *Pleurotus ostreatus,* M – PH – mycoremediation with *Phanerochaete chrysosporium*, M-IR – mycoremediation with *Irpex lacteus,* PSS – planted sewage sludge amended soil, PM-PL – plant assisted mycoremediation *Pleurotus ostreatus,* PM-PH – plant assisted mycoremediation with *Phanerochaete chrysosporium*, PM-IR – plant assisted mycoremediation with *Irpex lacteus.*

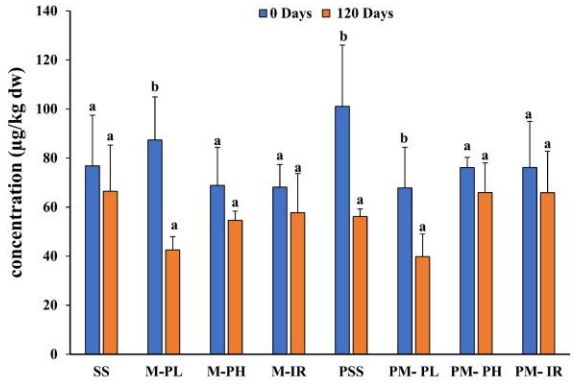


Fig. 1. Concentration of Galaxolide (HHCB) in the soil at each treatment during initial (0 days) and at the end of bioremediation (120 days).

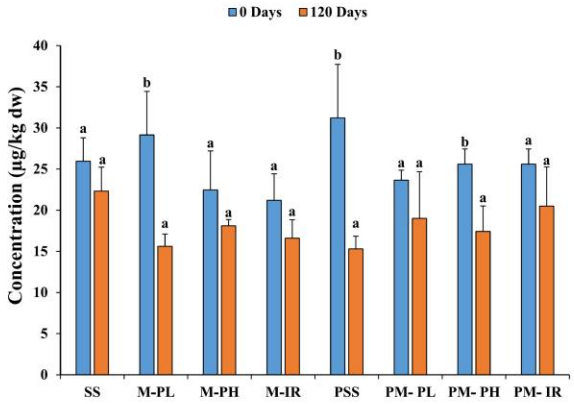


Fig. 2. Concentration of Tonalide (AHTN) in the soil at each treatment during initial (0 days) and at the end of bioremediation (120 days).

because of the weak volatilisation ability, or weak activity of indigenous soil microorganisms might contribute, as was suggested by Rodarte--Morales et al. (2011). DiFrancesco et al. (2004) observed a slow degradation rate of HHCB and AHTN that remained in soils amended with sludge for 3 months and reported the dissipation of both compounds as ranging from 39 to 58% at 6 months from the application. After 12 months of sludge soil application, AHTN was still present. Yang and Metcalfe (2006) reported that after sewage sludge amendment in an agricultural field, the concentrations of both compounds decreased over time, and only HHCB was detected after 6 months. The investigation of enzymatic activity (Table 2) reveals that lipase activity was decreased at the end of the study in biosolid-amended soil (SS treatment) from its respective initial value. The decrease was related to no fungal inoculation in this treatment, in which lipase activity will be decreased due to less organic matter that degrades over time (Wang et al., 2009). Balaji et al. (2014) as well as Ogbolosingha et al. (2015) found that the activity of lipase declined as the bioavailability of contaminants reduced at the end of their experiments. Qiao et al. (2014) also found a decrease in lipase activity in their natural attenuation treatments, with a low percentage of up to 6% of degradation of organic pollutants, like in this study. The fungal biomass content for SS treatment (Table 3) was not significantly different ($P < 0.05$) to its respective initial value, which means that indigenous soil fungi were not increased during the natural attenuation of biosolid-amended soil.

Table 2
Enzymatic activities in the biosolid amended soils at initial (0 days) and final (120 days) of the bioremediation experiment.

Treatment	Lipase		Laccase		Mn-peroxidase	
	0 days (U/g dw)	120 days (U/g dw)	0 days (mU/g dw)	120 days (mU/g dw)	0 days (mU/g dw)	120 days (mU/g dw)
SS	953 ± 0.3 ABa	864 ± 0.4 Ab	ND	0.4 ± 0.1 ABC	0.9 ± 0.7 Aa	0.5 ± 0.2 Aa
M-PL	1724 ± 0.6 ABa	1998 ± 1 ABa	0.7 ± 0.1 ABCb	0.3 ± 0.1 ABCa	0.4 ± 0.3 Aa	0.3 ± 0.2 Aa
M-PH	1354 ± 0.1ABa	1554 ± 0.4 ABa	1.3 ± 0.9 Ca	0.5 ± 0.3 ABCa	0.7 ± 0.3 Aa	0.5 ± 0.1 Aa
M-IR	1677 ± 0.2 ABa	1958 ± 0.6 Ba	1.3 ± 0.5 Cb	0.5 ± 0.3 ABCa	0.6 ± 0.2 Aa	0.5 ± 0.2 Aa
PSS	815 ± 0.3 Aa	1209 ± 0.9 ABa	ND	0.3 ± 0.4 ABC	0.7 ± 0.5 Aa	0.4 ± 0.3 Aa
PM- PL	1461 ± 0.3 ABa	1659 ± 0.2 ABa	1.1 ± 0.8 BCa	0.5 ± 0.5 ABCa	1.0 ± 0.8 Aa	0.5 ± 0.3 Aa
PM- PH	1681 ± 0.5 ABa	1843 ± 0.7 ABa	0.4 ± 0.4 ABCa	0.1 ± 0.1 ABa	0.4 ± 0.7 Aa	0.1 ± 0.2 Aa
PM- IR	1998 ± 0.1ABa	2170 ± 0.6 Ba	0.7 ± 0.3 ABCa	0.3 ± 0.1 ABCa	0.7 ± 0.5 Aa	0.3 ± 0.3 Aa

Means ± standard deviations calculated from four replications of respective treatments. ND = not detected. Different uppercase letters within the same column indicate significant differences ($P < 0.05$) among each treatment as determined by Tukey's test. Different lowercase letters within the same row indicate that significant differences ($P < 0.05$) between 0 and 120th days of experiment as determined by Tukey's test. Abbreviations: SS – sewage sludge amended soil (control), M-PL – mycoremediation with *Pleurotus ostreatus,* M – PH – mycoremediation with *Phanerochaete chrysosporium*, M-IR – mycoremediation with *Irpex lacteus,* PSS – planted sewage sludge amended soil, PM-PL – plant assisted mycoremediation *Pleurotus ostreatus,* PM-PH – plant assisted mycoremediation with *Phanerochaete chrysosporium*, PM-IR – plant assisted mycoremediation with *Irpex lacteus.*

Table 3
Ergosterol concentration in the biosolid amended soils at initial (0 days) and final (120 days) of the bioremediation experiment.

Treatment	0 days	120 days
	mg/kg dw	mg/kg dw
SS	6 ± 0.8 Ba	9.6 ± 0.8 Ba
M-PL	12.7 ± 2.5 Aa	15.2 ± 2.7 ABCa
M-PH	13 ± 2.3 Aa	15.5 ± 2.1 ABCa
M-IR	14.5 ± 1.8 Aa	17.3 ± 1.9 ACb
PSS	6.4 ± 0.9 Ba	10.5 ± 2.4 BCb
PM- PL	13.1 ± 1.0 Aa	18.7 ± 1.9 Ab
PM- PH	13.8 ± 3.6 Aa	19.7 ± 5.2 Aa
PM- IR	15.3 ± 3.1 Aa	21.7 ± 4.3 Aa

Means ± standard deviations calculated from four replications of individual treatments. Different uppercase letters within the same column indicate significant differences ($P < 0.05$) among each treatment as determined by Tukey's test. Different lowercase letters within the same row indicate that significant differences ($P < 0.05$) between 0 and 120th days of experiment as determined by Tukey's test. Abbreviations: SS – sewage sludge amended soil (control), M-PL – mycoremediation with *Pleurotus ostreatus,* M – PH – mycoremediation with *Phanerochaete chrysosporium*, M-IR – mycoremediation with *Irpex lacteus,* PSS – planted sewage sludge amended soil, PM-PL – plant assisted mycoremediation *Pleurotus ostreatus,* PM-PH – plant assisted mycoremediation with *Phanerochaete chrysosporium*, PM-IR – plant assisted mycoremediation with *Irpex lacteus.*

3.1.2. Mycoremediation of sludge-amended soil

During the mycoremediation-only treatment, the removal of HHCB showed the highest removal percentage, with 58.9% on average (Table 1) when *P. ostreatus* grown on substrates was added to the soil compared to control and other mycoremediation treatments. That treatment showed a significant decrease ($P < 0.05$) in HHCB concentration from its initial value as well (Fig. 1). In the time frame of the experiment, in M − PH and M-IR treatments, the HHCB content decreased similarly, by 21.6% in both treatments. The removal of AHTN in M-PL (Table 1) was significantly ($P < 0.05$) higher compared to other M and SS treatments, with a removal percentage of 49.7% after 120 days and a remaining concentration of 15.6 μg/kg dw. *Pleurotus ostreatus* showed better removal capacity compared with the other fungal strains, similar to the soil contaminated with different congeners of polychlorinated biphenyls (Kubátová et al., 2001; Stella et al., 2017). Rodarte-Morales et al. (2011) studied the degradation of AHTN and HHCB by using *P. chrysosporium* and other ligninolytic fungi, while both contaminants were removed effectively and were not detected at the end of the experiment. Balk and Ford (1999a) studied the degradation of selected musks in pure cultures of the ligninolytic fungus *P. chrysosporium*, where HHCB and AHTN were removed after 72 and 144 h, respectively. Another study by Stella et al. (2017) on *P. ostreatus* and *I. lacteus* fungal strains showed, after 12 weeks, similar trends to the current study for the dissipation of other organic contaminants, such as PCBs, in the soil, with removal percentages of 41.3 and 39.4%, respectively. In the current study, lipase activity in M-PL and M − PH treatments (Table 2) was higher than in the control (SS) treatment after 120 days, which led to greater degradation of the target musks in mycoremediation treatments. The Lac and MnP activities were not significantly different ($P < 0.05$) during the time frame of the experiment in M treatments, as shown in Table 2. Lac was involved in the degradation of SMCs in the current study through its oxidation mechanisms of the Lac mediator system, which is effective in selecting the appropriate redox mediator to obtain efficient degradation (oxidation) of compounds with a high redox potential, such as our target compounds HHCB and AHTN (Madhavi and Lele, 2009). However, the fungal biomass was increased significantly ($P < 0.05$) from the respective initial values after 120 days of the experiment in M treatments (Table 3). This leads to the suggestion that the present fungi were able to grow in soil in the current experiment, but without the ability for higher production of the tested ligninolytic enzymes in the time frame of the experiment.

3.1.3. Phytoremediation of sludge-amended soil

The concentration of HHCB decreased significantly ($P < 0.05$) in the sludge-amended soil phytoremediation (PSS) treatment, by 46.3% compared to its respective initial value (Table 1), with a final residual concentration of 56.2 μg/kg dw in soil. For AHTN, in the PSS treatment, 44.8% was removed and the final concentration was decreased significantly ($P < 0.05$) from the initial value to 15.3 μg/kg dw after 120 days of the experiment. Rivier et al. (2019) found that the presence of roots led to no additional decrease in soil concentration compared to non-vegetated treatments. The greater removal of HHCB and AHTN in our study could be caused by the presence of maize able to stimulate the indigenous microbial biomass and/or enzymatic activity in the rhizosphere, as suggested by Segura and Ramos (2013). This could be supported by the higher lipase activity obtained at the end of the experiment, as shown in Table 2.

Böhmer (2007) studied the phytoremediation of HHCB and AHTN using lettuce; musks were decreased by 65 and 46%, respectively, after 12 weeks of the experiment. However, in the same study, carrot roots showed less degradation of HHCB and AHTN, with removal percentages of 30 and 33%, respectively, after 14 weeks of the experiment. Ramos et al. (2021) found higher HHCB and AHTN degradation efficiency compared to the current study on biosolid-amended soils using tomatoes, resulting in 83 and 76% degradation, respectively, at the end of their experiment. Rivier et al. (2019) observed no plant enhancement in the degradation of HHCB; however, our study showed a significant plant enhancement nearly by 38% in the removal of HHCB in phytoremediation treatments using maize in comparison to non-planted treatment. Košnář et al. (2019) found higher fungal biomass and lipase activity during a phytoremediation experiment with maize which could be stimulated by root exudates, resulting in increased removal of total PAHs. The lipase activity and the fungal biomass in the PSS treatment of the current study were increased significantly ($P < 0.05$) after 120 days of the experiment (Tables 3 and 4). This points to the suggestion that maize can promote the activity of the autochthonous microbiome in soil by its root exudates which probably could be involved in the phytoremediation of the target musks.

3.1.4. Plant-assisted mycoremediation of sludge-amended soil

Greater dissipation of the target polycyclic musk fragrances in plant-assisted mycoremediation (PM treatments, Table 1) was found in the treatment with *P. ostreatus* (PM-PL), when compared to its initial content, in the case of HHCB with the removal of 44.7%. In that treatment, the significant removal ($P < 0.05$) of HHCB concentration was observed in comparison to the other plant-assisted mycoremediation treatments with *P. chrysosporium* and *I. lacteus* (Table 1). Čvančarová et al. (2012) reported that *P. ostreatus* combined with alfalfa is effective in the degradation of phenanthrene, with a removal efficiency of 85%. Moreover, after 120 days of the experiment in the present study, the content of HHCB was found to be 65.9 and 65.8 μg/kg dw in PM-PH and PM-IR treatments, respectively. That decrease of musks in soil was not statistically significant ($P < 0.05$) compared to their respective values at the beginning of the experiment (Fig. 1) and approximately 15–24% of both musks were removed (Table 1). In terms of AHTN removal rate (Table 1), the PM treatments followed the same trend of dissipation as for HHCB. However, 34.5% of AHTN was removed by plant-assisted mycoremediation with *P. chrysosporium* (PM-PH), which resulted in the concentration in soil being significantly ($P < 0.05$) lower after 120 days of the experiment than its respective initial value (Fig. 2). In the case of AHTN, the plant-assisted mycoremediation by *P. chrysosporium* improved the removal efficiency of AHTN compared to the mycoremediation-only treatment, and significantly more AHTN was removed than by natural attenuation in sludge-amended soil. We observed an increased ergosterol content and enzymatic activity of lipase in soil in PM treatments at the end of the experiment (Tables 3 and 4). The lipase enzymatic activity in the PM-IR treatment was also conclusively higher ($P < 0.05$) compared to other PM treatments after 120 days, but the degradation of SMCs was not satisfactory. Moreover, the activity of Lac and MnP enzymes in PM treatments found, in the range from 0.1 to 0.5 mU/g dw (Table 2), was not statistically different to that in the other studied treatments. Asif et al. (2017) studied the

Table 4
Contents and bioconcentration factor (BCF) of Galaxolide (HHCB) and Tonalide (AHTN) in the roots and shoots of maize after 120 days of bioremediation experiment. Means ± standard deviations represented four replicate measurements.

Treatments	Galaxolide (HHCB)				Tonalide (AHTN)	
	Shoots (μg/kg dw)	BCF	Roots (μg/kg dw)	BCF	Roots (μg/kg dw)	BCF
PSS	3.4 ± 2.3 a	0.06	8.1 ± 5.7 a	0.15	3.8 ± 2.2 a	0.25
PM- PL	2.1 ± 2.4 ab	0.06	9.9 ± 4.8 a	0.24	1.3 ± 1.7 b	0.08
PM- PH	0.7 ± 1.4 b	0.02	3.4 ± 4.0 b	0.06	1.6 ± 1.9 b	0.09
PM- IR	ND[a]	–[b]	1.8 ± 3.2 b	0.02	0.9 ± 1.8 b	0.05

Abbreviations: PSS – planted sewage sludge amended soil, PM-PL – plant assisted mycoremediation *Pleurotus ostreatus,* PM-PH – plant assisted mycoremediation with *Phanerochaete chrysosporium*, PM-IR – plant assisted mycoremediation with *Irpex lacteus.*

[a] ND = not detected.

[b] Not estimated. Different letters within the same column indicate significant differences ($P < 0.05$) among each treatment as determined by Tukey's test.

performance of ligninolytic fungi and their particular enzymes in the degradation of pharmaceuticals and personal care products (PPCPs) under different operating conditions, finding that *P. chrysosporium* with its Lip and MnP enzymatic system showed removal efficiency higher than 25%. This trend is in agreement with the current study, but *P. ostreatus* investigated by Asif et al. (2017) showed much higher removal efficiency, in the case of selected PPCPs, up to 98%. Nevertheless, in our study, *P. ostreatus* was the most effective fungal strain in the bioremediation of SMCs. This is consistent with the results of Wang et al. (2009) and Magan et al. (2010) who found higher Lac and MnP activity for *P. ostreatus* due to a better colonising ability of the fungus in the soil which helps to degrade contaminants. Hestbjerg et al. (2003) also reported that *P. ostreatus* is more effective than other studied fungal strains, probably due to less competition with native soil organisms and lower sensitivity to temperature requirements for growth and enzyme production. Unfortunately, the increased ergosterol content and enzymatic activity of lipase in the soil in the current experiment did not lead to an improved rate of removal of the tested musk compounds from the soil, as was expected when combining the mycoremediation and phytoremediation treatments in the time frame of the experiment. We can speculate that greater dissipation could occur with a prolonged duration of the experiment; therefore, further investigation for a longer period is needed.

3.2. Uptake of musks by maize, bioconcentration factor and environmental risk assessment

The studied HHCB was found in both root and shoot samples of maize, with a higher concentration of HHCB found in the roots ($P < 0.05$) except for the PM-IR treatment (Table 4). AHTN was detected only in maize roots, at a lower concentration, and was not found in the shoots. Fernandes et al. (2022) reported similar results, finding only HHCB in the above-ground biomass and both compounds in a higher concentration in the roots. This might be because AHTN has a higher log K_{ow} value than HHCB (Table S1), which could make it more lipophilic and more easily sorbed on soil organic matter. The concentration of HHCB found in the maize roots was higher ($P < 0.05$) in PSS and PM-PL (8.1 and 9.9 μg/kg dw, respectively) compared to other treatments. The relatively high log K_{ow} of HHCB and AHTN might limit transport from roots to shoots. We also found correspondingly higher removal of HHCB from the soil in both PSS and PM-PL treatments (Table 1), which might be related to root uptake or its combined effect with white rot fungi in microbial-assisted phytoremediation. Previous studies have reported that AHTN is more resistant to degradation by white rot fungus enzymes (Balk and Ford, 1999b; Chen et al., 2009; Vallecillos et al., 2017). The uptake of HHCB by roots in other treatments (PM-PH and PM-IR) was not higher than 3.4 μg/kg dw (Table 4). In the PSS treatment, the concentration of HHCB in the maize shoots and roots was higher than for other treatments. HHCB concentration in shoots of maize was lower ($P < 0.05$) than in roots compared to their respective treatments, with the concentration lower than 3.4 μg/kg dw (Table 4).

AHTN was taken up by maize roots with a concentration of 3.8 μg/kg dw in the PSS treatment, which was significantly higher ($P < 0.05$) compared to other treatments and related to a lower soil concentration after 120 days of the experiment. Surprisingly, concentrations of AHTN in maize roots of plant-assisted treatments (PM-PL, PM-PH and PM-IR) were similar and ranged from 0.9 to 1.6 μg/kg dw, respectively. We hypothesize, that due to the dissipation of the bioavailable contaminants by white rot fungi in the phytomycoremediation (PM) treatments, there could be less uptake of target musk by the maize plants compared to the PSS treatment. Böhmer (2007) showed the accumulation of HHCB and AHTN in roots and shoots at higher concentrations compared to the current study. They observed that lettuce (leaves) grown on soil spiked with 30 mg/kg dw of each musks accumulated 78–110 and 209–275 μg/kg dw, respectively. Another study reported that HHCB and AHTN were determined in higher contents in barley (0.81 and 6.9 mg/kg dw, respectively), meadow fescue roots (1.77 and 12.66 mg/kg dw, respectively) and carrots (0.86 and 2.45 mg/kg dw, respectively) (Macherius et al., 2012). In another study by Rivier et al. (2019) HHCB and AHTN were detected in a grain of barley grown in soil amended with 60 t/ha sewage sludge. The concentrations of HHCB and AHTN were found to be in the range from <LOQ to 0.5 and <LOQ to 0.9 μg/kg dw, respectively. Ramos et al. (2020) detected in different varieties of tomatoes in the range of 1–53 μg/kg dw. Ribeiro et al. (2017) tested the coastline plant species on their ability to accumulate 11 synthetic musk compounds. Their results showed that SMCs were detected in the range from 1.56 to 350 μg/kg dw.

The bioconcentration factor (BCF) was calculated based on the concentration ratio of contaminant in the plant biomass to soil concentrations in the harvest period (C_{plant} μg/kg dw/C_{soil} μg/kg dw) (Böhmer, 2007; Macherius et al., 2012). The BCF of HHCB was comparable to that of AHTN in the maize roots and ranged from 0.02 to 0.24 in the PM-PL and PM-IP treatments, as shown in Table 4. A considerable value of BCF for AHTN of 0.25 was also found in the PSS treatment, but in other treatments, it was lower than 0.09. Macherius et al. (2012) found considerably higher BCF values than in the current study in barley roots for both HHCB and AHTN, with values of 0.83 and 1.49, respectively. Böhmer (2007) reported BCF values comparable to those in the current study, ranging from 0.095 to 0.48 (HHCB) and 0.082 to 0.37 (AHTN) in carrot roots grown on biosolid-amended soils with 30 mg/kg dw background contamination of each musk. Chen et al. (2015) observed very low transferable capabilities of synthetic musks by root uptake in aloe, where the BCFs in aloe leaf ranged from 0.12 to 0.27, which were more than 100 times lower than in fish muscle. Based on the study of Hijosa-Valsero et al. (2016) HHCB and AHTN are taken up by plants and further incorporated into the cell wall of plant where can follow their aerobic biodegradation. Nevertheless, further studies are needed to suggest degradation products of plant metabolism of tested musk compounds.

To evaluate the possible environmental risk, the hazard quotient (HQ) was calculated. Based on European guidelines (European Commission, 2003), the HQ was estimated as $HQ = MEC/PNEC_{soil}$, where MEC is the environmental concentration and $PNEC_{soil}$ stands for the predicted no-effect concentration in the soil. If $HQ > 1$, it is considered that the concentration could be harmful to the living organisms in soil (Sánchez-Bayo et al., 2002). The value of HQ should be less than 1 to say there is no risk to the soil environment. There have been toxicity test studies to estimate the environmental risks of HHCB and AHTN on soil microorganisms (Balk and Ford, 1999b; Federle et al., 2014). Wang et al. (2013b) determined $PNEC_{soil}$ derived from the solid–solution equilibrium by including soil ingestion of the organisms, with values of HHCB (0.38 mg/kg dw) and AHTN (0.17 mg/kg dw). We used the maximum concentrations in soil for both compounds found at the beginning and end of the bioremediation experiment to calculate the HQ; the results were 0.26 and 0.17 for HHCB and 0.18 and 0.13 for AHTN, respectively. Therefore, the investigated musks namely Galaxolide and Tonalide in soil amended by sewage sludge do not present a risk to the environment since their HQ is far below 1.

4. Conclusion

Work was performed to study the bioremediation of SMCs (HHCB and AHTN) in biosolid-amended soil by white rot (lignolytic) fungi, namely *P. ostreatus*, *I. lacteus* and *P. chrysosporium*, combined with phytoremediation and without phytoremediation. *P. ostreatus* can be seen as the most effective fungal strain based on the removal of both tested musks compared to other mycoremediation treatments. Similar proportions of Galaxolide were removed in mycoremediation using only *P. ostreatus* and phytoremediation using maize (*Z. mays*): 58.9 and 46.3%, respectively. When *P. ostreatus* was used for mycoremediation as well in phyto-mycoremediation treatments, significantly higher removal of Galaxolide ($P < 0.05$) occurred than in the control treatment

represented by natural attenuation of sludge-amended soil. Tonalide was prone to be removed similarly to Galaxolide in the studied bioremediation approaches. Moreover, when *P. chrysosporium* was used in the plant-assisted mycoremediation it improved the removal of Tonalide by 34.5% which was significantly higher than for natural attenuation. This result could be caused by the increased enzymatic activity of lipase when *P. chrysosporium* substrate was used in plant-assisted mycoremediation after 120 days of the experiment. Galaxolide and Tonalide were found in maize roots, and only Galaxolide was observed in shoots. The calculated BCFs of both musks were very low. The residual concentration of tested fragrances remaining at the end of the experiment was not found to pose any environmental risk, as the estimated HQ values were significantly lower than 1.

Credit author statement

Abraham Demelash Chane: Investigation, Formal analysis, Conceptualization, Writing – original draft. Zdeněk Košnář: Methodology, Validation, Writing – review & editing. **Tereza Hřebečková**: Formal analysis. **Lucie Wiesnerová**: Formal analysis. **Miroslav Jozífek**: Methodology. **Petr Doležal**: Formal analysis. **Lukáš Praus**: Formal analysis. **Pavel Tlustoš**: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

Acknowledgment

This work was financial supported by Czech Ministry of Agriculture (QK1710379 and QK1910235) and by the European Regional Development Fund (project NUTRISK no. CZ.02.1.01/0.0/0.0/16_019/0000845).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.138605.

Different letters indicate significant differences ($P < 0.05$) among each treatment as determined by Tukey's test. Abbreviations: SS – sewage sludge amended soil (control), M-PL – mycoremediation with *Pleurotus ostreatus,* M – PH – mycoremediation with *Phanerochaete chrysosporium*, M-IR – mycoremediation with *Irpex lacteus,* PSS – planted sewage sludge amended soil, PM-PL – plant assisted mycoremediation *Pleurotus ostreatus,* PM-PH – plant assisted mycoremediation with *Phanerochaete chrysosporium*, PM-IR – plant assisted mycoremediation with *Irpex lacteus.*

Different letters indicate significant differences ($P < 0.05$) among each treatment as determined by Tukey's test. Abbreviations: SS – sewage sludge amended soil (control), M-PL – mycoremediation with *Pleurotus ostreatus,* M – PH – mycoremediation with *Phanerochaete chrysosporium*, M-IR – mycoremediation with *Irpex lacteus,* PSS – planted sewage sludge amended soil, PM-PL – plant assisted mycoremediation *Pleurotus ostreatus,* PM-PH – plant assisted mycoremediation with *Phanerochaete chrysosporium*, PM-IR – plant assisted mycoremediation with *Irpex lacteus.*

References

Asif, M.B., Hai, F.I., Singh, L., Price, W.E., Nghiem, L.D., 2017. Degradation of pharmaceuticals and personal care products by white-rot fungi—a critical review. Curr. Pollution Rep. 3, 88–103. https://doi.org/10.1007/s40726-017-0049-5.

Balaji, V., Arulazhagan, P., Ebenezer, P., 2014. Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. J. Environ. Biol. 35, 521.

Balci, E., Genisoglu, M., Sofuoglu, S.C., Sofuoglu, A., 2020. Indoor air partitioning of synthetic musk compounds: gas, particulate matter, house dust, and window film. Sci. Total Environ. 729, 138798 https://doi.org/10.1016/j.scitotenv.2020.138798.

Balk, F., Ford, R.A., 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU: I. Fate and exposure assessment. Toxicol. Lett. 111, 57–79. https://doi.org/10.1016/S0378-4274(99)00169-1.

Balk, F., Ford, R.A., 1999b. Environmental risk assessment for the polycyclic musks, AHTN and HHCB: II. Effect assessment and risk characterisation. Toxicol. Lett. 111, 81–94. https://doi.org/10.1016/S0378-4274(99)00170-8.

Biel-Maeso, M., Corada-Fernández, C., Lara-Martín, P.A., 2019. Removal of personal care products (PCPs) in wastewater and sludge treatment and their occurrence in receiving soils. Water Res. 150, 129–139. https://doi.org/10.1016/j.watres.2018.11.045.

Böhmer, W., 2007. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants. Part 2: investigation of polycyclic musks in Soils and Plants (9 pp). J. Soils Sediments 7, 36–44. https://doi.org/10.1065/jss2006.10.187.2.

Cajthaml, T., Křesinová, Z., Svobodová, K., Möder, M., 2009. Biodegradation of endocrine-disrupting compounds and suppression of estrogenic activity by ligninolytic fungi. Chemosphere 75, 745–750. https://doi.org/10.1016/j.chemosphere.2009.01.034.

Chen, G., iang, R., Qiu, J., Cai, S., Zhu, F., Ouyang, G., 2015. Environmental fates of synthetic musks in animal and plant: an in vivo study. Chemosphere 138, 584–591. https://doi.org/10.1016/j.chemosphere.2015.07.003.

Chen, F., Ying, G.G., Ma, Y.B., Chen, Z.F., Lai, H.J., Peng, F.J., 2014. Field dissipation and risk assessment of typical personal care products TCC, TCS, AHTN and HHCB in biosolid-amended soils. Sci. Total Environ. 470, 1078–1086. https://doi.org/10.1016/j.scitotenv.2013.10.080. –471.

Chen, X., Pauly, U., Rehfus, S., Bester, K., 2009. Personal care compounds in a reed bed sludge treatment system. Chemosphere 76, 1094–1101. https://doi.org/10.1016/j.chemosphere.2009.04.023.

Čvančarová, M., Křesinová, Z., Filipová, A., Covino, S., Cajthaml, T., 2012. Biodegradation of PCBs by ligninolytic fungi and characterization of the degradation products. Chemosphere 88, 1317–1323. https://doi.org/10.1016/j.chemosphere.2012.03.107.

DiFrancesco, A.M., Chiu, P.C., Standley, L.J., Allen, H.E., Salvito, D.T., 2004. Dissipation of fragrance materials in sludge-amended soils. Environ. Sci. Technol. 38, 194–201. https://doi.org/10.1021/es034618v.

Djajakirana, G., Joergensen, R.G., Meyer, B., 1996. Ergosterol and microbial biomass relationship in soil. Biol. Fertil. Soils 22, 299–304. https://doi.org/10.1007/BF00334573.

Federle, T., Sun, P., Dyer, S., Kiel, B., 2014. Probabilistic assessment of environmental exposure to the polycyclic musk, HHCB and associated risks in wastewater treatment plant mixing zones and sludge amended soils in the United States. Sci. Total Environ. 493, 1079–1087. https://doi.org/10.1016/j.scitotenv.2014.03.058.

Fernandes, A.S., Azevedo, T., Rocha, F., Nunes, E., Homem, V., 2022. Uptake and translocation of synthetic musk fragrances by pea plant grown in sewage sludge-amended soils. Environ. Pollut. 310, 119908 https://doi.org/10.1016/j.envpol.2022.119908.

Gao, D., Du, L., Yang, J., Wu, W.M., Liang, H., 2010. A critical review of the application of white rot fungus to environmental pollution control. Crit. Rev. Biotechnol. 30, 70–77. https://doi.org/10.3109/07388550903427272.

Hestbjerg, H., Willumsen, P.A., Christensen, M., Andersen, O., Jacobsen, C.S., 2003. Bioaugmentation of tar-contaminated soils under field conditions using *Pleurotus ostreatus* refuse from commercial mushroom production. Environ. Toxicol. Chem. 22, 692–698. https://doi.org/10.1002/etc.5620220402.

Hijosa-Valsero, M., Reyes-Contreras, C., Domínguez, C., Bécares, E., Bayona, J.M., 2016. Behaviour of pharmaceuticals and personal care products in constructed wetland compartments: influent, effluent, pore water, substrate and plant roots. Chemosphere 145, 508–517. https://doi.org/10.1016/j.chemosphere.2015.11.090.

Homem, V., Magalhães, I., Alves, A., Santos, L., 2017. Assessing seasonal variation of synthetic musks in beach sands from Oporto coastal area: a case study. Environ. Pollut. 226, 190–197. https://doi.org/10.1016/j.envpol.2017.04.022.

Hu, Z., Shi, Y., Cai, Y., 2011. Reprint of: concentrations, distribution, and bioaccumulation of synthetic musks in the Haihe River of China. Chemosphere 85, 262–267. https://doi.org/10.1016/j.chemosphere.2011.09.002.

Kinney, C.A., Heuvel, B.V., 2020. Translocation of pharmaceuticals and personal care products after land application of biosolids. Curr. Opin. Environ. Sci. Health. 14, 23–30. https://doi.org/10.1016/j.coesh.2019.11.004.

Košnář, Z., Částková, T., Wiesnerová, L., Praus, L., Jablonský, I., Koudela, M., Tlustoš, P., 2019. Comparing the removal of polycyclic aromatic hydrocarbons in soil after different bioremediation approaches in relationto the extracellular enzyme activities. J. Environ. Sci. 76, 249–258. https://doi.org/10.1016/j.jes.2018.05.007.

Košnář, Z., Mercl, F., Chane, A.D., Pierdonà, L., Míchal, P., Tlustoš, P., 2021. Occurrence of synthetic polycyclic and nitro musk compounds in sewage sludge from municipal wastewater treatment plants. Sci. Total Environ. 801, 149777 https://doi.org/10.1016/j.scitotenv.2021.149777.

Kubátová, A., Erbanová, P., Eichlerová, I., Homolka, L., Nerud, F., Šašek, V., 2001. PCB congener selective biodegradation by the white rot fungus *Pleurotus ostreatus* in

contaminated soil. Chemosphere 43, 207–215. https://doi.org/10.1016/S0045-6535(00)00154-5.
Lange, C., Kuch, B., Metzger, J.W., 2015. Occurrence and fate of synthetic musk fragrances in a small German river. J. Hazard Mater. 282, 34–40. https://doi.org/10.1016/j.jhazmat.2014.06.027.
Li, W., Nanaboina, V., Chen, F., Korshin, G.V., 2016. Removal of polycyclic synthetic musks and antineoplastic drugs in ozonated wastewater: quantitation based on the data of differential spectroscopy. J. Hazard Mater. 304, 242–250. https://doi.org/10.1016/j.jhazmat.2015.10.035.
Lou, Y., Wang, J., Wang, L., Shi, L., Yu, Y., Zhang, M., 2016. Determination of synthetic musks in sediments of Yellow river delta wetland, China. Bull. Environ. Contam. Toxicol. 97, 78–83. https://doi.org/10.1007/s00128-016-1814-7.
Macherius, A., Eggen, T., Lorenz, W.G., Reemtsma, T., Winkler, U., Moeder, M., 2012. Uptake of galaxolide, tonalide, and triclosan by carrot, barley, and meadow fescue plants. J. Agric. Food Chem. 60, 7785–7791. https://doi.org/10.1021/jf301917q.
Madhavi, V., Lele, S.S., 2009. Laccase: properties and applications. Bioresources 4, 1694–1717.
Magan, N., Fragoeiro, S., Bastos, C., 2010. Environmental factors and bioremediation of xenobiotics using white rot fungi. MYCOBIOLOGY 38, 238–248. https://doi.org/10.4489/MYCO.2010.38.4.238.
Mir-Tutusaus, J.A., Baccar, R., Caminal, G., Sarrà, M., 2018. Can white-rot fungi be a real wastewater treatment alternative for organic micropollutants removal? A review. Water Res. 138, 137–151. https://doi.org/10.1016/j.watres.2018.02.056.
Nakata, H., Hinosaka, M., Yanagimoto, H., 2015. Macrocyclic-, polycyclic-, and nitro musks in cosmetics, household commodities and indoor dusts collected from Japan: implications for their human exposure. Ecotoxicol. Environ. Saf. 111, 248–255. https://doi.org/10.1016/j.ecoenv.2014.09.032.
Ogbolosingha, A.J., Essien, E.B., Ohiri, R.C., 2015. Variation of lipase, catalase and dehydrogenase activities during bioremediation of crude oil polluted soil. J. Environ. Earth Sci. 5, 128–141.
Parrish, Z.D., Banks, M.K., Schwab, A.P., 2004. Effectiveness of phytoremediation as a secondary treatment for polycyclic aromatic hydrocarbons (PAHs) in composted soil. Int. J. Phytoremediation 6, 119–137. https://doi.org/10.1080/16226510490454803.
Qiao, J., Zhang, C., Luo, S., Chen, W., 2014. Bioremediation of highly contaminated oilfield soil: bioaugmentation for enhancing aromatic compounds removal. Front. Environ. Sci. Eng. 8, 293–304. https://doi.org/10.1007/s11783-013-0561-9.
Ramos, S., Homem, V., Santos, L., 2019. Development and optimization of a QuEChERS-GC–MS/MS methodology to analyse ultraviolet-filters and synthetic musks in sewage sludge. Sci. Total Environ. 651, 2606. https://doi.org/10.1016/j.scitotenv.2018.10.143. –2614.
Ramos, S., Homem, V., Santos, L., 2020. Analytical methodology to screen UV-filters and synthetic musk compounds in market tomatoes. Chemosphere 238, 124605. https://doi.org/10.1016/j.chemosphere.2019.124605.
Ramos, S., Homem, V., Santos, L., 2021. Uptake and translocation of UV-filters and synthetic musk compounds into edible parts of tomato grown in amended soils. Sci. Total Environ. 792, 148482 https://doi.org/10.1016/j.scitotenv.2021.148482.
Ribeiro, H., Ramos, S., Homem, V., Santos, L., 2017. Can coastline plant species be used as biosamplers of emerging contaminants? - UV-filters and synthetic musks as case studies. Chemosphere 184, 1134–1140.
Rivier, P.A., Havranek, I., Coutris, C., Norli, H.R., Joner, E.J., 2019. Transfer of organic pollutants from sewage sludge to earthworms and barley under field conditions. Chemosphere 222, 954–960. https://doi.org/10.1016/j.chemosphere.2019.02.010.
Rodarte-Morales, A.I., Feijoo, G., Moreira, M.T., Lema, J.M., 2011. Degradation of selected pharmaceutical and personal care products (PPCPs) by white-rot fungi. World J. Microbiol. Biotechnol. 27, 1839–1846. https://doi.org/10.1007/s11274-010-0642-x.
Sánchez-Bayo, F., Baskaran, S., Kennedy, I.R., 2002. Ecological relative risk (EcoRR): another approach for risk assessment of pesticides in agriculture. Agric. Ecosyst. Environ. 91, 37–57. https://doi.org/10.1016/S0167-8809(01)00258-4.
Segura, A., Ramos, J.L., 2013. Plant–bacteria interactions in the removal of pollutants. Curr. Opin. Biotechnol. 24, 467–473. https://doi.org/10.1016/j.copbio.2012.09.011.
Seleiman, M.F., Santanen, A., Mäkelä, P.S.A., 2020. Recycling sludge on cropland as fertilizer – advantages and risks. Resour. Conserv. Recycl. 155, 104647 https://doi.org/10.1016/j.resconrec.2019.104647.
Stella, T., Covino, S., Čvančarová, M., Filipová, A., Petruccioli, M., D'Annibale, A., Cajthaml, T., 2017. Bioremediation of long-term PCB-contaminated soil by white-rot fungi. J. Hazard Mater. 324, 701–710. https://doi.org/10.1016/j.jhazmat.2016.11.044.
Sun, P., Casteel, K., Dai, H., Wehmeyer, K.R., Kiel, B., Federle, T., 2014. Distributions of polycyclic musk fragrance in wastewater treatment plant (WWTP) effluents and sludges in the United States. Sci. Total Environ. 493, 1073–1078. https://doi.org/10.1016/j.scitotenv.2014.04.038.
Vallecillos, L., Pocurull, E., Borrull, F., 2015. Influence of pre-treatment process on matrix effect for the determination of musk fragrances in fish and mussel. Talanta 134, 690–698.
Vallecillos, L., Sadef, Y., Borrull, F., Pocurull, E., Bester, K., 2017. Degradation of synthetic fragrances by laccase-mediated system. J. Hazard Mater. 334, 233–243. https://doi.org/10.1016/j.jhazmat.2017.04.003.
Wang, C., Sun, H., Li, J., Li, Y., Zhang, Q., 2009. Enzyme activities during degradation of polycyclic aromatic hydrocarbons by white rot fungus *Phanerochaete chrysosporium* in soils. Chemosphere 77, 733–738. https://doi.org/10.1016/j.chemosphere.2009.08.028.
Wang, M., Peng, C., Chen, W., Markert, B., 2013a. Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicol. Environ. Saf. 97, 242–247. https://doi.org/10.1016/j.ecoenv.2013.07.032.
Wang, M., Peng, C., Chen, W., Markert, B., 2013b. Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicol. Environ. Saf. 97, 242–247. https://doi.org/10.1016/j.ecoenv.2013.07.032.
Xie, Z., Ebinghaus, R., Temme, C., Heemken, O., Ruck, W., 2007. Air–sea exchange fluxes of synthetic polycyclic musks in the North Sea and the Arctic. Environ. Sci. Technol. 41, 5654–5659. https://doi.org/10.1021/es0704434.
Yang, J.J., Metcalfe, C.D., 2006. Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. Sci. Total Environ. 363, 149–165. https://doi.org/10.1016/j.scitotenv.2005.06.022.
Yang, Y., Liu, Y., Li, Z., Wang, Z., Li, C., Wei, H., 2020. Significance of soil microbe in microbial-assisted phytoremediation: an effective way to enhance phytoremediation of contaminated soil. Int. J. Environ. Sci. Technol. 17, 2477–2484. https://doi.org/10.1007/s13762-020-02668-2.
Zhang, C., Feng, Y., Liu, Y., Chang, H., Li, Z., Xue, J., 2017. Uptake and translocation of organic pollutants in plants: a review. J. Integr. Agric. 16, 1659–1668. https://doi.org/10.1016/S2095-3119(16)61590-3.
Zhang, H., Bu, Q., Wu, D., Yu, G., 2020. Polycyclic musks in surface water and sediments from an urban catchment in the megacity Beijing, China. Environ. Pollut. 263, 114548 https://doi.org/10.1016/j.envpol.2020.114548.

4.5. Fate of selected polycyclic aromatic hydrocarbons and synthetic musks during composting of sludge with bioaugmentation of solid state white-rot fungi strains

Authors: Abraham Demelash Chane, Zdeněk Košnář, Tereza Hřebečková, Miroslav Jozífek, Petr Doležal, Pavel Tlustoš

Information about the magazine: Submitted to a journal with impact factor

Fate of selected polycyclic aromatic hydrocarbons and synthetic musk compounds during composting of sludge with bioaugmentation of solid state white-rot fungi strains

Abraham Demelash Chane[a], Zdeněk Košnář[a]*, Tereza Hřebečková[a], Miroslav Jozífek[b], Petr Doležal[a], Pavel Tlustoš[a]

A Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha-Suchdol, Czech Republic

B Department of Horticulture, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha-Suchdol, Czech Republic

*Corresponding author: Zdeněk Košnář (kosnarz@af.czu.cz)

Abstract

Sewage sludge composting is a widely employed technique aimed at reducing contaminant concentrations and enhancing nutrient content. Given the increasing urbanization, proper management and disposal of sewage sludge are essential due to contamination concerns. The objective of this study was to evaluate the effectiveness of white-rot fungi inoculation at various stages of sludge composting in removing PAHs. Results revealed that the pH of the final sewage sludge ranged slightly acidic, between 6.39 and 6.49. Additionally, the electrical conductivity (EC) ranging from 4.04 mS /cm to 4.6 mS /cm and carbon-to-nitrogen (C/N) ratio ranging 12.3 to 12.9, respectively. A significant negative correlation (r = -0.64) was observed between temperature and PAH removal. Low-molecular-weight (LMW) PAHs were removed in the highest rates from 87.2% to 90.1%, PAH removal decreased with increasing molecular weight. Notably, Naphthalene and Acenaphthylene were highly degraded within this group, while Benzo[a]pyrene and Dibenz[a,h]anthracene were completely removed from high-molecular-weight (HMW) PAHs. The total PAH degradation after 90 days of composting ranged from 56% to 69%. *P. chrysosporium* exhibited significantly lower residual total PAH content compared to other treatments, indicating its effectiveness in PAH removal. Compared to the control, treatments inoculated with white-rot

fungi demonstrated higher PAH degradation at the end of composting. During sludge composting, synthetic musk compounds showed lower degradation in the control treatment compared to other treatments, with galaxolide and tonalide degrading by 13.2% and 18.8%, respectively. Treatments using substrates of white-rot fungi, such as *Phanerochaete*, *Bjerkandera*, *Irpex*, and *Trametes*, exhibited significantly higher degradation rates. Galaxolide degradation ranged from 32.7% to 37.3%, while tonalide degradation ranged from 31.4% to 36.4%, indicating the effectiveness of white-rot fungi in enhancing the breakdown of these compounds.

Keywords: Organic pollutants; Bioremediation**;** Composting**;** Biosolids**;** White-rot fungi

Abbreviations: ACE, Acenaphthylene; ACEN, Acenaphthene; AHTN, Tonalide; ANT, Anthracene; BaA, Benzo[a]anthracene; BbF, Benzo[b]fluoranthene; BkF, Benzo[k]fluoranthene; BghiP, Benzo[g,h,i]perylene; BaP, Benzo[a]pyrene; CHR, Chrysene; DBA, Dibenz[a,h]anthracene; FLU, Fluorene; FLUO, Fluoranthene; IYP, Indeno[l,2,3-c,d] pyrene; NAP,Naphthalene, HHCB, Galaxolide

Introduction

Sewage sludge (SS) is an important biodegradable waste material byproduct of the municipal wastewater treatment plant (Yu et al., 2018). With urbanization on the rise, the scale and number of urban sewage treatment plants are constantly expanding, thereby resulting in the generation of a large volume of sewage sludge (H. Chen et al., 2012). At present, the main representative disposal ways of sewage sludge include landfill, land application, drying-incineration, composting, and recycling as building materials (Lü et al., 2021b).

Sewage sludge (SS) serves as a valuable resource for soil amendments, as it contains rich organic matter and essential nutrients such as phosphorus and nitrogen (Chen et al., 2021; Verlicchi and Zambello, 2015). Application of sludge improves soil structure, enhances aggregate stability, increases water retention capacity, and promotes soil aeration. Additionally, it mitigates

salinization effects in arid regions, supports plant root development, and plays a crucial role in carbon sequestration within the soil. Consequently, this helps in reducing atmospheric CO_2 levels and potential global warming impacts (Annabi et al., 2007; Boldrin et al., 2009).

The main obstacle for sludge-soil utilization is a presence of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides residues, pharmaceutical and personal care product (PPCPs) residues including synthetic musk compounds (SMCs), and many other organic contaminants in it. Many of these compounds result from domestic household use or from small industrial facilities, and it is therefore difficult to avoid the contamination of sludge by these substances (Lapen et al., 2008; Ozaki et al., 2017).

Composting is widely used technique for treating sewage sludge, and it is an accepted method for preparing sewage sludge for land application as a soil amendment (Peltre et al., 2015). Composted sewage sludge is increasingly used due to its ability for rebuilding soil organic matter, provide nutrients, and suppress plant diseases (Sadef et al., 2016). Aerobic composting has been globally applied as an effective and cost-efficient process for its management and reuse (Wang et al., 2019). Many organic micropollutants could be degraded microbially, either aerobically (oxidation) or anaerobically (dehalogenation) for instance. Currently, it's well known that persistent organic compounds (POPs) such as PAHs, PCBs, polychlorinated dibenzodioxins/furans (PCDD/F), synthetic musk compounds (SMCs) and others can be degraded by the composting while anaerobic digestion has a much less effect (Ozaki et al., 2017; Muñoz et al., 2018; Lü et al., 2021).

The aim of this study was to assess the removal efficiency of PAHs and SMCs at different stages of the composting process and to investigate the impact of individually inoculated white-rot fungi on compound degradation. Furthermore, we analyzed how the relationship between physicochemical properties of sludge and the degradation of PAHs over the composting duration.

1. Materials and methods

2.1. Sewage sludge and fungal substrates

Experimental sewage sludge was obtained from a wastewater treatment plant serving a population equivalent to 40,000 individuals. The sludge underwent anaerobic stabilization with a wastewater load of 38%, while the hydraulic retention time in the WWTP was 35 days. Measured removal efficiencies for biochemical oxygen demand and chemical oxygen demand were 96.8% and 95.4%, respectively.

Five fungal substrates, namely *Pleurotus ostreatus* (Jacq.) P. Kumm., strain HK35 (*P. ostreatus*), *Phanerochaete chrysosporium* (Burds.) (*P. chrysosporium*) and *Irpex lacteus* (Fr.), strain 931 (*I. lacteus*), *Bjerkandera adusta* (*B. adusta*) and *Trametes versicolor* (*T. versicolor*), were prepared by the Department of Horticulture, CZU Prague. As briefly outlined by Chane et al. (2023), fungi were cultivated on malt extract-glucose agar to obtain mycelium, which was subsequently utilized as a fungal spawn on wheat grain. The resulting substrate, enriched with gypsum, underwent autoclaving, inoculation with mycelia, and cultivation. Sterilized wheat straw pellets with 60% moisture content were then inoculated with the substrate for each culture, and substrates containing white rot fungi were cultivated for 4 weeks until the straw pellets were fully colonized by respective fungal mycelium.

2.2. The experimental setup

The composting of sewage sludge was conducted in 70-liter plastic laboratory fermenters, with the outer vessel wall insulated to regulate temperature. Aeration was facilitated through an air inlet positioned at the bottom of the vessel, where an aeration device (Atmos, Czech Republic) operated to aerate the composting vessels at 4 l/min per hour for 5 minutes. To prevent condensation within

the vessels, hoses were connected at the top of the plastic and linked to condensation flasks. The composting process spanned 90 days, with treatments organized in 7 treatments, each with a replication. The treatment groups comprised: 1) sewage sludge (S) = control; 2) sewage sludge + autoclaved fungal substrate (SS); 3) sewage sludge + *Pleurotus* substrate (SS-PL); 4) sewage sludge + *Phanerochaete* substrate (SS-PH); 5) sewage sludge + *Bjerkandera* substrate (SS-BJ); 6) sewage sludge + *Irpex* substrate (SS-IR); and 7) sewage sludge + *Trametes* substrate (SS-TR). Substrates were added to the sludge with the ratio of 10:1 (sludge: substrate). Sampling occurred four times during composting: at the experiment's initiation (t = 0 days), at the end of the thermophilic step (t = 30 days), during the stable temperature period midway through the experiment (t = 60 days), and at the maturation step (t = 90 days). Before sampling, the compost was thoroughly mixed to ensure a representative sample and promote aeration. Fresh samples weighing 200 grams were collected in glass bottles and stored in a freezer at -20°C prior to further processes.

2.3. Temperature monitoring and physiochemical analysis

The temperature throughout the composting period was monitored using a PT-100 type sensor enclosed in a stainless-steel casing measuring 0.5 meters in length and 200 millimeters in diameter. The sensor probe traversed the composting material from top to bottom, allowing for comprehensive temperature profiling throughout the process. The sensor probe cable was connected to a computer system for monitoring and recording purposes, enabling remote temperature control. The ambient temperature within the laboratory was maintained at 25 °C.

The dryness content of the composted sewage sludge was determined gravimetrically by lyophilizing the samples. Electrical conductivity and pH were assessed using a 1:5 ratio

(composting sample wet weight: demi water volume). The percentage of organic matter content in the composting piles was determined through gravimetric loss-on-ignition of oven-dried samples (at 105℃ for 24 hours), followed by ashing in a muffle furnace at 550 ℃ for 24 hours. Additionally, the moisture content of the composting materials was determined by drying the samples at 105 ℃ for 24 hours.

2.4. Extraction of organic compounds

The extraction process for determining PAHs and SMCs in composted materials followed the method outlined by (Chane et al., 2023). Initially, 1g of dried composted sludge sample, along with a surrogate standard, was combined with 10 mL each of ethanol, sodium acetate buffer, and hexane. This mixture underwent 30 minutes of rotary shaking (DKSH, Switzerland), followed by 30 minutes of ultrasonication (Bandelin Sonorex, DT510H, Germany) and centrifugation (1328×g) using a Rotina 420 R centrifuge (Hettich, Germany) for 10 minutes. The resulting extracts were filtered using 150 mm filter paper (Whatman®, Grade 40) before being evaporated to near dryness using nitrogen. Subsequently, the samples underwent solid phase extraction (SPE) described by Chane et al., (2023). After evaporation to near dryness, the final 2 mL of n-hexane was added, and following the addition of the internal standard, the samples were ready for PAH and SMCs analysis.

2.5. GC/MS/MS analysis

Instrumental analysis was conducted utilizing an Agilent 8890 gas chromatograph with 7000D Triple Quadrupole (Agilent Technologies, USA), coupled with an automatic sampler system PAL RTC (CTC Analytics AG, Switzerland), following the methodology outlined in a detail by Košnář

et al., (2021). Quantification of target compounds utilized the dynamic multiple reaction monitoring mode. The average recovery of the p-terphenyl-d14 used as a surrogate standard from each sample extracted was 94%. The limit of detection for individual PAHs and SMCs ranged from 0.1 to 5.0 µg/kg dry weight (dw).

2.6. Statistical analysis

Statistical differences among treatments were evaluated using one-way analysis of variance, followed by a *post-hoc* Tukey's test, with a significance level set at $P < 0.05$ for comparisons involving experimental groups. These analyses were carried out using the Statistica 12.0 software package (StatSoft, USA). Pearson correlation was utilized to investigate the relationship between the physicochemical properties of composting sludge and compound degradation during composting. Tables and figures were prepared using Excel 2010 (Microsoft Corporation, USA).

3. Result and discussion

3.1. Physicochemical parameters of sludge during the composting process

Table 1 presents the average changes in physicochemical properties during composting experiment. The initial pH values ranged from 6.85 to 6.75 value and exhibited a slight decrease by the end of the study, ranging from 6.39 to 6.49. Consistent with our findings, Liu et al. (2019) also observed a decrease in pH at the end of their sewage sludge composting experiment (day 60), with values ranging from 6.7−7.1. Value of pH plays a crucial role in the composting process, influencing microbial growth and metabolism.

The carbon-to-nitrogen (C/N) ratio is a vital parameter in composting as it offers valuable insights into the likely rate of organic matter decomposition. Microorganisms typically utilize

approximately 30 parts of carbon for each part of nitrogen (Khalil et al., 2011). Sewage sludge typically exhibits a C/N ratio of less than 15. In our current study, the C/N ratio ranged from 12.8 to 14.1 initially, with the final compost displaying lower values ranging from 12.3 to 12.9. The lower C/N ratio observed in our study could be attributed to the absence of any bulking agent addition during composting. Ramdani et al. (2015) reported a higher C/N ratio of 15.4 after 150 days of composting, while Wang et al. (2013) reported a more similar C/N value at the beginning of composting to our study.

The electrical conductivity (EC) of all treatments exhibited a significant increase from initial values ranging from 2.5 to 3.02 mS cm^{-1} to final compost values ranging from 4.04 to 4.62 mS cm^{-1}. EC serves as an indicator of the salinity level in the composting mixture, which may affect plant growth adversely upon application to soil (Sánchez-Monedero et al., 2001; Lin, 2008). The EC of compost holds considerable agricultural significance, influencing plant growth and seed germination. Elevated EC levels exceeding 8 mS cm^{-1} have been found to negatively impact soil microbial populations and the bio-transformation of organic matter (Ammari et al., 2012). However, it is worth noting that the EC of the final compost products remains below 2.5 mS cm^{-1}.

3.2. Degradation of PAHs and SMCs during sludge composting

During composting, the removal of 16 priority PAHs was measured. At the end of the 90-day period, the concentration of total 16 PAHs significantly decreased compared to the initial concentrations across all treatments (Table 2, Fig. 2). The loss of total PAHs was observed to decrease with increasing hydrophobicity of the PAHs. Composting exhibited higher degradation rates across all treatments for low molecular weight (LMW) PAHs, with removal percentages ranging from 87.2% to 90.1% (Fig. 2). Conversely, medium molecular weight (MMW) and high molecular weight (HMW) PAHs showed lower degradation rates, ranging from 37.4% to 61.6%

and 36% to 53.2%, respectively (Fig. 2). Oleszczuk and Baran (2003) also reported rapid loss of LMW PAHs after composting, while HMW molecules exhibited greater persistence. Despite this trend, BaP and DBA were completely removed in all treatments after 90 days of sludge composting. This finding is consistent with the results of Hafidi et al. (2008), who observed 100% removal of higher molecular weight PAHs, possibly due to less reversible adsorption with an increased number of fused aromatic rings or greater hydrophobicity.

Following 90 days of composting, the residual total PAH content in the composted sludge ranged from 2.35 to 3.32 mg/kg dw, all significantly below the regulatory limit of 6 mg/kg set by EU regulations (Suciu et al., 2015b). The highest final PAH concentration was observed in the control sludge treatment, whereas the *P. chrysosporium* strain (SSPH) treatment exhibited the lowest concentration.

NAP, ACEN, and FLU exhibited the highest degradation rates among LMW PAHs, with percentages exceeding 95 % after 90 days of sludge composting. Siebielska (2014) reported a lower removal percentage for NAP during sludge composting compared to the current study, which utilized a longer composting time and achieved a reduction percentage of approximately 70%. ACE displayed a lower degradation rate among LMW PAHs at the end of experiment, with degradation percentages ranging from 71.5 % to 76.5 %. PHE also exhibited a relatively high degradation rate, ranging from 66.7 % to 88.1 % at the end of the study. Han et al., (2004) similarly reported the degradation of PHE by *T. versicolor* during their incubation experiment, achieving a removal percentage of 65 %. These hydrocarbon groups, characterized by weak sorption and low hydrophobicity, are more readily decomposed by microorganisms (Ozaki et al., 2017). Due to the higher degradation of LMW PAHs, their residual content did not significantly differ between treatments at the end of the study (Table 2).

The sludge treatment (control) exhibited notably lower degradation of MMW PAHs compared to other treatments, with a degradation efficiency of 37% (Fig. 2). Conversely, the *B. adusta* (SS-BJ) variant demonstrated the most significant degradation of total MMW PAHs, with a percentage of 61.6%. Additionally, treatments *T. versicolor* (SS-TR) and *P. chrysosporium* (SS-PH) also showed high degradation efficiencies, reaching 60.2% and 59.4%, respectively (Fig. 2). Among MMW PAHs, PYR emerged as the most resilient, persisting as the primary residual PAH in the composted sludge. Its final concentration ranged from 1167 to 1750 µg/kg dw, potentially impacting the suitability of the composted material for agricultural soil use. Chen and Ding (2012) found that *P. chrysosporium* degraded PYR from sludge-soil by 51.9% after 90 days of incubation. In the current study using a similar strain, the degradation rate was comparable, at 55% after 90 days. The residual content of CHR and BaA in the SS-BJ, SS-IR, and SS-TR treatments at the end of the study was significantly lower than in other treatments, ranging from 3.75 to 4.98 and 10.53 to 11.76 µg/kg dw, respectively (Table 2). Additionally, the degradation percentage at day 90 for both compounds was significantly higher in these three treatments compared to others, ranging from 97% to 97.8% and 90.4% to 91.7%, respectively (Fig. 2a). Cajthaml et al. (2006) reported that *I. lacteus* culture was able to degrade 70% of initially spiked BaA to the culture after just 14 days of the experiment.

As anticipated, the HMW PAHs showed the lowest degradation rates during the 90-day sludge composting period (Figure 2). This phenomenon may be attributed to reduced evaporation rates or diminished biological decomposition due to stronger bonding to solid surfaces (Ozaki et al., 2017). In our study the residual total content of HMW PAHs at the end of composting ranged from 2328 to 3302 µg/kg dw (Table 2). Furthermore, at the end of the study, the concentration of PAHs in all treatments was significantly lower compared to their initial levels. The SS-PH and SS-TR

treatments exhibited the highest degradation percentages of total HMW PAHs at the end of composting, both at 53.2%. For instance this outcome could be attributed to *T. versicolor's* ability to secrete all three enzymes of Lac, MnP, and LiP that facilitate the degradation of HMW PAHs, unlike other treatments (Cajthaml et al., 2009).

During the SS-IR and SS-TR treatments, BghiP was found in significantly lower concentrations compared to the sludge (control) or other treatments at the end of the study, with residual contents of 409 and 397 µg/kg dw, respectively. After 90 days of composting, nearly all PAHs were still detectable, except for DahA and BaP, which were not found in any treatments. Similarly, Cai et al., (2007) reported the absence of DahA and ACY after 56 days of sludge composting, while Hua et al., (2008) observed their absence after 50 days of sludge composting with a degradation percentage of 92%. Another study by Hafidi et al., (2008) reported approximately 99.5% removal of BaP from sludge composting after 90 days. BaP is known to be lipophilic and easily adsorbed on colloids, minerals, and humus (Semple et al., 2003). In the current study, BkF and BbF exhibited the removal percentages ranging from 30% to 61% and 30% to 61%, respectively. The lowest degradation in this group was observed during BghiP, with removal efficiencies ranging from 16% to 49% in all treatments after 90 days.

The removal of total PAHs from sludge composting after 90 days ranged from 55.9% to 69%. Similar results have been reported in other studies, such as Lu et al. (2019), who found removal rates ranging from 58.7% to 76% after 39 days, and Cai et al. (2012), who reported removal rates of 54.6% to 75.9% after 56 days of composting. However, Oleszczuk (2008) reported lower removal rates compared to the current study, ranging from 29.3% to 46.8% after 76 days of sludge composting. The degradation of total PAHs at the end of the study was significantly higher during *P. chrysosporium* (SS-PH) treatment compared to other treatments with 70% of degradation (Fig.

3). Other treatments with white-rot fungi inoculation also exhibited significantly higher degradation of total PAHs compared to the control (SS) treatment. Taha et al. (2018) reported that the removal of total PAHs from biosolids with the application of *P. chrysosporium* was approximately 62% higher after 14 days, compared to natural attenuation treatment, which only achieved 40% degradation. In the current study, the SS-PH, SS-TR, SS-BJ, and SS-IR treatments showed the highest degradation efficiencies, ranging from 63% to 68%.

Synthetic musk compounds (SMCs) were studied for their possible degradation over a 90-day of sludge composting period. The results showed that only galaxolide and tonalide were detected at any stage of the study. The control sludge (S) treatment exhibited statistically lower degradation for both compounds. Similarly, the sludge substrate (SS) and SS-PL treatments also showed lower degradation rates for tonalide, at 22% and 18.6%, and for galaxolide, at 15.4% and 12.8%, respectively. Significantly higher degradation rates for galaxolide and tonalide were observed in the *P. chrysosporium* (SS-PH), B. adusta (SS-BJ), *Irpex lacteus* (SS-IR), and *T. versicolor's* (SS-TR)treatments compared to control, with degradation for galaxolide and tonalide ranging from 32.7% to 37.3% and 31.4% to 36.4%, respectively. This indicates the microbial contribution to contaminant removal. Vallecillos et al. (2017) reported similar results, with a 42% removal of tonalide during sewage sludge composting. Poulsen and Bester. (2010) reported higher degradation rates for galaxolide and tonalide at 89% and 68%, respectively, during 24 days of sludge composting. The higher temperature during the early stages of composting likely contributed to this relatively high degradation. Ozaki et al., (2017) also reported the removal of HHCB and AHTN during composting, registering a median degradation of 65% for both compounds over the span of one year.

The concentration of galaxolide was higher than that of tonalide in the raw material and at the end of the study. After 90 days of composting, during the S, SS, and SS-PL treatments, the concentrations of galaxolide and tonalide were significantly higher than in other treatments, ranging from 6506 to 5742.1 µg/kg and 579.7 to 515.5 µg/kg, respectively (Table 5). In contrast, for treatments with white-rot fungi substrates, including *P. chrysosporium* (SS-PH), B. adusta (SS-BJ), *Irpex lacteus* (SS-IR), and *T. versicolor's* (SS-TR), the remaining concentrations after 90 days of sludge composting were significantly lower than the control, ranging from 4515.8 to 4885.5 µg/kg for galaxolide and from 430.5 to 459.5 µg/kg for tonalide.

3.3. Temperature and removal of PAHs

In the present study, the temperature of sewage sludge composting was ranging from the temperature of 5°C to 41°C. At the beginning of composting experiment, the temperature reaches its maximum 41°C after 4 days afterwards the temperature slowly decreased until it reached around 26°C at the end of composting. Due to depletion of organic matter, the degradation process becomes less resulted with dropped temperature (Wu et al., 2017). Temperature has been previously mentioned as a critical indicator of variations in the microbial activity during the composting process (Huang et al., 2017). From a biological point of view, there are three intervals that govern the different aspects: temperature above 55°C to maximize sanitisation, between 45°C and 55°C to improve the degradation rate and between 35°C and 40°C to increase microbial diversity (Khalil et al., 2011).

The PAHs degradation during the first stage (30 days) of sludge composting was very high compared to the later stages of composting which has slower degradation stages, this could be most likely due to the depletion of the nutrients needed for the microbial activity the possible

natural PAH degraders (Sayara et al., 2011). As shown on Fig. 1, in the current study, the temperature raised to its maximum at the beginning of composting and the degradation of total PAHs was high during that stage. The correlation coefficient between temperature and total PAHs removal for 90 days composting was r = -0.64, which indicated as temperature raised during the first 7 days the concentration of PAHs was also show a significant decreased at the early stages. Later after 40 days of composting, the temperature to stabilize and reached a constant level, where the degradation also seems slower degradation. Antizar-ladislao et al. (2004) also highlight another reason, increased temperatures at the start will increase the solubility and mass transfer rates of the contaminants, thereby making them more available to be metabolised. At the start of composting of the current study, the temperature was raised to reach around 41 °C, because of high organic matter at the beginning of the experiment (Figure 3), according to Antizar-Ladislao et al. (2005) the 38 °C was the optimum temperature for both PAH removal and microbial activity.

4. Conclusion

The study investigated the removal of 16 priority polycyclic aromatic hydrocarbons (PAHs) during vessel sludge composting over a 90-day period. The results revealed significant reductions in total PAH concentrations across all treatments, with LMW PAHs exhibiting higher degradation rates compared to MMW and HMW PAHs. Notably, BaP and DBA were completely removed in all treatments, consistent with previous findings indicating the efficacy of composting in eliminating higher molecular weight PAHs. Significant degradation of B[a]A and CHR occurred during the treatments of *B. adusta* (SS-BJ), *I. lacteus* (SS-IR), and *T. versicolor* (SS-TR) with removal

percentages ranging from 91.6% to 97.8%. *P. chrysosporium* (SS-PH) showed higher degradation of B[b]F, with a removal percentage of 61.2% at the end of composting. *P. chrysosporium* (SS-PH) also demonstrated the highest degradation of total PAHs, underscoring the potential of white-rot fungi (WRF) inoculation to enhance PAH removal during sludge composting processes. For HHCB and AHTN, the treatments with substrates of *P. chrysosporium* (SS-PH), *B. adusta* (SS-BJ), *I. lacteus* (SS-IR), and *T. versicolor* (SS-TR) showed higher degradation at the end of study compared to the control. These findings suggest that composting can be an effective method for reducing PAH, HHCB, and AHTN contamination in sewage sludge, thereby mitigating potential environmental and health risks associated with PAH exposure. However, further research is needed to optimize composting conditions, assess the long-term stability of PAH removal, and evaluate the potential impacts of composted sludge application on soil quality and ecosystem health.

Acknowledgements

The authors gratefully acknowledge the support given by the Czech Ministry of Agriculture (Project QK1710379) and by the European Regional Development Fund (Project NUTRISK: No. CZ.02.1.01/0.0/0.0/16_019/0000845).

5. References

Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egypt. J. Pet. 25, 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011

Aguirre, J., Bizkarguenaga, E., Iparraguirre, A., Fernández, L.Á., Zuloaga, O., Prieto, A., 2014. Development of stir-bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry for the analysis of musks in vegetables and amended soils. Anal. Chim. Acta 812, 74–82. https://doi.org/10.1016/j.aca.2013.12.036

Akhtar, A.B.T., Naseem, S., Yasar, A., Naseem, Z., 2021. Persistent Organic Pollutants (POPs): Sources, Types, Impacts, and Their Remediation, in: Prasad, R. (Ed.), Environmental Pollution and Remediation, Environmental and Microbial Biotechnology. Springer, Singapore, pp. 213–246. https://doi.org/10.1007/978-981-15-5499-5_8

Amir, S., Hafidi, M., Merlina, G., Hamdi, H., Revel, J.C., 2005. Fate of polycyclic aromatic hydrocarbons during composting of lagooning sewage sludge. Chemosphere 58, 449–458. https://doi.org/10.1016/j.chemosphere.2004.09.039

Ammari, T.G., Al-Omari, Q., Abbassi, B.E., 2012. Composting sewage sludge amended with different sawdust proportions and textures and organic waste of food industry--assessment of quality. Environ. Technol. 33, 1641–1649. https://doi.org/10.1080/09593330.2011.641589

An, J., Zhou, Q., Sun, Y., Xu, Z., 2009. Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (Triticum aestivum L.). Chemosphere 76, 1428–1434. https://doi.org/10.1016/j.chemosphere.2009.06.004

Annabi, M., Houot, S., Francou, C., Poitrenaud, M., Bissonnais, Y.L., 2007. Soil Aggregate Stability Improvement with Urban Composts of Different Maturities. Soil Sci. Soc. Am. J. 71, 413–423. https://doi.org/10.2136/sssaj2006.0161

Ansari, F., Momina, Ahmad, A., Rafatullah, M., 2023. Review on bioremediation technologies of polycyclic aromatic hydrocarbons (PAHs) from soil: Mechanisms and future perspective. Int. Biodeterior. Biodegrad. 179, 105582. https://doi.org/10.1016/j.ibiod.2023.105582

ANTIZAR-LADISLAO, B., LOPEZ-REAL, J., BECK, A., 2004. Bioremediation of Polycyclic Aromatic Hydrocarbon (PAH)-Contaminated Waste Using Composting Approaches. Crit. Rev. Environ. Sci. Technol. 34, 249–289. https://doi.org/10.1080/10643380490434119

Antizar-Ladislao, B., Lopez-Real, J., Beck, A.J., 2005. Laboratory studies of the remediation of polycyclic aromatic hydrocarbon contaminated soil by in-vessel composting. Waste Manag. 25, 281–289. https://doi.org/10.1016/j.wasman.2005.01.009

Arruda, V., Simões, M., Gomes, I.B., 2022. Synthetic Musk Fragrances in Water Systems and Their Impact on Microbial Communities. Water 14, 692.

Ayuk-Takem, L., Amissah, F., Aguilar, B.J., Lamango, N.S., 2014. Inhibition of polyisoprenylated methylated protein methyl esterase by synthetic musks induces cell degeneration. Environ. Toxicol. 29, 466–477. https://doi.org/10.1002/tox.21773

Bagheri, M., Bauer, T., Burgman, L.E., Wetterlund, E., 2023. Fifty years of sewage sludge management research: Mapping researchers' motivations and concerns. J. Environ. Manage. 325, 116412. https://doi.org/10.1016/j.jenvman.2022.116412

Balk, F., Ford, R.A., 1999. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU: I. Fate and exposure assessment. Toxicol. Lett. 111, 57–79. https://doi.org/10.1016/S0378-4274(99)00169-1

Biel-Maeso, M., Corada-Fernández, C., Lara-Martín, P.A., 2019. Removal of personal care products (PCPs) in wastewater and sludge treatment and their occurrence in receiving soils. Water Res. 150, 129–139. https://doi.org/10.1016/j.watres.2018.11.045

Böhmer, W., 2007. Occurrence of Polycyclic Musks in Sewage Sludge and their Behaviour in Soils and Plants. Part 2: Investigation of Polycyclic Musks in Soils and Plants (9 pp). J. Soils Sediments 7, 36–44. https://doi.org/10.1065/jss2006.10.187.2

Bolden, A.L., Rochester, J.R., Schultz, K., Kwiatkowski, C.F., 2017. Polycyclic aromatic hydrocarbons and female reproductive health: A scoping review. Reprod. Toxicol. 73, 61–74. https://doi.org/10.1016/j.reprotox.2017.07.012

Boldrin, A., Andersen, J.K., Møller, J., Christensen, T.H., Favoino, E., 2009. Composting and compost utilization: accounting of greenhouse gases and global warming contributions. Waste Manag. Res. 27, 800–812. https://doi.org/10.1177/0734242X09345275

Burchiel, S.W., Luster, M.I., 2001. Signaling by Environmental Polycyclic Aromatic Hydrocarbons in Human Lymphocytes. Clin. Immunol. 98, 2–10. https://doi.org/10.1006/clim.2000.4934

Cai, Q.-Y., Mo, C.-H., Lü, H., Zeng, Q.-Y., Wu, Q.-T., Li, Y.-W., 2012. Effect of composting on the removal of semivolatile organic chemicals (SVOCs) from sewage sludge. Bioresour. Technol., Advances in Biological Waste Treatment and Bioconversion Technologies 126, 453–457. https://doi.org/10.1016/j.biortech.2011.11.039

Cai, Q.-Y., Mo, C.-H., Wu, Q.-T., Zeng, Q.-Y., 2008. Polycyclic aromatic hydrocarbons and phthalic acid esters in the soil–radish (Raphanus sativus) system with sewage sludge and compost application. Bioresour. Technol. 99, 1830–1836. https://doi.org/10.1016/j.biortech.2007.03.035

Cai, Q.-Y., Mo, C.-H., Wu, Q.-T., Zeng, Q.-Y., Katsoyiannis, A., Férard, J.-F., 2007a. Bioremediation of polycyclic aromatic hydrocarbons (PAHs)-contaminated sewage sludge by different composting processes. J. Hazard. Mater. 142, 535–542. https://doi.org/10.1016/j.jhazmat.2006.08.062

Cai, Q.-Y., Mo, C.-H., Wu, Q.-T., Zeng, Q.-Y., Katsoyiannis, A., Férard, J.-F., 2007b. Bioremediation of polycyclic aromatic hydrocarbons (PAHs)-contaminated sewage sludge by different composting processes. J. Hazard. Mater. 142, 535–542. https://doi.org/10.1016/j.jhazmat.2006.08.062

Cajthaml, T., Erbanová, P., Šašek, V., Moeder, M., 2006. Breakdown products on metabolic pathway of degradation of benz[*a*]anthracene by a ligninolytic fungus. Chemosphere 64, 560–564. https://doi.org/10.1016/j.chemosphere.2005.11.034

Cajthaml, T., Křesinová, Z., Svobodová, K., Möder, M., 2009. Biodegradation of endocrine-disrupting compounds and suppression of estrogenic activity by ligninolytic fungi. Chemosphere 75, 745–750. https://doi.org/10.1016/j.chemosphere.2009.01.034

Calderón-Preciado, D., Matamoros, V., Bayona, J.M., 2011. Occurrence and potential crop uptake of emerging contaminants and related compounds in an agricultural irrigation network. Sci. Total Environ. 412–413, 14–19. https://doi.org/10.1016/j.scitotenv.2011.09.057

Chane, A.D., Košnář, Z., Hřebečková, T., Wiesnerová, L., Jozífek, M., Doležal, P., Praus, L., Tlustoš, P., 2023. Bioremediation of the synthetic musk compounds Galaxolide and Tonalide by white rot fungal strain-assisted phytoremediation in biosolid-amended soil. Chemosphere 328, 138605. https://doi.org/10.1016/j.chemosphere.2023.138605

Chase, D.A., Karnjanapiboonwong, A., Fang, Y., Cobb, G.P., Morse, A.N., Anderson, T.A., 2012. Occurrence of synthetic musk fragrances in effluent and non-effluent impacted

environments. Sci. Total Environ. 416, 253–260. https://doi.org/10.1016/j.scitotenv.2011.11.067

Chen, B., Ding, J., 2012. Biosorption and biodegradation of phenanthrene and pyrene in sterilized and unsterilized soil slurry systems stimulated by *Phanerochaete chrysosporium*. J. Hazard. Mater. 229–230, 159–169. https://doi.org/10.1016/j.jhazmat.2012.05.090

Chen, C., Zhou, Q., Bao, Y., Li, Y., Wang, P., 2010. Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (Triticum aestivum). J. Environ. Sci. 22, 1966–1973. https://doi.org/10.1016/S1001-0742(09)60347-8

Chen, F., Gao, J., Zhou, Q., 2012. Toxicity assessment of simulated urban runoff containing polycyclic musks and cadmium in Carassius auratus using oxidative stress biomarkers. Environ. Pollut. 162, 91–97. https://doi.org/10.1016/j.envpol.2011.10.016

Chen, F., Ying, G.-G., Ma, Y.-B., Chen, Z.-F., Lai, H.-J., Peng, F.-J., 2014. Field dissipation and risk assessment of typical personal care products TCC, TCS, AHTN and HHCB in biosolid-amended soils. Sci. Total Environ. 470–471, 1078–1086. https://doi.org/10.1016/j.scitotenv.2013.10.080

Chen, H., Yan, S.-H., Ye, Z.-L., Meng, H.-J., Zhu, Y.-G., 2012. Utilization of urban sewage sludge: Chinese perspectives. Environ. Sci. Pollut. Res. 19, 1454–1463. https://doi.org/10.1007/s11356-012-0760-0

Chen, Z., Li, Y., Peng, Y., Ye, C., Zhang, S., 2021. Effects of antibiotics on hydrolase activity and structure of microbial community during aerobic co-composting of food waste with sewage sludge. Bioresour. Technol. 321, 124506. https://doi.org/10.1016/j.biortech.2020.124506

Cristaldi, A., Conti, G.O., Jho, E.H., Zuccarello, P., Grasso, A., Copat, C., Ferrante, M., 2017. Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. Environ. Technol. Innov. 8, 309–326. https://doi.org/10.1016/j.eti.2017.08.002

Deselnicu, D., Militaru, G., Deselnicu, V., ZĂINESCU, G., A., L., 2018. Towards a Circular Economy– a Zero Waste Programme for Europe. https://doi.org/10.24264/icams-2018.XI.4

Diggs, D.L., Huderson, A.C., Harris, K.L., Myers, J.N., Banks, L.D., Rekhadevi, P.V., Niaz, M.S., Ramesh, A., 2011. Polycyclic Aromatic Hydrocarbons and Digestive Tract Cancers: A Perspective. J. Environ. Sci. Health Part C 29, 324–357. https://doi.org/10.1080/10590501.2011.629974

Dodgen, L.K., Ueda, A., Wu, X., Parker, D.R., Gan, J., 2015. Effect of transpiration on plant accumulation and translocation of PPCP/EDCs. Environ. Pollut. 198, 144–153. https://doi.org/10.1016/j.envpol.2015.01.002

Domínguez-Morueco, N., Carvalho, M., Sierra, J., Schuhmacher, M., Domingo, J.L., Ratola, N., Nadal, M., 2018. Multi-component determination of atmospheric semi-volatile organic compounds in soils and vegetation from Tarragona County, Catalonia, Spain. Sci. Total Environ. 631–632, 1138–1152. https://doi.org/10.1016/j.scitotenv.2018.03.074

Edokpayi, J.N., Odiyo, J.O., Popoola, O.E., Msagati, T.A.M., 2016. zhend. Int. J. Environ. Res. Public. Health 13, 387. https://doi.org/10.3390/ijerph13040387

Feng, L.-J., Zhang, L.-Q., Feng, L., Li, J.-L., 2019. Dissipation of polycyclic aromatic hydrocarbons (PAHs) in soil amended with sewage sludge and sludge compost. Environ. Sci. Pollut. Res. 26, 34127–34136. https://doi.org/10.1007/s11356-018-3383-2

Fernandes, A.S., Azevedo, T., Rocha, F., Nunes, E., Homem, V., 2022. Uptake and translocation of synthetic musk fragrances by pea plant grown in sewage sludge-amended soils. Environ. Pollut. 310, 119908. https://doi.org/10.1016/j.envpol.2022.119908

Fussell, R.J., Garcia Lopez, M., Mortimer, D.N., Wright, S., Sehnalova, M., Sinclair, C.J., Fernandes, A., Sharman, M., 2014. Investigation into the Occurrence in Food of Veterinary Medicines, Pharmaceuticals, and Chemicals Used in Personal Care Products. J. Agric. Food Chem. 62, 3651–3659. https://doi.org/10.1021/jf4052418

Gao, N., Kamran, K., Quan, C., Williams, P.T., 2020. Thermochemical conversion of sewage sludge: A critical review. Prog. Energy Combust. Sci. 79, 100843. https://doi.org/10.1016/j.pecs.2020.100843

Gawryluk, A., Stępniowska, A., Lipińska, H., 2022. Effect of soil contamination with polycyclic aromatic hydrocarbons from drilling waste on germination and growth of lawn grasses. Ecotoxicol. Environ. Saf. 236, 113492. https://doi.org/10.1016/j.ecoenv.2022.113492

Ghahari, Sajjad, Ghahari, Somayeh, Ghahari, Saeid, Nematzadeh, G., Sarma, H., 2021. Integrated remediation approaches for selected pharmaceutical and personal care products in urban soils for a sustainable future. Energy Ecol. Environ. https://doi.org/10.1007/s40974-021-00218-1

Guo, Y., Rene, E.R., Wang, J., Ma, W., 2020. Biodegradation of polyaromatic hydrocarbons and the influence of environmental factors during the co-composting of sewage sludge and green forest waste. Bioresour. Technol. 297, 122434. https://doi.org/10.1016/j.biortech.2019.122434

Gupta, S., Pathak, B., 2020. Chapter 6 - Mycoremediation of polycyclic aromatic hydrocarbons, in: Singh, P., Kumar, A., Borthakur, A. (Eds.), Abatement of Environmental Pollutants. Elsevier, pp. 127–149. https://doi.org/10.1016/B978-0-12-818095-2.00006-0

Haddaoui, I., Mahjoub, O., Mahjoub, B., Boujelben, A., Di Bella, G., 2016. Occurrence and distribution of PAHs, PCBs, and chlorinated pesticides in Tunisian soil irrigated with treated wastewater. Chemosphere 146, 195–205. https://doi.org/10.1016/j.chemosphere.2015.12.007

Hafidi, M., Amir, S., Jouraiphy, A., Winterton, P., El Gharous, M., Merlina, G., Revel, J.-C., 2008. Fate of polycyclic aromatic hydrocarbons during composting of activated sewage sludge with green waste. Bioresour. Technol. 99, 8819–8823. https://doi.org/10.1016/j.biortech.2008.04.044

Han, M.-J., Park, H.-T., Song, H.-G., 2004. Degradation of Phenanthrene by Trametes versicolor and Its Laccase. J. Microbiol. 42, 94–98.

Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. J. Hazard. Mater. 169, 1–15. https://doi.org/10.1016/j.jhazmat.2009.03.137

Heidler, J., Halden, R.U., 2008. Meta-Analysis of Mass Balances Examining Chemical Fate during Wastewater Treatment. Environ. Sci. Technol. 42, 6324–6332. https://doi.org/10.1021/es703008y

Ho, J., Chambers, L.G., 2019. Altered soil microbial community composition and function in two shrub-encroached marshes with different physicochemical gradients. Soil Biol. Biochem. 130, 122–131. https://doi.org/10.1016/j.soilbio.2018.12.004

Homem, V., Magalhães, I., Alves, A., Santos, L., 2017. Assessing seasonal variation of synthetic musks in beach sands from Oporto coastal area: A case study. Environ. Pollut. 226, 190–197. https://doi.org/10.1016/j.envpol.2017.04.022

Houshani, M., Salehi-Lisar, S.Y., Motafakkerazad, R., Movafeghi, A., 2019. Uptake and distribution of phenanthrene and pyrene in roots and shoots of maize (Zea mays L.). Environ. Sci. Pollut. Res. 26, 9938–9944. https://doi.org/10.1007/s11356-019-04371-3

Hua, L., Wu, W., Liu, Y., Chen, Y., McBride, M.B., 2008. Effect of composting on polycyclic aromatic hydrocarbons removal in sewage sludge. Water. Air. Soil Pollut. 193, 259–267. https://doi.org/10.1007/s11270-008-9687-y

Huang, C., Zeng, G., Huang, D., Lai, C., Xu, P., Zhang, C., Cheng, M., Wan, J., Hu, L., Zhang, Y., 2017. Effect of *Phanerochaete chrysosporium* inoculation on bacterial community and metal stabilization in lead-contaminated agricultural waste composting. Bioresour. Technol. 243, 294–303. https://doi.org/10.1016/j.biortech.2017.06.124

Hurtado, C., Domínguez, C., Pérez-Babace, L., Cañameras, N., Comas, J., Bayona, J.M., 2016. Estimate of uptake and translocation of emerging organic contaminants from irrigation water concentration in lettuce grown under controlled conditions. J. Hazard. Mater. 305, 139–148. https://doi.org/10.1016/j.jhazmat.2015.11.039

Inglezakis, V.J., Zorpas, A.A., Karagiannidis, A., Samaras, P., Voukkali, I., Sklari, S., 2014. EUROPEAN UNION LEGISLATION ON SEWAGE SLUDGE MANAGEMENT. Fresenius Environ. Bull. 23, 6.

Iticescu, C., Georgescu, L.P., Murariu, G., Circiumaru, A., Timofti, M., 2018. The characteristics of sewage sludge used on agricultural lands. AIP Conf. Proc. 2022, 020001. https://doi.org/10.1063/1.5060681

Jadlovec, M., Výtisk, J., Honus, S., Pospišilík, V., Bassel, N., 2023. Pollutants production, energy recovery and environmental impact of sewage sludge co-incineration with biomass pellets. Environ. Technol. Innov. 32, 103400. https://doi.org/10.1016/j.eti.2023.103400

Jin, B., Liu, Y., Liu, G., Zhai, H., Niu, J., Jia, Y., Wang, B., Du, J., Yin, Z., Pang, L., Ji, J., Cao, X., 2024. Toxic effects of polycyclic aromatic hydrocarbons on the two-sludge system: Combined functional gene, resistance gene and microbial community assessment. Process Saf. Environ. Prot. 185, 53–63. https://doi.org/10.1016/j.psep.2024.03.018

Jones, V., Gardner, M., Ellor, B., 2014. Concentrations of trace substances in sewage sludge from 28 wastewater treatment works in the UK. Chemosphere 111, 478–484. https://doi.org/10.1016/j.chemosphere.2014.04.025

Kanungo, J., Sahoo, T., Swain, L.P., Behera, I.D., 2023. Toxicity of Persistent Hydrocarbon Pollutants, Sources and Sustainable Remediation Process, in: Behera, I.D., Das, A.P. (Eds.), Impact of Petroleum Waste on Environmental Pollution and Its Sustainable Management Through Circular Economy. Springer Nature Switzerland, Cham, pp. 39–65. https://doi.org/10.1007/978-3-031-48220-5_2

Katsou, E., Malamis, S., Mamais, D., Bolzonella, D., Fatone, F., 2015. Occurrence, fate, and removal of PAHs and VOCs in WWTPs using activated sludge processes and membrane bioreactors: Results from Italy and Greece, in: Wastewater Treatment: Occurrence and Fate of Polycyclic Aromatic Hydrocarbons (PAHs). pp. 113–140.

Kelessidis, A., Stasinakis, A.S., 2012. Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. Waste Manag. 32, 1186–1195. https://doi.org/10.1016/j.wasman.2012.01.012

Khalid, M., Abdollahi, M., 2021. Environmental Distribution of Personal Care Products and Their Effects on Human Health. Iran. J. Pharm. Res. IJPR 20, 216–253. https://doi.org/10.22037/ijpr.2021.114891.15088

Khalil, A.I., Hassouna, M.S., El-Ashqar, H.M.A., Fawzi, M., 2011. Changes in physical, chemical and microbial parameters during the composting of municipal sewage sludge. World J. Microbiol. Biotechnol. 27, 2359–2369. https://doi.org/10.1007/s11274-011-0704-8

Kim, K.-H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ. Int. 60, 71–80. https://doi.org/10.1016/j.envint.2013.07.019

Kinney, C.A., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner, S.L., Bossio, J.P., Benotti, M.J., 2008. Bioaccumulation of Pharmaceuticals and Other Anthropogenic Waste Indicators in Earthworms from Agricultural Soil Amended With Biosolid or Swine Manure. Environ. Sci. Technol. 42, 1863–1870. https://doi.org/10.1021/es702304c

Kinney, C.A., Furlong, E.T., Zaugg, S.D., Burkhardt, M.R., Werner, S.L., Cahill, J.D., Jorgensen, G.R., 2006. Survey of Organic Wastewater Contaminants in Biosolids Destined for Land Application. Environ. Sci. Technol. 40, 7207–7215. https://doi.org/10.1021/es0603406

Kinney, C.A., Heuvel, B.V., 2020. Translocation of pharmaceuticals and personal care products after land application of biosolids. Curr. Opin. Environ. Sci. Health, Environmental Pollution: Biosolids 14, 23–30. https://doi.org/10.1016/j.coesh.2019.11.004

Kleinteich, J., Seidensticker, S., Marggrander, N., Zarfl, C., 2018. Microplastics Reduce Short-Term Effects of Environmental Contaminants. Part II: Polyethylene Particles Decrease the

Effect of Polycyclic Aromatic Hydrocarbons on Microorganisms. Int. J. Environ. Res. Public. Health 15, 287. https://doi.org/10.3390/ijerph15020287

Kodešová, R., Švecová, H., Klement, A., Fér, M., Nikodem, A., Fedorova, G., Rieznyk, O., Kočárek, M., Sadchenko, A., Chroňáková, A., Grabic, R., 2024. Contamination of water, soil, and plants by micropollutants from reclaimed wastewater and sludge from a wastewater treatment plant. Sci. Total Environ. 907, 167965. https://doi.org/10.1016/j.scitotenv.2023.167965

Košnář, Z., Mercl, F., Chane, A.D., Pierdonà, L., Míchal, P., Tlustoš, P., 2021. Occurrence of synthetic polycyclic and nitro musk compounds in sewage sludge from municipal wastewater treatment plants. Sci. Total Environ. 801, 149777. https://doi.org/10.1016/j.scitotenv.2021.149777

Lapen, D.R., Topp, E., Metcalfe, C.D., Li, H., Edwards, M., Gottschall, N., Bolton, P., Curnoe, W., Payne, M., Beck, A., 2008. Pharmaceutical and personal care products in tile drainage following land application of municipal biosolids. Sci. Total Environ. 399, 50–65. https://doi.org/10.1016/j.scitotenv.2008.02.025

Lee, B.-K., 2010. Sources, Distribution and Toxicity of Polyaromatic Hydrocarbons (PAHs) in Particulate Matter, in: Air Pollution. IntechOpen. https://doi.org/10.5772/10045

Li, H., Ma, Y., 2016. Field study on the uptake, accumulation, translocation and risk assessment of PAHs in a soil-wheat system with amendments of sewage sludge. Sci. Total Environ. 560–561, 55–61. https://doi.org/10.1016/j.scitotenv.2016.04.017

Lin, C., 2008. A negative-pressure aeration system for composting food wastes. Bioresour. Technol. 99, 7651–7656. https://doi.org/10.1016/j.biortech.2008.01.078

Litz, W., 2007. Occurrence of Polycyclic Musks in Sewage Sludge and their Behaviour in Soils and Plants. Part 2: investigation of Polycyclic Musks in Soils and Plants (9 pp). J. Soils Sediments 7, 36–44.

Liu, H., Yin, H., Tang, S., Wei, K., Peng, H., Lu, G., Dang, Z., 2019. Effects of benzo [a] pyrene (BaP) on the composting and microbial community of sewage sludge. Chemosphere 222, 517–526. https://doi.org/10.1016/j.chemosphere.2019.01.180

Liu, J., Zhang, W., Zhou, Q., Zhou, Q., Zhang, Y., Zhu, L., 2020. Polycyclic musks in the environment: A review of their concentrations and distribution, ecological effects and behavior, current concerns and future prospects. Crit. Rev. Environ. Sci. Technol. https://doi.org/10.1080/10643389.2020.1724748

Liu, N., Shi, Y., Li, W., Xu, L., Cai, Y., 2014. Concentrations and distribution of synthetic musks and siloxanes in sewage sludge of wastewater treatment plants in China. Sci. Total Environ. 476–477, 65–72. https://doi.org/10.1016/j.scitotenv.2013.12.124

Liu, Q., Xu, X., Lin, L., Wang, D., 2021. Occurrence, distribution and ecological risk assessment of polycyclic aromatic hydrocarbons and their derivatives in the effluents of wastewater treatment plants. Sci. Total Environ. 789, 147911. https://doi.org/10.1016/j.scitotenv.2021.147911

Liu, Z., Li, Q., Wu, Q., Kuo, D.T.F., Chen, S., Hu, X., Deng, M., Zhang, H., Luo, M., 2017. Removal Efficiency and Risk Assessment of Polycyclic Aromatic Hydrocarbons in a Typical Municipal Wastewater Treatment Facility in Guangzhou, China. Int. J. Environ. Res. Public. Health 14, 861. https://doi.org/10.3390/ijerph14080861

Lixandru, G., 2005. The use of sewage sludge as a fertilizer in agriculture, Pedogenic Factors and Processes in the Temperate Zone. Al Cuza Univ.

Lü, H., Chen, X.-H., Mo, C.-H., Huang, Y.-H., He, M.-Y., Li, Y.-W., Feng, N.-X., Katsoyiannis, A., Cai, Q.-Y., 2021. Occurrence and dissipation mechanism of organic pollutants during the composting of sewage sludge: A critical review. Bioresour. Technol. 328, 124847. https://doi.org/10.1016/j.biortech.2021.124847

Lu, Y., Zheng, G., Zhou, W., Wang, J., Zhou, L., 2019. Bioleaching conditioning increased the bioavailability of polycyclic aromatic hydrocarbons to promote their removal during co-composting of industrial and municipal sewage sludges. Sci. Total Environ. 665, 1073–1082. https://doi.org/10.1016/j.scitotenv.2019.02.174

Lucid, J.D., Fenton, O., Grant, J., Healy, M.G., 2014. Effect of Rainfall Time Interval on Runoff Losses of Biosolids and Meat and Bone Meal when Applied to a Grassland Soil. Water. Air. Soil Pollut. 225, 2042. https://doi.org/10.1007/s11270-014-2042-6

Luo, N., Gao, Y., Chen, X., Wang, M., Niu, X., Li, G., An, T., 2023. A critical review of environmental exposure, metabolic transformation, and the human health risks of synthetic musks. Crit. Rev. Environ. Sci. Technol. 0, 1–18. https://doi.org/10.1080/10643389.2023.2217068

LüZe, XiaoMin, H., Jing, A., Wei, W., 2017. Joint effects of galaxolide and cadmium on soil microbial community function and abundance. J. Agro-Environ. Sci. 36, 66–75.

Lv, N., Wang, B., Wang, H., Xiao, T., Dong, B., Xu, Z., 2024. The occurrence characteristics, removal efficiency, and risk assessment of polycyclic aromatic hydrocarbons in sewage sludges from across China. Chemosphere 351, 141033. https://doi.org/10.1016/j.chemosphere.2023.141033

Lv, Y., Yuan, T., Hu, J., Wang, W., 2010. Seasonal occurrence and behavior of synthetic musks (SMs) during wastewater treatment process in Shanghai, China. Sci. Total Environ. 408, 4170–4176. https://doi.org/10.1016/j.scitotenv.2010.05.003

Macherius, A., Eggen, T., Lorenz, W.G., Reemtsma, T., Winkler, U., Moeder, M., 2012. Uptake of Galaxolide, Tonalide, and Triclosan by Carrot, Barley, and Meadow Fescue Plants. J. Agric. Food Chem. 60, 7785–7791. https://doi.org/10.1021/jf301917q

Mailler, R., Gasperi, J., Chebbo, G., Rocher, V., 2014. Priority and emerging pollutants in sewage sludge and fate during sludge treatment. Waste Manag. 34, 1217–1226. https://doi.org/10.1016/j.wasman.2014.03.028

McErlean, C., Marchant, R., Banat, I.M., 2006. An Evaluation of Soil Colonisation Potential of Selected Fungi and their Production of Ligninolytic Enzymes for Use in Soil Bioremediation Applications. Antonie Van Leeuwenhoek 90, 147–158. https://doi.org/10.1007/s10482-006-9069-7

Meng, X., Liu, B., Zhang, H., Wu, J., Yuan, X., Cui, Z., 2019. Co-composting of the biogas residues and spent mushroom substrate: Physicochemical properties and maturity assessment. Bioresour. Technol. 276, 281–287. https://doi.org/10.1016/j.biortech.2018.12.097

Muñoz, M., Garrido, M.A., Gomez-Rico, M.F., Font, R., 2018. PCDD/F determination in sewage sludge composting. Influence of aeration and the presence of PCP. Sci. Total Environ. 616–617, 763–773. https://doi.org/10.1016/j.scitotenv.2017.10.249

Nam, T.-H., Jeon, H.-J., Mo, H., Cho, K., Ok, Y.-S., Lee, S.-E., 2015. Determination of biomarkers for polycyclic aromatic hydrocarbons (PAHs) toxicity to earthworm (Eisenia fetida). Environ. Geochem. Health 37, 943–951. https://doi.org/10.1007/s10653-015-9706-z

Ning, X.-A., Lin, M.-Q., Shen, L.-Z., Zhang, J.-H., Wang, J.-Y., Wang, Y.-J., Yang, Z.-Y., Liu, J.-Y., 2014. Levels, composition profiles and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in sludge from ten textile dyeing plants. Environ. Res. 132, 112–118. https://doi.org/10.1016/j.envres.2014.03.041

Novotný, Č., Erbanová, P., Cajthaml, T., Rothschild, N., Dosoretz, C., Šašek, V., 2000. Irpex lacteus, a white rot fungus applicable to water and soil bioremediation. Appl. Microbiol. Biotechnol. 54, 850–853. https://doi.org/10.1007/s002530000432

Oleszczuk, P., 2009. Application of three methods used for the evaluation of polycyclic aromatic hydrocarbons (PAHs) bioaccessibility for sewage sludge composting. Bioresour. Technol. 100, 413–420. https://doi.org/10.1016/j.biortech.2008.05.039

Oleszczuk, P., 2008. Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. Ecotoxicol. Environ. Saf. 69, 496–505. https://doi.org/10.1016/j.ecoenv.2007.04.006

Oleszczuk, P., Baran, S., 2003. Degradation of Individual Polycyclic Aromatic Hydrocarbons (PAHs) in Soil Polluted With Aircraft Fuel. Pol. J. Environ. Stud. 12.

Olofsson, U., Brorström-Lundén, E., Kylin, H., Haglund, P., 2013. Comprehensive mass flow analysis of Swedish sludge contaminants. Chemosphere 90, 28–35. https://doi.org/10.1016/j.chemosphere.2012.07.002

Osemwengie, L.I., Steinberg, S., 2001. On-site solid-phase extraction and laboratory analysis of ultra-trace synthetic musks in municipal sewage effluent using gas chromatography–mass spectrometry in the full-scan mode. J. Chromatogr. A 932, 107–118. https://doi.org/10.1016/S0021-9673(01)01216-X

Ozaki, N., Nakazato, A., Nakashima, K., Kindaichi, T., Ohashi, A., 2017. Loading and removal of PAHs, fragrance compounds, triclosan and toxicity by composting process from sewage sludge. Sci. Total Environ. 605–606, 860–866. https://doi.org/10.1016/j.scitotenv.2017.06.165

Ozcan, S., Tor, A., Aydin, M.E., 2013. Investigation on the Levels of Heavy Metals, Polycyclic Aromatic Hydrocarbons, and Polychlorinated Biphenyls in Sewage Sludge Samples and Ecotoxicological Testing. CLEAN – Soil Air Water 41, 411–418. https://doi.org/10.1002/clen.201100187

Panneerselvam, P., Mitra, D., Mohanty, B., Rittick, M., Shadangi, S., 2022. Soil pollution and their impact on soil microorganisms. Environ. Pollut. 70–85.

Paraíba, L.C., Queiroz, S.C.N., Maia, A. de H.N., Ferracini, V.L., 2010. Bioconcentration factor estimates of polycyclic aromatic hydrocarbons in grains of corn plants cultivated in soils treated with sewage sludge. Sci. Total Environ. 408, 3270–3276. https://doi.org/10.1016/j.scitotenv.2010.04.026

Parolini, M., Magni, S., Traversi, I., Villa, S., Finizio, A., Binelli, A., 2015. Environmentally relevant concentrations of galaxolide (HHCB) and tonalide (AHTN) induced oxidative and genetic damage in Dreissena polymorpha. J. Hazard. Mater. 285, 1–10. https://doi.org/10.1016/j.jhazmat.2014.11.037

Patel, A.B., Shaikh, S., Jain, K.R., Desai, C., Madamwar, D., 2020. Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. Front. Microbiol. 11.

Pedersen, J.A., Soliman, M., Suffet, I.H., 2005. Human pharmaceuticals, hormones, and personal care product ingredients in runoff from agricultural fields irrigated with treated wastewater. J. Agric. Food Chem. 53, 1625–1632. https://doi.org/10.1021/jf049228m

Peltre, C., Nyord, T., Bruun, S., Jensen, L.S., Magid, J., 2015. Repeated soil application of organic waste amendments reduces draught force and fuel consumption for soil tillage. Agric. Ecosyst. Environ. 211, 94–101. https://doi.org/10.1016/j.agee.2015.06.004

Peng, F.-J., Kiggen, F., Pan, C.-G., Bracewell, S.A., Ying, G.-G., Salvito, D., Selck, H., Van den Brink, P.J., 2019. Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicol. Environ. Saf. 169, 902–910. https://doi.org/10.1016/j.ecoenv.2018.11.092

Phillips, P.J., Chalmers, A.T., Gray, J.L., Kolpin, D.W., Foreman, W.T., Wall, G.R., 2012. Combined Sewer Overflows: An Environmental Source of Hormones and Wastewater Micropollutants. Environ. Sci. Technol. 46, 5336–5343. https://doi.org/10.1021/es3001294

Pinkas, A., Gonçalves, C.L., Aschner, M., 2017. Neurotoxicity of fragrance compounds: A review. Environ. Res. 158, 342–349. https://doi.org/10.1016/j.envres.2017.06.035

Poluszyńska, J., Jarosz-Krzemińska, E., Helios-Rybicka, E., 2017. Studying the Effects of Two Various Methods of Composting on the Degradation Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in Sewage Sludge. Water. Air. Soil Pollut. 228, 305. https://doi.org/10.1007/s11270-017-3481-7

Poulsen, T.G., Bester, K., 2010. Organic Micropollutant Degradation in Sewage Sludge during Composting under Thermophilic Conditions. Environ. Sci. Technol. 44, 5086–5091. https://doi.org/10.1021/es9038243

Prakash, V., Saxena, S., Sharma, A., Singh, S., Singh, S.K., 2015. Treatment of oil sludge contamination by composting. J. Bioremediation Biodegredation 6, 1–6.

Pulkrabová, J., Černý, J., Száková, J., Švarcová, A., Gramblička, T., Hajšlová, J., Balík, J., Tlustoš, P., 2019. Is the long-term application of sewage sludge turning soil into a sink for organic pollutants?: evidence from field studies in the Czech Republic. J. Soils Sediments 19, 2445–2458. https://doi.org/10.1007/s11368-019-02265-y

Qadir, M., Mateo-Sagasta, J., Jiménez, B., Siebe, C., Siemens, J., Hanjra, M.A., 2015. Environmental Risks and Cost-Effective Risk Management in Wastewater Use Systems, in: Drechsel, P., Qadir, M., Wichelns, D. (Eds.), Wastewater: Economic Asset in an Urbanizing World. Springer Netherlands, Dordrecht, pp. 55–72. https://doi.org/10.1007/978-94-017-9545-6_4

Ramdani, N., Hamou, A., Lousdad, A., Al-Douri, Y., 2015. Physicochemical characterization of sewage sludge and green waste for agricultural utilization. Environ. Technol. 36, 1594–1604. https://doi.org/10.1080/09593330.2014.998716

Ramos, S., Homem, V., Santos, L., 2021a. Modified dispersive solid-phase extraction and cleanup followed by GC-MS/MS analysis to quantify ultraviolet filters and synthetic musk compounds in soil samples. J. Sep. Sci. 44, 3107–3116. https://doi.org/10.1002/jssc.202100281

Ramos, S., Homem, V., Santos, L., 2021b. Uptake and translocation of UV-filters and synthetic musk compounds into edible parts of tomato grown in amended soils. Sci. Total Environ. 792, 148482. https://doi.org/10.1016/j.scitotenv.2021.148482

Reiner, J.L., Berset, J.D., Kannan, K., 2007. Mass Flow of Polycyclic Musks in Two Wastewater Treatment Plants. Arch. Environ. Contam. Toxicol. 52, 451–457. https://doi.org/10.1007/s00244-006-0203-3

Rivier, P.-A., Havranek, I., Coutris, C., Norli, H.R., Joner, E.J., 2019. Transfer of organic pollutants from sewage sludge to earthworms and barley under field conditions. Chemosphere 222, 954–960. https://doi.org/10.1016/j.chemosphere.2019.02.010

Rodarte-Morales, A.I., Feijoo, G., Moreira, M.T., Lema, J.M., 2011. Degradation of selected pharmaceutical and personal care products (PPCPs) by white-rot fungi. World J. Microbiol. Biotechnol. 27, 1839–1846. https://doi.org/10.1007/s11274-010-0642-x

Sadef, Y., Poulsen, T.G., Habib, K., Iqbal, T., Nizami, A.S., 2016. Uncertainty in degradation rates for organic micro-pollutants during full-scale sewage sludge composting. Waste Manag. 56, 396–402. https://doi.org/10.1016/j.wasman.2016.06.018

Sánchez-Monedero, M.A., Roig, A., Paredes, C., Bernal, M.P., 2001. Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. Bioresour. Technol. 78, 301–308. https://doi.org/10.1016/S0960-8524(01)00031-1

Saravanan, A., Thamarai, P., Deivayanai, V.C., Karishma, S., Shaji, A., Yaashikaa, P.R., 2024. Current strategies on bioremediation of personal care products and detergents: Sustainability and life cycle assessment. Chemosphere 354, 141698. https://doi.org/10.1016/j.chemosphere.2024.141698

Sayara, T., Borràs, E., Caminal, G., Sarrà, M., Sánchez, A., 2011. Bioremediation of PAHs-contaminated soil through composting: Influence of bioaugmentation and biostimulation on contaminant biodegradation. Int. Biodeterior. Biodegrad. 65, 859–865. https://doi.org/10.1016/j.ibiod.2011.05.006

Segura, A., Ramos, J.L., 2013. Plant–bacteria interactions in the removal of pollutants. Curr. Opin. Biotechnol., Energy biotechnology • Environmental biotechnology 24, 467–473. https://doi.org/10.1016/j.copbio.2012.09.011

Semple, K.T., Morriss, A.W.J., Paton, G.I., 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. Eur. J. Soil Sci. 54, 809–818. https://doi.org/10.1046/j.1351-0754.2003.0564.x

Sharma, H., Jain, V.K., Khan, Z.H., 2007. Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in the urban environment of Delhi. Chemosphere 66, 302–310. https://doi.org/10.1016/j.chemosphere.2006.05.003

Siebielska, I., 2014. Comparison of changes in selected polycyclic aromatic hydrocarbons concentrations during the composting and anaerobic digestion processes of municipal waste and sewage sludge mixtures. Water Sci. Technol. 70, 1617–1624. https://doi.org/10.2166/wst.2014.417

Smyth, S.A., Lishman, L., McBean, E., Kleywegt, S., Yang, J.-J., Svoboda, M., Lee, H.-B., Seto, P., 2007. Fate of Polycyclic and Nitro Musks during Aerobic and Anaerobic Sludge Digestion. https://www.researchgate.net/profile/Shirley-Anne-Smyth/publication/228495803_Fate_of_Polycyclic_and_Nitro_Musks_during_Aerobic_and_Anaerobic_Sludge_Digestion/links/5469546a0cf2f5eb1804ed22/Fate-of-Polycyclic-and-Nitro-Musks-during-Aerobic-and-Anaerobic-Sludge-Digestion.pdf

Srogi, K., 2007. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ. Chem. Lett. 5, 169–195. https://doi.org/10.1007/s10311-007-0095-0

Stella, T., Covino, S., Čvančarová, M., Filipová, A., Petruccioli, M., D'Annibale, A., Cajthaml, T., 2017. Bioremediation of long-term PCB-contaminated soil by white-rot fungi. J. Hazard. Mater. 324, 701–710. https://doi.org/10.1016/j.jhazmat.2016.11.044

Suciu, N.A., Lamastra, L., Trevisan, M., 2015. PAHs content of sewage sludge in Europe and its use as soil fertilizer. Waste Manag. 41, 119–127. https://doi.org/10.1016/j.wasman.2015.03.018

Sun, P., Casteel, K., Dai, H., Wehmeyer, K.R., Kiel, B., Federle, T., 2014. Distributions of polycyclic musk fragrance in wastewater treatment plant (WWTP) effluents and sludges in the United States. Sci. Total Environ. 493, 1073–1078. https://doi.org/10.1016/j.scitotenv.2014.04.038

Sun, S.-J., Zhao, Z.-B., Li, B., Ma, L.-X., Fu, D.-L., Sun, X.-Z., Thapa, S., Shen, J.-M., Qi, H., Wu, Y.-N., 2019. Occurrence, composition profiles and risk assessment of polycyclic aromatic hydrocarbons in municipal sewage sludge in China. Environ. Pollut. 245, 764–770. https://doi.org/10.1016/j.envpol.2018.11.067

Taha, M., Shahsavari, E., Aburto-Medina, A., Foda, M.F., Clarke, B., Roddick, F., Ball, A.S., 2018. Bioremediation of biosolids with *Phanerochaete chrysosporium* culture filtrates enhances the degradation of polycyclic aromatic hydrocarbons (PAHs). Appl. Soil Ecol. 124, 163–170. https://doi.org/10.1016/j.apsoil.2017.11.002

Tavera Busso, I., Tames, F., Silva, J.A., Ramos, S., Homem, V., Ratola, N., Carreras, H., 2018. Biomonitoring levels and trends of PAHs and synthetic musks associated with land use in urban environments. Sci. Total Environ. 618, 93–100. https://doi.org/10.1016/j.scitotenv.2017.10.295

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38, 4075–4084. https://doi.org/10.1016/j.watres.2004.07.015

Thacharodi, A., Hassan, S., Singh, T., Mandal, R., Chinnadurai, J., Khan, H.A., Hussain, M.A., Brindhadevi, K., Pugazhendhi, A., 2023. Bioremediation of polycyclic aromatic hydrocarbons: An updated microbiological review. Chemosphere 328, 138498. https://doi.org/10.1016/j.chemosphere.2023.138498

Tomasi Morgano, M., Leibold, H., Richter, F., Stapf, D., Seifert, H., 2018. Screw pyrolysis technology for sewage sludge treatment. Waste Manag. 73, 487–495. https://doi.org/10.1016/j.wasman.2017.05.049

Tomczyk, B., Siatecka, A., Jędruchniewicz, K., Sochacka, A., Bogusz, A., Oleszczuk, P., 2020. Polycyclic aromatic hydrocarbons (PAHs) persistence, bioavailability and toxicity in sewage sludge- or sewage sludge-derived biochar-amended soil. Sci. Total Environ. 747, 141123. https://doi.org/10.1016/j.scitotenv.2020.141123

Vallecillos, L., Borrull, F., Pocurull, E., 2012. Determination of musk fragrances in sewage sludge by pressurized liquid extraction coupled to automated ionic liquid-based headspace single-drop microextraction followed by GC-MS/MS. J. Sep. Sci. 35, 2735–2742. https://doi.org/10.1002/jssc.201200326

Vallecillos, L., Sadef, Y., Borrull, F., Pocurull, E., Bester, K., 2017. Degradation of synthetic fragrances by laccase-mediated system. J. Hazard. Mater. 334, 233–243. https://doi.org/10.1016/j.jhazmat.2017.04.003

Vecchiato, M., Bonato, T., Barbante, C., Gambaro, A., Piazza, R., 2021. Organic pollutants in protected plain areas: The occurrence of PAHs, musks, UV-filters, flame retardants and hydrocarbons in woodland soils. Sci. Total Environ. 796, 149003. https://doi.org/10.1016/j.scitotenv.2021.149003

Verlicchi, P., Zambello, E., 2015. Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil — A critical review. Sci. Total Environ. 538, 750–767. https://doi.org/10.1016/j.scitotenv.2015.08.108

Wang, C., Sun, H., Li, J., Li, Y., Zhang, Q., 2009. Enzyme activities during degradation of polycyclic aromatic hydrocarbons by white rot fungus Phanerochaete chrysosporium in soils. Chemosphere 77, 733–738. https://doi.org/10.1016/j.chemosphere.2009.08.028

Wang, M., Peng, C., Chen, W., Markert, B., 2013. Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicol. Environ. Saf. 97, 242–247. https://doi.org/10.1016/j.ecoenv.2013.07.032

Wang, X., Zheng, G., Chen, T., Shi, X., Wang, Y., Nie, E., Liu, J., 2019. Effect of phosphate amendments on improving the fertilizer efficiency and reducing the mobility of heavy metals during sewage sludge composting. J. Environ. Manage. 235, 124–132. https://doi.org/10.1016/j.jenvman.2019.01.048

Wang, Z., Gao, M., Wang, Zhe, She, Z., Hu, B., Wang, Y., Zhao, C., 2013. Comparison of physicochemical parameters during the forced-aeration composting of sewage sludge and maize straw at different initial C/N ratios. J. Air Waste Manag. Assoc. 63, 1130–1136. https://doi.org/10.1080/10962247.2013.800616

Ward, O., Singh, A., Van Hamme, J., 2003. Accelerated biodegradation of petroleum hydrocarbon waste. J. Ind. Microbiol. Biotechnol. 30, 260–270. https://doi.org/10.1007/s10295-003-0042-4

Wei, H., Song, S., Tian, H., Liu, T., 2014. Effects of phenanthrene on seed germination and some physiological activities of wheat seedling. C. R. Biol. 337, 95–100. https://doi.org/10.1016/j.crvi.2013.11.005

Wluka, A.-K., Huang, Y., Coenen, L., Dsikowitzky, L., Schwarzbauer, J., 2021. Structural diversity of organic contaminants in sewage sludge: a comparison of sewage fingerprints from Germany and China. Discov. Water 1, 4. https://doi.org/10.1007/s43832-021-00004-4

Wong, F., Robson, M., Melymuk, L., Shunthirasingham, C., Alexandrou, N., Shoeib, M., Luk, E., Helm, P., L. Diamond, M., Hung, H., 2019. Urban sources of synthetic musk compounds to the environment. Environ. Sci. Process. Impacts 21, 74–88. https://doi.org/10.1039/C8EM00341F

Wu, H., Lai, C., Zeng, G., Liang, J., Chen, J., Xu, J., Dai, J., Li, X., Liu, J., Chen, M., Lu, L., Hu, L., Wan, J., 2017. The interactions of composting and biochar and their implications for soil amendment and pollution remediation: a review. Crit. Rev. Biotechnol. 37, 754–764. https://doi.org/10.1080/07388551.2016.1232696

Wu, W., Ma, M., Hu, Y., Yu, W., Liu, H., Bao, Z., 2021. The fate and impacts of pharmaceuticals and personal care products and microbes in agricultural soils with long term irrigation with reclaimed water. Agric. Water Manag. 251, 106862. https://doi.org/10.1016/j.agwat.2021.106862

Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Sci. Total Environ. 536, 655–666. https://doi.org/10.1016/j.scitotenv.2015.07.129

Xia, K., Bhandari, A., Das, K., Pillar, G., 2005. Occurrence and Fate of Pharmaceuticals and Personal Care Products (PPCPs) in Biosolids. J. Environ. Qual. 34, 91–104. https://doi.org/10.2134/jeq2005.0091

Yu, Z., Tang, J., Liao, H., Liu, X., Zhou, P., Chen, Z., Rensing, C., Zhou, S., 2018. The distinctive microbial community improves composting efficiency in a full-scale hyperthermophilic composting plant. Bioresour. Technol. 265, 146–154. https://doi.org/10.1016/j.biortech.2018.06.011

Zafra, G., Absalón, Á.E., Anducho-Reyes, M.Á., Fernandez, F.J., Cortés-Espinosa, D.V., 2017. Construction of PAH-degrading mixed microbial consortia by induced selection in soil. Chemosphere 172, 120–126. https://doi.org/10.1016/j.chemosphere.2016.12.038

Zafra, G., Cortés-Espinosa, D.V., 2015. Biodegradation of polycyclic aromatic hydrocarbons by Trichoderma species: a mini review. Environ. Sci. Pollut. Res. 22, 19426–19433. https://doi.org/10.1007/s11356-015-5602-4

Zhang, S., Yao, H., Lu, Y., Yu, X., Wang, J., Sun, S., Liu, M., Li, D., Li, Y.-F., Zhang, D., 2017. Uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by maize from soil irrigated with wastewater. Sci. Rep. 7, 12165. https://doi.org/10.1038/s41598-017-12437-w

Zheng, M., Hu, S., Liu, X., Wang, W., Yin, X., Zheng, L., Wang, L., Lou, Y., 2019. Levels and distribution of synthetic musks in farmland soils from the Three Northeast Provinces of

China. Ecotoxicol. Environ. Saf. 172, 303–307. https://doi.org/10.1016/j.ecoenv.2019.01.100

Table 1. The physyo- chemical property of composted sewage sludge.

		Sludge (S)	**Substrate (SS)**	**P.ostreatus (SS-PL)**	**P.chrysosporium (SS-PH)**	**B. adusta (SS-BJ)**	**Irpex lacteus (SS-IR)**	**T.versicolor (SS-IR)**
C/N	T0	12.8±1.2A	14.1±0.2A	13.7±0.4A	13.7±2.1A	13.9±1A	13.9±1.5A	13.5±.05A
	T3	12.3±1A	12.7±0.5A	12.8±1.1A	12.9±0.5A	12.9±0.9A	12.8±2A	12.9±1.2A
pH ($CaCl_2$)	T0	6.85±0A	6.84±0.02A	6.78±0.01A	6.79±0.07A	6.81±0.04A	6.77±0.05A	6.75±0.07A
	T3	6.39±0.01A	6.45±0.02A	6.42±0.02A	6.41±0.02A	6.49±0.01A	6.42±0.03A	6.47±0.04A
EC (µS/cm)	T0	3029.3±28A	2774.3±35A	2874.3±47A	2929.3±90A	2886.8±245A	2951.8±23A	2456.8±234A
	T3	4428.4±42A	4248.3±466A	4445.8±326A	4040.9±920A	4488.3±19A	4518.5±229A	4620.8±100A

Means ± standard deviations calculated from four replications of respective treatments. Different letters across the row indicates significant differences among the variants (P <0.05) in each treatment.

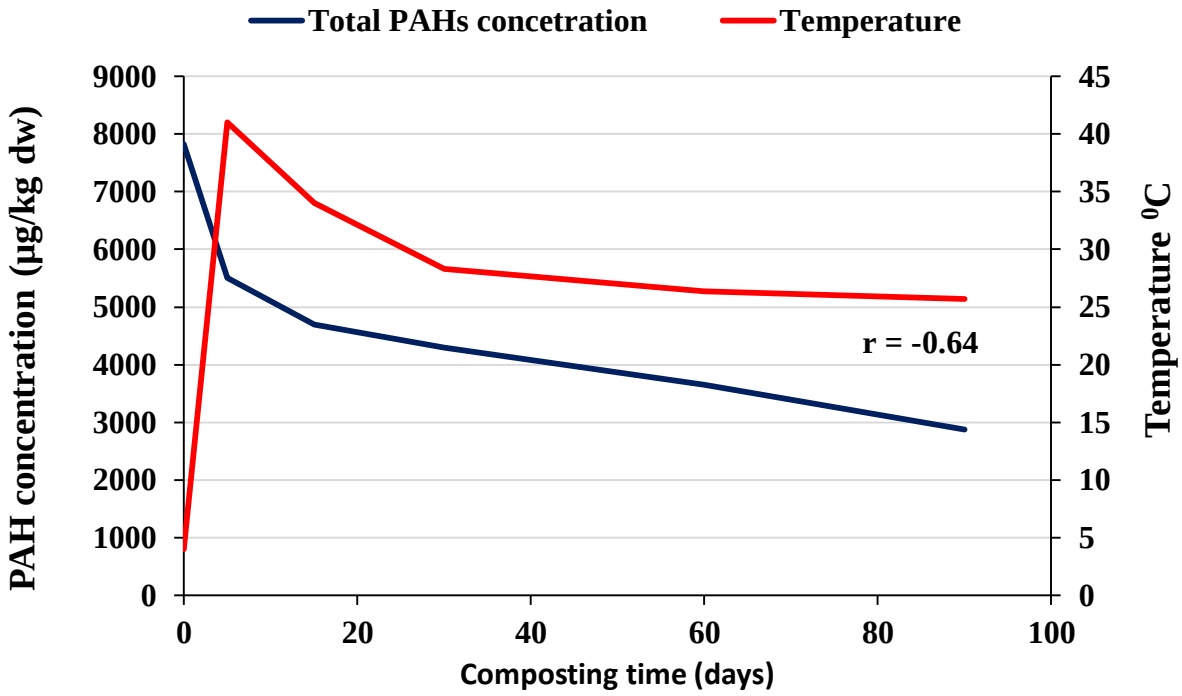
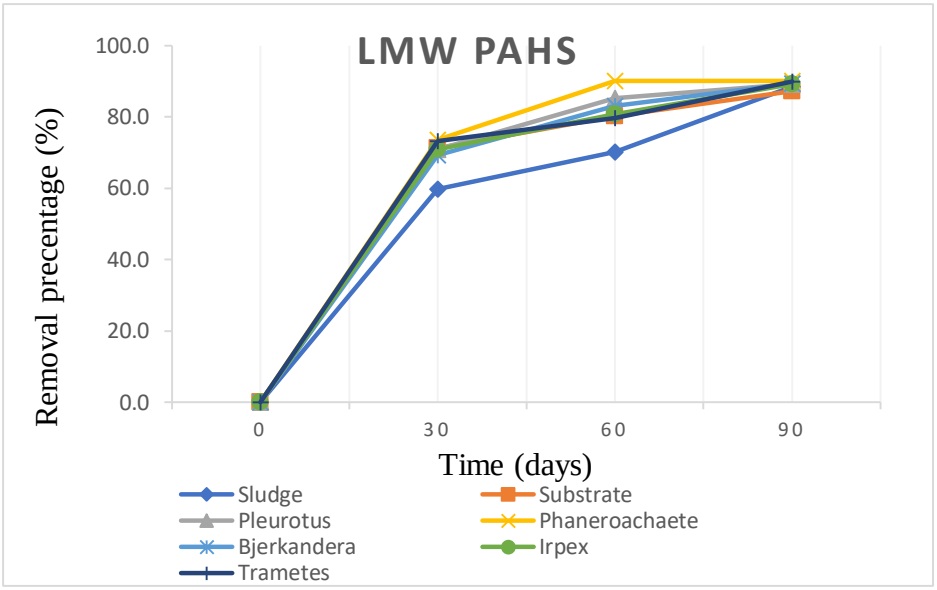
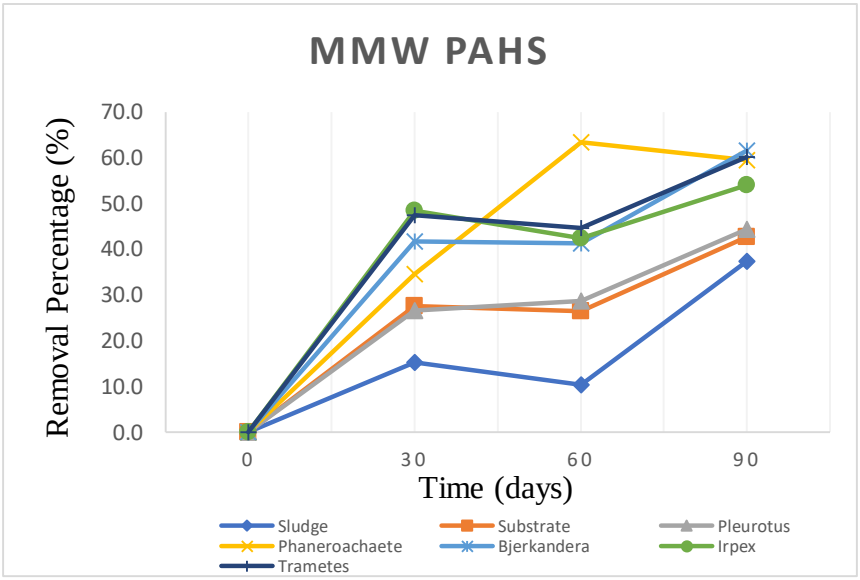
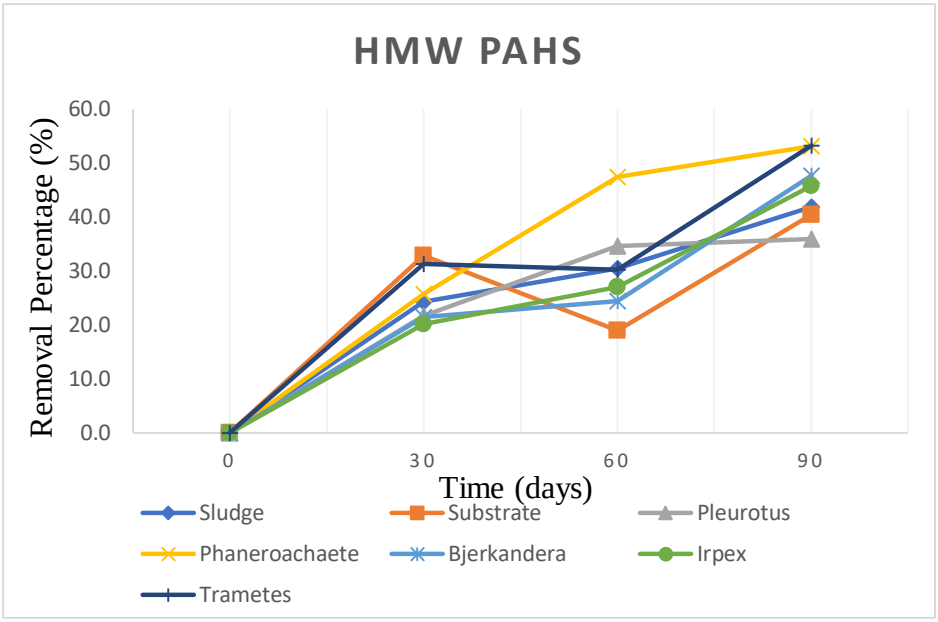


Fig. 1. The relationship between average temperature and total PAHs concentrations and derived Pearson Correlation Coefficient (r)


LMW PAHS
Removal precentage (%)
100.0
80.0
60.0
40.0
20.0
0.0
0
30
60
90
Time (days)
Sludge
Substrate
Pleurotus
Phaneroachaete
Bjerkandera
Irpex
Trametes


MMW PAHS
Removal Percentage (%)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
30
60
90
Time (days)
Sludge
Substrate
Pleurotus
Phaneroachaete
Bjerkandera
Irpex
Trametes



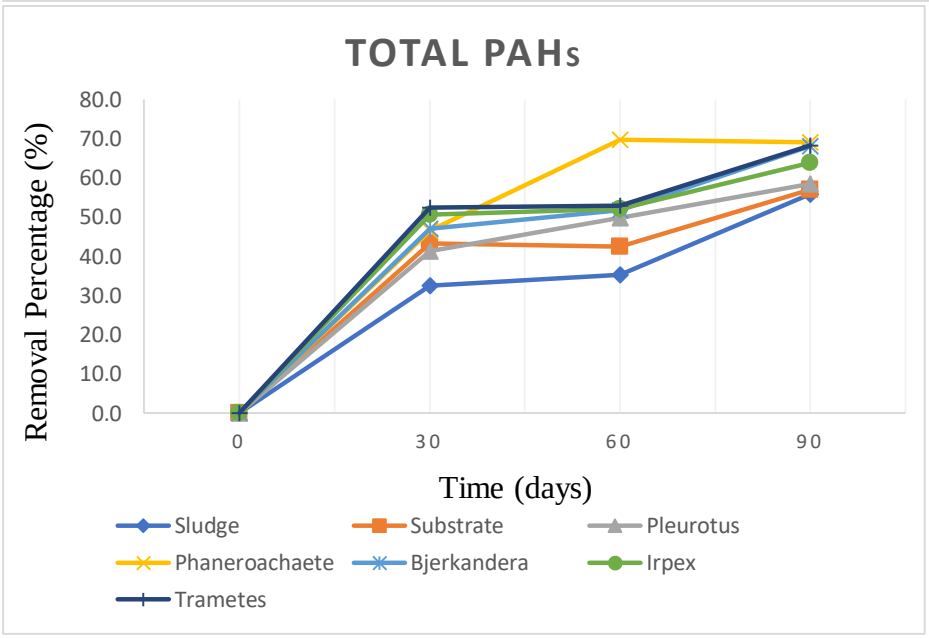


Fig. 2. Removal percentages of lower, medium, high and total polycyclic aromatic hydrocarbons (PAHs) versus time of composting sewage sludge

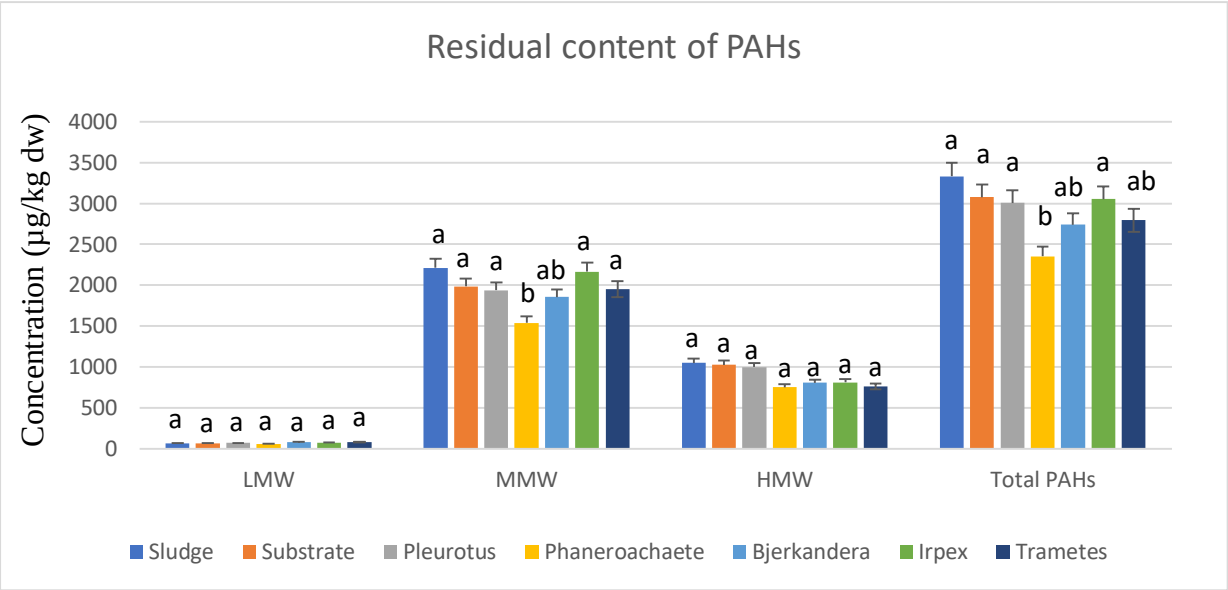


Fig. 3. Residual content of PAHs after 90 days of composting

The data represent means (±SD) of four replicate measurements. The same lowercase letters above bars indicate that differences among treatments were not significant at $P < 0.05$ as determined by Turkey's test. Abbreviations: **LMW PAHs** (low molecular weight PAHs) = sum of naphthalene, acenaphthylene, acenaphthene, and fluorene; **MMW PAHs** (medium molecular weight PAHs) = sum of phenanthrene, anthracene, fluoranthene, and pyrene; **HMW PAHs** (high molecular weight PAHs) = sum of benzo [b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene; **Total PAHs** (total sum PAHs) = sum of LMW, MMW, and HMW PAHs.

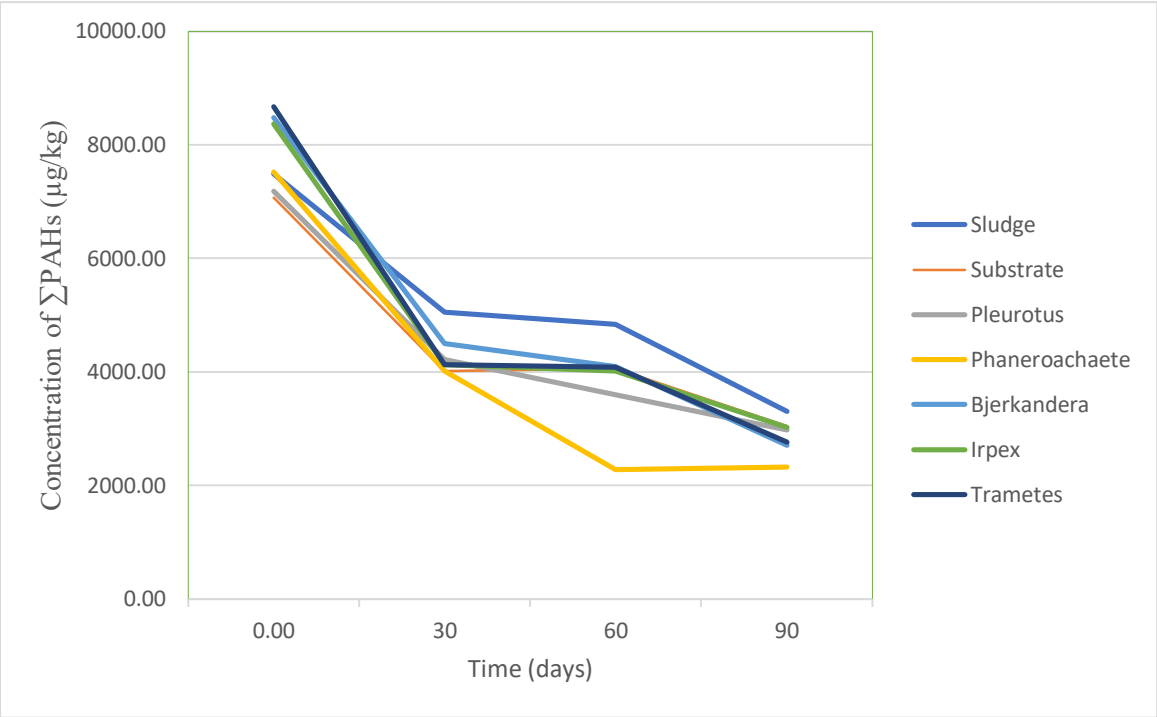


Fig. 4. The effect of individual Fungi species on the degradation of total PAH

Table 2. Residual content of PAHs at the end of composting.

	Sludge	*Substrate*	*Pleurotus*	*Phaneroachaete*	*Bjerkandera*	*Irpex*	*Trametes*
Naphthalene	29.3±4.1 A	29.9± 7 A	31.6 ±16 A	27.93±20A	35.2± 8.3 B	33.3±14 AB	36.8±13 B
Acenaphthylene	0.9 ±6 A	0.5±0.1 A	0.7±0.2 A	0.67±0.4 A	0.82±0.1 A	0.9 ±0.2 A	0.8±0.3 A
Acenaphthene	2.6 ±0.4 A	1.8 ±0.2 A	2.1 ±0.2 A	1.2 ±1.1 A	2.3 ±0.4 A	2.3 ±0.3 A	2.2 ±0.5 A
Fluorene	3.8 ±0.3 A	2.8 ±0.1 A	3.6 ±1.3 A	3.22 ±0.7 A	3.77 ±0.9 A	4.1 ±0.3 D	3.8 ±0.3 C
Anthracene	255±44 A	260.1 ±50 A	234±139 A	228±151 A	250 ±26 A	245± 50A	225 ±47 A
Phenanthrene	2.6 ±0.6 A	3.4 ± 0.1A	2.95± 1A	3.3 ±0.5 A	3.2 ±1 A	4.8 ± 2A	4.6 ± 0.7A
Fluoranthene	171±0.4 A	134.8±10AB	137.4±41 AB	116.1 ± 69B	129.4±14 AB	160.1±57 A	125 ± 29 AB
Pyrene	1739.1±145A	1538.8±85A	1516.9±223A	1167.37 ±644 A	1466.4 ±147 A	1750.1±128A	1586.±137 A
Benzo[a]anthracene	48.1 ±5 A	49.9 ±1 A	49.1±20A	29.1 ±17 AB	10.53 ±2 B	10.6 ±2 B	11.7 ±1.2 B
Chrysene	70.4 ± A	66.83 ± A	71.81 ± A	55.37 ± A	3.75 ± B	4.98 ± B	4.20 ± B
Benzo[b]fluoranthene	165.2±13 A	167.4 ±3 A	157.3 ±22 A	108.8 ±31 A	173.7 ±0.5 A	198.8 ±2 A	180.1 ±0.4 A
Benzo[k]fluoranthene	165.2 ±2 A	167.5±11 A	157.3 ±31 A	108.8 ±62 A	173.8 ±11 A	198.8 ±67 A	180.2 ±46 A
Benzo[a]pyrene	ND	ND	ND	ND	ND	ND	ND
Dibenz[a,h]anthracene	ND	ND	ND	ND	ND	ND	ND
Indeno[1,2,3-cd]pyrene	ND	ND	ND	ND	ND	ND	ND
Benzo[g,h,i]perylene	649.3 ±9 A	624.4±17 A	612.3±85 A	477.9 ±263 A	456.3 ±38 A	408.8±54A	397.4 ±38A

Means ± standard deviations calculated from four replications of respective treatments. Different letters across the row indicates significant differences among the variants (P <0.05) in each treatment. ND = not detected.

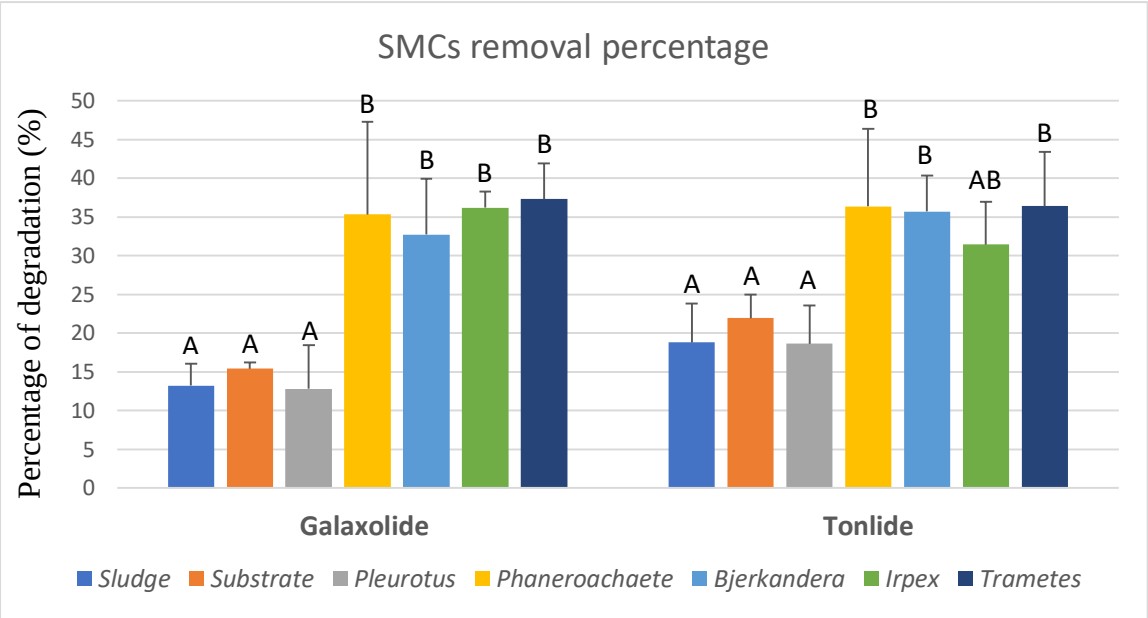


Fig. 5. The percentage change of galaxolide and tonalide at the end of composting

The data represent means (±SD) of four replicate measurements. The different uppercase letters above bars indicate that differences among treatments were significant at (P <0.05) as determined by Turkey's test.

Table 3: Residual content of galaxolide and tonalide (µg/kg) at 0, 30, 60 and 90 days

	Galaxolide				Tonalide			
Treatments	0	30	60	90	0	30	60	90
Sludge	7502.4±340 A	6741.6±737 AB	7448.2±560 A	6506.1±44 B	722.4±128 B	574.8±78 A	675.5±0.9 AB	579.8±27 A
Substrate	68023±126 AB	6198.9±109 A	6840.5±200 B	5753.9±54 C	663.5±40 C	574.6±26B	724.1±24 D	517.1±6 A
Pleurotus	6585.2±324 A	6194.9±527 A	5723.2±420A	5742.1±494 A	632.6±32 A	587.5±66 A	586.7±299 A	515.5±54 A
Phaneroachaete	6747.4±634 A	5879.5±732 A	4567.9±181 B	4515.9±244 B	655.6±60 A	552.3±42 AB	364.8±122 A	430.4±245 AB
Bjerkandera	7150.3±465 A	7035.5±390 A	6116.5±646 AB	4808.5±607 B	693.6±31 B	628.8±31 AB	611.1±13 A	446.8±47 C
Irpex	7232.9±463 A	6480.6±501 AB	6061.1±374 B	4611.3±274 C	672.8±123 A	613.9±65 AB	684.1±65 A	459.5±79 B
Trametes	7487.6±514 A	6348.4±377 A	6048.4±330 B	4695.1±486 C	710.4±71 A	620.9±48 A	628.6±7 A	448.1±59 B

Means ± standard deviations calculated from four replications of respective treatments. Different letters across the row indicates significant differences among the variants (P <0.05) in each treatment.

5. General Discussion

The investigation commenced by probing the prevalence of persistent organic pollutants (POPs) and personal care products (PCPs) within sewage sludge collected from municipal wastewater treatment plants (WWTPs) across the Czech Republic. The concentration of persistent organic pollutants (POPs) in sewage sludge was studied, particularly focusing on polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), in relation to various WWTP parameters and sludge stabilization techniques. Sampling from 40 different WWTPs revealed the ubiquitous presence of PAHs in all examined sludge samples, averaging at 3096 μg/kg dw for sum of 16 PAHs, with concentrations exceeding threshold values outlined for recycling in only a few samples. The average PAH content in sewage sludge samples was sorted in the following order: HMW PAHs = MMW PAHs > LMW PAHs. All 16 PAHs were detected in the analyzed samples, with concentrations ranging from 2.10 μg/kg dw of acenaphthene to 3077 μg/kg dw of benzo[a]anthracene. The average content of the carcinogenic benzo[a]pyrene was 190 μg/kg dw. Furthermore, seven PCB congeners were identified, with PCB 138 and PCB 153 consistently detected across all samples, indicating their predominant presence. The average concentration of 7 PCBs in the 40 sewage sludge samples was 95.7 μg/kg dw. In the current study, the average total amount of the 11 OCPs was 76.1 μg/kg dw; almost 82% were DDT congeners, and the rest were HCB and HCH isomers. The correlation between PAHs and PCB concentrations and WWTP design capacity highlighted the potential impact of urbanization on pollutant levels in sewage sludge, agreed with findings from (Wluka et al., 2021). Moreover, the moderate positive correlations observed between the total sum of PCBs and PAHs underscored the widespread presence of contaminants within the sludge. Certain studies have reported a lack of distinct differences in the content of PAHs and PCBs between aerobic and anaerobic treatments (Olofsson et al., 2013; Ning et al., 2014). However, the current study revealed a notably higher ($P < 0.05 – 0.001$) concentration of

most investigated PAHs in sewage sludge's treated under anaerobic conditions compared to those treated aerobically.

Concurrently, a comprehensive analysis of 55 sewage sludge samples showed the prevalent occurrence of approximately 11 synthetic musk compounds, while synthetic polycyclic musk compounds (SPMCs) were found in higher concentrations than synthetic nitro musk compounds (SNMCs) in concentration. The predominant compounds identified were galaxolide and tonalide, with the nitro musk, musk ketone emerging as the most frequently detected compound in the majority of sludge samples. Notably, the concentrations of these compounds did not correlate with the percentage of wastewater load or the hydraulic retention time of wastewater, suggesting their resistance to degradation within WWTPs. This observation aligns with previous findings by Vallecillos et al., (2012) (2012) and Liu et al., (2014), who noted the persistence of synthetic musk compounds in wastewater treatment processes.

Moving forward, after investigation of the occurrence of target contaminants and their concentrations in the sewage sludge, the focus turned to PAHs and synthetic musks bioremediation study after their composting or application at agricultural soil. Various bioremediation techniques, including mycoremediation, phytoremediation, their combination (phyto-mycoremediation), were evaluated for their efficacy in reducing contaminant levels. Results showed that, during the natural attenuation (SS) treatment, 16 PAHs showed only a slight degradation at the end of study, which was not statistically significant compared to the initial concentration, with only 18% decrease. Houshani et al. (2019) also reported lower total PAHs about 31 % from non-planted treatment after their 120 days of experiment. In terms of individual PAHs, 2–3-ring PAHs exhibited a higher removal percentage. Specifically, ACY, NAP, and ACE had removal values of 33.6 %, 56.7 % and 78.6 %, respectively. In contrast, degradation of 4–6-ring PAHs was relatively lower, as PYR, CHR and BkF were negligibly removed with values

of 7.1 %, 10.1 % and 11.9 %, respectively. Mycoremediation showed the most effective degradation of total PAHs, notably by *P. chrysosporium* that accounted for a removal percentage of 52 %. Both LMW PAHs and HMW PAHs degraded more rapidly *by P. chrysosporium*, with showing the highest degradation rate. The degradation sequence of PAHs at the end of the current study was as follows: LMW > MMW > HMW. The phytoremediated sludge-soil (PSS) treatment demonstrated a higher removal efficiency compared to natural attenuation but lower than mycoremediation. Among the plant-assisted mycoremediation (PMR) treatments, particularly PMR-IR (*I. lacteus*) and PMR-PL (*P. ostreatus*), exhibited the highest overall removal rates for total PAHs, achieving average reductions of 60 % and 57 %, respectively. In the case of plant-assisted mycoremediation (PMR) treatments, the concentration of total PAHs in the soil at the end of the experiment showed a significant decrease compared to their respective initial concentrations. Specifically, the plant-assisted mycoremediation treatments using *P. ostreatus* substrate (PMR-PL) and *P. chrysosporium* substrate (PMR-PH) were capable of completely removing ACE and FLU (100 %). Enzymatic activities in PMR treatments were significantly higher than SS (control) treatment and just phytoremediation (PSS) treatments, probably due to root exudates stimulating soil microorganism growth (Segura and Ramos, 2013).

Regarding the bioremediation of SMCs, among the 11 screened SMCs, only galaxolide (HHCB) and tonalide (AHTN) were detected in the soil amended with sludge at any stage of the experiment. Similar to PAHs degradation, during natural attenuation (control) treatment, the degradation of both compounds at the end of study (after 120 days) were not statistically different compared to the initial concentrations. Böhmer. (2007) similarly on natural attenuation treatment, found slower degradation of both compounds from sludge amended soils after 259 days of their experiment. This could be due to the lipophilic characteristics of target compounds and as suggested by Rodarte-Morales et al. (2011), the weak microbial activities during control treatment could also contribute the lower degradation. The

mycoremediation treatments, especially those using *P. ostreatus* substrates, showed a substantial degradation of HHCB and AHTN, with removal percentages of 58.9 % and 49.7 %, respectively. In contrast, in the other mycoremediation treatments, specifically M − PH (*P. chrysosporium*) and M-IR (*I. lacteus)* treatments, the HHCB content decreased less at a rate of 21.6 % in both treatments. Phytoremediation treatments, enhanced with maize, also exhibited promising results in reducing both group of contaminant concentrations with removal percentages of 46.3 % and 44.8 %, for HHCB and AHTN, respectively. Our findings are with the agreement of Stella et al., (2017) and Muratova et al., (2009), studies demonstrated the effectiveness of fungal bioremediation and plant-assisted remediation in reducing contaminant levels in soil environments. During the plant–assisted mycoremediation, the degradation of HHCB and AHTN was comparable to other treatments, with maximum removal for HHCB in the treatment with *P. ostreatus* (PM-PL) was 44.7 % and for AHTN in the *P. chrysosporium* (PM-PH) treatment was 34.5 %. The results revealed HQ values below 1 for synthetic musk compounds, suggesting that the addition of sewage sludge to the soil does not present a risk to the environment.

Furthermore, the study investigated the degradation of PAHs during sewage sludge composting, a commonly employed method for sludge management. Physicochemical analyses conducted throughout the composting process revealed dynamic changes in pH, electrical conductivity (EC), and temperature. The observed decline in pH and C/N ratio, alongside the stabilization of temperature at the end of study around 26°C, indicated the maturation of the composting process. When temperature were high during the early stages of composting, the removal of PAHs increased significantly. This phenomenon aligns with previous studies by Khalil et al. (2011), which highlighted the influence of temperature on the degradation of organic pollutants during composting. The highest final total PAH concentrations were observed in the control sludge treatment, whereas the *P. chrysosporium* strain (SSPH) treatment

exhibited the lowest concentration. The degradation of 16 PAHs varied with their hydrophobicity, with LMW PAHs showing the highest degradation rates form 87.2 % to 90.1 %. Comparatively, MMW and HMW PAHs exhibited lower degradation percentages ranging from 37.4 % to 61.6 % and 36 % to 53.2 %, respectively. Among MMW PAHs, PYR emerged as the most resilient, persisting as the primary residual PAH in the composted sludge. Its final concentration ranged from 1167 to 1750 µg/kg dw, potentially impacting the suitability of the composted material for agricultural soil use. The *B. adusta* treatment showed significantly higher degradation of MMW PAHs (61.6%). while treatments SS-TR (*T. versicolor*) and SS-PH (*P. chrysosporium*) also showed high degradation efficiencies, reaching 60.2 % and 59.4 %, respectively. The *P. chrysosporium* and *T. versicolor* treatments had the highest degradation of HMW PAHs (both 53.2 %). Among HMW PAHs, the lower degradation was observed during BkF, with removal efficiencies ranging from 19 % to 41 % in all treatments after 90 days. The total removal of PAHs from sludge composting after 90 days ranged from 55.9 % to 69 %. Similar results have been reported in other studies, such as Lu et al. (2019), who found removal rates ranging from 58.7 % to 76 % after 39 days, and Cai et al. (2012), who reported removal rates of 54.6 % to 75.9 % after 56 days of composting. In the current study, following 90 days of composting, the residual total PAH content in the composted sludge ranged from 3.32 to 2.35 mg/kg dw, all significantly below the regulatory limit of 5 mg/kg set by EU regulations (Suciu et al., 2015). Regarding the removal SMCs, galaxolide and tonalide showed a significantly higher degradation rates in the *P. chrysosporium* (SS-PH), B. adusta (SS-BJ), *Irpex lacteus* (SS-IR), and *T. versicolor's* (SS-TR) treatments compared to the control. The degradation ranged from 32.7% to 37.3% for galaxolide and 31.4% to 36.4% for tonalide. At the end of study for similar treatments, the residual content of galaxolide and tonalide were ranging from 4515.8 to 4885.5 µg/kg and from 430.5 to 459.5 µg/kg, respectively.

In summary, the study provided valuable insights into the prevalence of synthetic musk compounds and polycyclic aromatic hydrocarbons in sewage sludge, as well as the efficacy of various bioremediation techniques, phytoremediation, mycoremediation including composting processes in reducing contaminant levels. Through a multidisciplinary approach encompassing analytical chemistry, environmental science, and biotechnology, the study shed light on the importance of effective remediation strategies for mitigating the environmental impact of sewage sludge contamination. By integrating findings from previous studies and leveraging innovative methodologies, the current study contributed to advancing knowledge in the field of environmental remediation and sustainable waste management

6. Conclusion

- Contaminants in sewage sludge threaten the environment and living organisms due to their persistence and toxicity.
- The study hypothesized that WWTP parameters and digestion technology impact SMC and PAH content in sewage sludge.
- Results showed higher concentrations of SMCs and PAHs in larger WWTPs, particularly galaxolide, tonalide, and different classes of PAHs.
- The concentration of most investigated PAHs in sewage sludge treated under anaerobic conditions was significantly higher ($P < 0.05 – 0.001$) compared to those treated aerobically.
- Mycoremediation with *P. ostreatus* substrates achieved the most significant degradation of HHCB and AHTN.
- Both LMW PAHs and HMW PAHs degraded more rapidly *by P. chrysosporium*, with showing the highest degradation rate.
- During phytoremediation of soil-sludge treatment, AHTN, HHCB, and total PAHs significantly reduced ($P < 0.05$) compared to control, with removal rates of 44.8%, 46.3%, and 32%, respectively.
- Plant-assisted mycoremediation treatments, especially with *I. lacteus* (PMR-IR) and *P. ostreatus* (PMR-PL), showed highest total PAH degradation, averaging 60% and 57%, respectively.
- During sludge composting, the *P. chrysosporium* (SS-PH), *B. adusta* (SS-BJ), *Irpex lacteus* (SS-IR), and *T. versicolor's* (SS-TR) treatments showed significantly higher degradation rates for galaxolide (32.7%-37.3%) and tonalide (31.4%-36.4%) compared to the control.

- LMW PAHs showed the highest degradation rates during composting (87.2% to 90.1%), while MMW and HMW PAHs had lower degradation percentages, ranging from 37.4% to 61.6% and 36% to 53.2%, respectively.
- Overall, mycoremediation treatments were most effective for AHTN and HHCB, while PAHs degraded best in plant-assisted mycoremediation treatments.

7. References

Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egypt. J. Pet. 25, 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011

Aguirre, J., Bizkarguenaga, E., Iparraguirre, A., Fernández, L.Á., Zuloaga, O., Prieto, A., 2014. Development of stir-bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry for the analysis of musks in vegetables and amended soils. Anal. Chim. Acta 812, 74–82. https://doi.org/10.1016/j.aca.2013.12.036

Akhtar, A.B.T., Naseem, S., Yasar, A., Naseem, Z., 2021. Persistent Organic Pollutants (POPs): Sources, Types, Impacts, and Their Remediation, in: Prasad, R. (Ed.), Environmental Pollution and Remediation, Environmental and Microbial Biotechnology. Springer, Singapore, pp. 213–246. https://doi.org/10.1007/978-981-15-5499-5_8

Amir, S., Hafidi, M., Merlina, G., Hamdi, H., Revel, J.C., 2005. Fate of polycyclic aromatic hydrocarbons during composting of lagooning sewage sludge. Chemosphere 58, 449–458. https://doi.org/10.1016/j.chemosphere.2004.09.039

Ammari, T.G., Al-Omari, Q., Abbassi, B.E., 2012. Composting sewage sludge amended with different sawdust proportions and textures and organic waste of food industry--assessment of quality. Environ. Technol. 33, 1641–1649. https://doi.org/10.1080/09593330.2011.641589

An, J., Zhou, Q., Sun, Y., Xu, Z., 2009. Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (Triticum aestivum L.). Chemosphere 76, 1428–1434. https://doi.org/10.1016/j.chemosphere.2009.06.004

Annabi, M., Houot, S., Francou, C., Poitrenaud, M., Bissonnais, Y.L., 2007. Soil Aggregate Stability Improvement with Urban Composts of Different Maturities. Soil Sci. Soc. Am. J. 71, 413–423. https://doi.org/10.2136/sssaj2006.0161

Ansari, F., Momina, Ahmad, A., Rafatullah, M., 2023. Review on bioremediation technologies of polycyclic aromatic hydrocarbons (PAHs) from soil: Mechanisms and future perspective. Int. Biodeterior. Biodegrad. 179, 105582. https://doi.org/10.1016/j.ibiod.2023.105582

ANTIZAR-LADISLAO, B., LOPEZ-REAL, J., BECK, A., 2004. Bioremediation of Polycyclic Aromatic Hydrocarbon (PAH)-Contaminated Waste Using Composting Approaches. Crit. Rev. Environ. Sci. Technol. 34, 249–289. https://doi.org/10.1080/10643380490434119

Antizar-Ladislao, B., Lopez-Real, J., Beck, A.J., 2005. Laboratory studies of the remediation of polycyclic aromatic hydrocarbon contaminated soil by in-vessel composting. Waste Manag. 25, 281–289. https://doi.org/10.1016/j.wasman.2005.01.009

Arruda, V., Simões, M., Gomes, I.B., 2022. Synthetic Musk Fragrances in Water Systems and Their Impact on Microbial Communities. Water 14, 692.

Ayuk-Takem, L., Amissah, F., Aguilar, B.J., Lamango, N.S., 2014. Inhibition of polyisoprenylated methylated protein methyl esterase by synthetic musks induces cell degeneration. Environ. Toxicol. 29, 466–477. https://doi.org/10.1002/tox.21773

Bagheri, M., Bauer, T., Burgman, L.E., Wetterlund, E., 2023. Fifty years of sewage sludge management research: Mapping researchers' motivations and concerns. J. Environ. Manage. 325, 116412. https://doi.org/10.1016/j.jenvman.2022.116412

Balk, F., Ford, R.A., 1999. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU: I. Fate and exposure assessment. Toxicol. Lett. 111, 57–79. https://doi.org/10.1016/S0378-4274(99)00169-1

Biel-Maeso, M., Corada-Fernández, C., Lara-Martín, P.A., 2019. Removal of personal care products (PCPs) in wastewater and sludge treatment and their occurrence in receiving soils. Water Res. 150, 129–139. https://doi.org/10.1016/j.watres.2018.11.045

Böhmer, W., 2007. Occurrence of Polycyclic Musks in Sewage Sludge and their Behaviour in Soils and Plants. Part 2: Investigation of Polycyclic Musks in Soils and Plants (9 pp). J. Soils Sediments 7, 36–44. https://doi.org/10.1065/jss2006.10.187.2

Bolden, A.L., Rochester, J.R., Schultz, K., Kwiatkowski, C.F., 2017. Polycyclic aromatic hydrocarbons and female reproductive health: A scoping review. Reprod. Toxicol. 73, 61–74. https://doi.org/10.1016/j.reprotox.2017.07.012

Boldrin, A., Andersen, J.K., Møller, J., Christensen, T.H., Favoino, E., 2009. Composting and compost utilization: accounting of greenhouse gases and global warming contributions. Waste Manag. Res. 27, 800–812. https://doi.org/10.1177/0734242X09345275

Burchiel, S.W., Luster, M.I., 2001. Signaling by Environmental Polycyclic Aromatic Hydrocarbons in Human Lymphocytes. Clin. Immunol. 98, 2–10. https://doi.org/10.1006/clim.2000.4934

Cai, Q.-Y., Mo, C.-H., Lü, H., Zeng, Q.-Y., Wu, Q.-T., Li, Y.-W., 2012. Effect of composting on the removal of semivolatile organic chemicals (SVOCs) from sewage sludge. Bioresour. Technol., Advances in Biological Waste Treatment and Bioconversion Technologies 126, 453–457. https://doi.org/10.1016/j.biortech.2011.11.039

Cai, Q.-Y., Mo, C.-H., Wu, Q.-T., Zeng, Q.-Y., 2008. Polycyclic aromatic hydrocarbons and phthalic acid esters in the soil–radish (Raphanus sativus) system with sewage sludge and compost application. Bioresour. Technol. 99, 1830–1836. https://doi.org/10.1016/j.biortech.2007.03.035

Cai, Q.-Y., Mo, C.-H., Wu, Q.-T., Zeng, Q.-Y., Katsoyiannis, A., Férard, J.-F., 2007. Bioremediation of polycyclic aromatic hydrocarbons (PAHs)-contaminated sewage sludge by different composting processes. J. Hazard. Mater. 142, 535–542. https://doi.org/10.1016/j.jhazmat.2006.08.062

Cajthaml, T., Erbanová, P., Šašek, V., Moeder, M., 2006. Breakdown products on metabolic pathway of degradation of benz[*a*]anthracene by a ligninolytic fungus. Chemosphere 64, 560–564. https://doi.org/10.1016/j.chemosphere.2005.11.034

Cajthaml, T., Křesinová, Z., Svobodová, K., Möder, M., 2009. Biodegradation of endocrine-disrupting compounds and suppression of estrogenic activity by ligninolytic fungi. Chemosphere 75, 745–750. https://doi.org/10.1016/j.chemosphere.2009.01.034

Calderón-Preciado, D., Matamoros, V., Bayona, J.M., 2011. Occurrence and potential crop uptake of emerging contaminants and related compounds in an agricultural irrigation network. Sci. Total Environ. 412–413, 14–19. https://doi.org/10.1016/j.scitotenv.2011.09.057

Chase, D.A., Karnjanapiboonwong, A., Fang, Y., Cobb, G.P., Morse, A.N., Anderson, T.A., 2012. Occurrence of synthetic musk fragrances in effluent and non-effluent impacted environments. Sci. Total Environ. 416, 253–260. https://doi.org/10.1016/j.scitotenv.2011.11.067

Chen, B., Ding, J., 2012. Biosorption and biodegradation of phenanthrene and pyrene in sterilized and unsterilized soil slurry systems stimulated by *Phanerochaete chrysosporium*. J. Hazard. Mater. 229–230, 159–169. https://doi.org/10.1016/j.jhazmat.2012.05.090

Chen, C., Zhou, Q., Bao, Y., Li, Y., Wang, P., 2010. Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (Triticum aestivum). J. Environ. Sci. 22, 1966–1973. https://doi.org/10.1016/S1001-0742(09)60347-8

Chen, F., Gao, J., Zhou, Q., 2012. Toxicity assessment of simulated urban runoff containing polycyclic musks and cadmium in Carassius auratus using oxidative stress biomarkers. Environ. Pollut. 162, 91–97. https://doi.org/10.1016/j.envpol.2011.10.016

Chen, F., Ying, G.-G., Ma, Y.-B., Chen, Z.-F., Lai, H.-J., Peng, F.-J., 2014. Field dissipation and risk assessment of typical personal care products TCC, TCS, AHTN and HHCB in biosolid-amended soils. Sci. Total Environ. 470–471, 1078–1086. https://doi.org/10.1016/j.scitotenv.2013.10.080

Chen, H., Yan, S.-H., Ye, Z.-L., Meng, H.-J., Zhu, Y.-G., 2012. Utilization of urban sewage sludge: Chinese perspectives. Environ. Sci. Pollut. Res. 19, 1454–1463. https://doi.org/10.1007/s11356-012-0760-0

Chen, Z., Li, Y., Peng, Y., Ye, C., Zhang, S., 2021. Effects of antibiotics on hydrolase activity and structure of microbial community during aerobic co-composting of food waste with sewage sludge. Bioresour. Technol. 321, 124506. https://doi.org/10.1016/j.biortech.2020.124506

Cristaldi, A., Conti, G.O., Jho, E.H., Zuccarello, P., Grasso, A., Copat, C., Ferrante, M., 2017. Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. Environ. Technol. Innov. 8, 309–326. https://doi.org/10.1016/j.eti.2017.08.002

Deselnicu, D., Militaru, G., Deselnicu, V., ZĂINESCU, G., A., L., 2018. Towards a Circular Economy– a Zero Waste Programme for Europe. https://doi.org/10.24264/icams-2018.XI.4

Diggs, D.L., Huderson, A.C., Harris, K.L., Myers, J.N., Banks, L.D., Rekhadevi, P.V., Niaz, M.S., Ramesh, A., 2011. Polycyclic Aromatic Hydrocarbons and Digestive Tract Cancers: A Perspective. J. Environ. Sci. Health Part C 29, 324–357. https://doi.org/10.1080/10590501.2011.629974

Dodgen, L.K., Ueda, A., Wu, X., Parker, D.R., Gan, J., 2015. Effect of transpiration on plant accumulation and translocation of PPCP/EDCs. Environ. Pollut. 198, 144–153. https://doi.org/10.1016/j.envpol.2015.01.002

Domínguez-Morueco, N., Carvalho, M., Sierra, J., Schuhmacher, M., Domingo, J.L., Ratola, N., Nadal, M., 2018. Multi-component determination of atmospheric semi-volatile organic compounds in soils and vegetation from Tarragona County, Catalonia, Spain. Sci. Total Environ. 631–632, 1138–1152. https://doi.org/10.1016/j.scitotenv.2018.03.074

Edokpayi, J.N., Odiyo, J.O., Popoola, O.E., Msagati, T.A.M., 2016. zhend. Int. J. Environ. Res. Public. Health 13, 387. https://doi.org/10.3390/ijerph13040387

Feng, L.-J., Zhang, L.-Q., Feng, L., Li, J.-L., 2019. Dissipation of polycyclic aromatic hydrocarbons (PAHs) in soil amended with sewage sludge and sludge compost. Environ. Sci. Pollut. Res. 26, 34127–34136. https://doi.org/10.1007/s11356-018-3383-2

Fernandes, A.S., Azevedo, T., Rocha, F., Nunes, E., Homem, V., 2022. Uptake and translocation of synthetic musk fragrances by pea plant grown in sewage sludge-amended soils. Environ. Pollut. 310, 119908. https://doi.org/10.1016/j.envpol.2022.119908

Fussell, R.J., Garcia Lopez, M., Mortimer, D.N., Wright, S., Sehnalova, M., Sinclair, C.J., Fernandes, A., Sharman, M., 2014. Investigation into the Occurrence in Food of Veterinary Medicines, Pharmaceuticals, and Chemicals Used in Personal Care Products. J. Agric. Food Chem. 62, 3651–3659. https://doi.org/10.1021/jf4052418

Gao, N., Kamran, K., Quan, C., Williams, P.T., 2020. Thermochemical conversion of sewage sludge: A critical review. Prog. Energy Combust. Sci. 79, 100843. https://doi.org/10.1016/j.pecs.2020.100843

Gawryluk, A., Stępniowska, A., Lipińska, H., 2022. Effect of soil contamination with polycyclic aromatic hydrocarbons from drilling waste on germination and growth of lawn grasses. Ecotoxicol. Environ. Saf. 236, 113492. https://doi.org/10.1016/j.ecoenv.2022.113492

Ghahari, Sajjad, Ghahari, Somayeh, Ghahari, Saeid, Nematzadeh, G., Sarma, H., 2021. Integrated remediation approaches for selected pharmaceutical and personal care products in urban soils for a sustainable future. Energy Ecol. Environ. https://doi.org/10.1007/s40974-021-00218-1

Guo, Y., Rene, E.R., Wang, J., Ma, W., 2020. Biodegradation of polyaromatic hydrocarbons and the influence of environmental factors during the co-composting of sewage sludge and green forest waste. Bioresour. Technol. 297, 122434. https://doi.org/10.1016/j.biortech.2019.122434

Gupta, S., Pathak, B., 2020. Chapter 6 - Mycoremediation of polycyclic aromatic hydrocarbons, in: Singh, P., Kumar, A., Borthakur, A. (Eds.), Abatement of Environmental Pollutants. Elsevier, pp. 127–149. https://doi.org/10.1016/B978-0-12-818095-2.00006-0

Haddaoui, I., Mahjoub, O., Mahjoub, B., Boujelben, A., Di Bella, G., 2016. Occurrence and distribution of PAHs, PCBs, and chlorinated pesticides in Tunisian soil irrigated with treated wastewater. Chemosphere 146, 195–205. https://doi.org/10.1016/j.chemosphere.2015.12.007

Hafidi, M., Amir, S., Jouraiphy, A., Winterton, P., El Gharous, M., Merlina, G., Revel, J.-C., 2008a. Fate of polycyclic aromatic hydrocarbons during composting of activated sewage sludge with green waste. Bioresour. Technol. 99, 8819–8823. https://doi.org/10.1016/j.biortech.2008.04.044

Han, M.-J., Park, H.-T., Song, H.-G., 2004. Degradation of Phenanthrene by Trametes versicolor and Its Laccase. J. Microbiol. 42, 94–98.

Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. J. Hazard. Mater. 169, 1–15. https://doi.org/10.1016/j.jhazmat.2009.03.137

Heidler, J., Halden, R.U., 2008. Meta-Analysis of Mass Balances Examining Chemical Fate during Wastewater Treatment. Environ. Sci. Technol. 42, 6324–6332. https://doi.org/10.1021/es703008y

Ho, J., Chambers, L.G., 2019. Altered soil microbial community composition and function in two shrub-encroached marshes with different physicochemical gradients. Soil Biol. Biochem. 130, 122–131. https://doi.org/10.1016/j.soilbio.2018.12.004

Homem, V., Magalhães, I., Alves, A., Santos, L., 2017. Assessing seasonal variation of synthetic musks in beach sands from Oporto coastal area: A case study. Environ. Pollut. 226, 190–197. https://doi.org/10.1016/j.envpol.2017.04.022

Houshani, M., Salehi-Lisar, S.Y., Motafakkerazad, R., Movafeghi, A., 2019. Uptake and distribution of phenanthrene and pyrene in roots and shoots of maize (Zea mays L.). Environ. Sci. Pollut. Res. 26, 9938–9944. https://doi.org/10.1007/s11356-019-04371-3

Hua, L., Wu, W., Liu, Y., Chen, Y., McBride, M.B., 2008. Effect of composting on polycyclic aromatic hydrocarbons removal in sewage sludge. Water. Air. Soil Pollut. 193, 259–267. https://doi.org/10.1007/s11270-008-9687-y

Huang, C., Zeng, G., Huang, D., Lai, C., Xu, P., Zhang, C., Cheng, M., Wan, J., Hu, L., Zhang, Y., 2017. Effect of *Phanerochaete chrysosporium* inoculation on bacterial community and metal stabilization in lead-contaminated agricultural waste composting. Bioresour. Technol. 243, 294–303. https://doi.org/10.1016/j.biortech.2017.06.124

Hurtado, C., Domínguez, C., Pérez-Babace, L., Cañameras, N., Comas, J., Bayona, J.M., 2016. Estimate of uptake and translocation of emerging organic contaminants from irrigation water concentration in lettuce grown under controlled conditions. J. Hazard. Mater. 305, 139–148. https://doi.org/10.1016/j.jhazmat.2015.11.039

Inglezakis, V.J., Zorpas, A.A., Karagiannidis, A., Samaras, P., Voukkali, I., Sklari, S., 2014. EUROPEAN UNION LEGISLATION ON SEWAGE SLUDGE MANAGEMENT. Fresenius Environ. Bull. 23, 6.

Iticescu, C., Georgescu, L.P., Murariu, G., Circiumaru, A., Timofti, M., 2018. The characteristics of sewage sludge used on agricultural lands. AIP Conf. Proc. 2022, 020001. https://doi.org/10.1063/1.5060681

Jadlovec, M., Výtisk, J., Honus, S., Pospišilík, V., Bassel, N., 2023. Pollutants production, energy recovery and environmental impact of sewage sludge co-incineration with biomass pellets. Environ. Technol. Innov. 32, 103400. https://doi.org/10.1016/j.eti.2023.103400

Jin, B., Liu, Y., Liu, G., Zhai, H., Niu, J., Jia, Y., Wang, B., Du, J., Yin, Z., Pang, L., Ji, J., Cao, X., 2024. Toxic effects of polycyclic aromatic hydrocarbons on the two-sludge system: Combined functional gene, resistance gene and microbial community assessment. Process Saf. Environ. Prot. 185, 53–63. https://doi.org/10.1016/j.psep.2024.03.018

Jones, V., Gardner, M., Ellor, B., 2014. Concentrations of trace substances in sewage sludge from 28 wastewater treatment works in the UK. Chemosphere 111, 478–484. https://doi.org/10.1016/j.chemosphere.2014.04.025

Kanungo, J., Sahoo, T., Swain, L.P., Behera, I.D., 2023. Toxicity of Persistent Hydrocarbon Pollutants, Sources and Sustainable Remediation Process, in: Behera, I.D., Das, A.P. (Eds.), Impact of Petroleum Waste on

Environmental Pollution and Its Sustainable Management Through Circular Economy. Springer Nature Switzerland, Cham, pp. 39–65. https://doi.org/10.1007/978-3-031-48220-5_2

Katsou, E., Malamis, S., Mamais, D., Bolzonella, D., Fatone, F., 2015. Occurrence, fate, and removal of PAHs and VOCs in WWTPs using activated sludge processes and membrane bioreactors: Results from Italy and Greece, in: Wastewater Treatment: Occurrence and Fate of Polycyclic Aromatic Hydrocarbons (PAHs). pp. 113–140.

Kelessidis, A., Stasinakis, A.S., 2012. Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. Waste Manag. 32, 1186–1195. https://doi.org/10.1016/j.wasman.2012.01.012

Khalid, M., Abdollahi, M., 2021. Environmental Distribution of Personal Care Products and Their Effects on Human Health. Iran. J. Pharm. Res. IJPR 20, 216–253. https://doi.org/10.22037/ijpr.2021.114891.15088

Khalil, A.I., Hassouna, M.S., El-Ashqar, H.M.A., Fawzi, M., 2011. Changes in physical, chemical and microbial parameters during the composting of municipal sewage sludge. World J. Microbiol. Biotechnol. 27, 2359–2369. https://doi.org/10.1007/s11274-011-0704-8

Kim, K.-H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ. Int. 60, 71–80. https://doi.org/10.1016/j.envint.2013.07.019

Kinney, C.A., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner, S.L., Bossio, J.P., Benotti, M.J., 2008. Bioaccumulation of Pharmaceuticals and Other Anthropogenic Waste Indicators in Earthworms from Agricultural Soil Amended With Biosolid or Swine Manure. Environ. Sci. Technol. 42, 1863–1870. https://doi.org/10.1021/es702304c

Kinney, C.A., Furlong, E.T., Zaugg, S.D., Burkhardt, M.R., Werner, S.L., Cahill, J.D., Jorgensen, G.R., 2006. Survey of Organic Wastewater Contaminants in Biosolids Destined for Land Application. Environ. Sci. Technol. 40, 7207–7215. https://doi.org/10.1021/es0603406

Kinney, C.A., Heuvel, B.V., 2020. Translocation of pharmaceuticals and personal care products after land application of biosolids. Curr. Opin. Environ. Sci. Health, Environmental Pollution: Biosolids 14, 23–30. https://doi.org/10.1016/j.coesh.2019.11.004

Kleinteich, J., Seidensticker, S., Marggrander, N., Zarfl, C., 2018. Microplastics Reduce Short-Term Effects of Environmental Contaminants. Part II: Polyethylene Particles Decrease the Effect of Polycyclic Aromatic Hydrocarbons on Microorganisms. Int. J. Environ. Res. Public. Health 15, 287. https://doi.org/10.3390/ijerph15020287

Kodešová, R., Švecová, H., Klement, A., Fér, M., Nikodem, A., Fedorova, G., Rieznyk, O., Kočárek, M., Sadchenko, A., Chroňáková, A., Grabic, R., 2024. Contamination of water, soil, and plants by micropollutants from reclaimed wastewater and sludge from a wastewater treatment plant. Sci. Total Environ. 907, 167965. https://doi.org/10.1016/j.scitotenv.2023.167965

Lapen, D.R., Topp, E., Metcalfe, C.D., Li, H., Edwards, M., Gottschall, N., Bolton, P., Curnoe, W., Payne, M., Beck, A., 2008. Pharmaceutical and personal care products in tile drainage following land application of municipal biosolids. Sci. Total Environ. 399, 50–65. https://doi.org/10.1016/j.scitotenv.2008.02.025

Lee, B.-K., 2010. Sources, Distribution and Toxicity of Polyaromatic Hydrocarbons (PAHs) in Particulate Matter, in: Air Pollution. IntechOpen. https://doi.org/10.5772/10045

Li, H., Ma, Y., 2016. Field study on the uptake, accumulation, translocation and risk assessment of PAHs in a soil-wheat system with amendments of sewage sludge. Sci. Total Environ. 560–561, 55–61. https://doi.org/10.1016/j.scitotenv.2016.04.017

Lin, C., 2008. A negative-pressure aeration system for composting food wastes. Bioresour. Technol. 99, 7651–7656. https://doi.org/10.1016/j.biortech.2008.01.078

Litz, W., 2007. Occurrence of Polycyclic Musks in Sewage Sludge and their Behaviour in Soils and Plants. Part 2: investigation of Polycyclic Musks in Soils and Plants (9 pp). J. Soils Sediments 7, 36–44.

Liu, H., Yin, H., Tang, S., Wei, K., Peng, H., Lu, G., Dang, Z., 2019. Effects of benzo [a] pyrene (BaP) on the composting and microbial community of sewage sludge. Chemosphere 222, 517–526. https://doi.org/10.1016/j.chemosphere.2019.01.180

Liu, J., Zhang, W., Zhou, Q., Zhou, Q., Zhang, Y., Zhu, L., 2020. Polycyclic musks in the environment: A review of their concentrations and distribution, ecological effects and behavior, current concerns and future prospects. Crit. Rev. Environ. Sci. Technol. https://doi.org/10.1080/10643389.2020.1724748

Liu, N., Shi, Y., Li, W., Xu, L., Cai, Y., 2014. Concentrations and distribution of synthetic musks and siloxanes in sewage sludge of wastewater treatment plants in China. Sci. Total Environ. 476–477, 65–72. https://doi.org/10.1016/j.scitotenv.2013.12.124

Liu, Q., Xu, X., Lin, L., Wang, D., 2021. Occurrence, distribution and ecological risk assessment of polycyclic aromatic hydrocarbons and their derivatives in the effluents of wastewater treatment plants. Sci. Total Environ. 789, 147911. https://doi.org/10.1016/j.scitotenv.2021.147911

Liu, Z., Li, Q., Wu, Q., Kuo, D.T.F., Chen, S., Hu, X., Deng, M., Zhang, H., Luo, M., 2017. Removal Efficiency and Risk Assessment of Polycyclic Aromatic Hydrocarbons in a Typical Municipal Wastewater Treatment Facility in Guangzhou, China. Int. J. Environ. Res. Public. Health 14, 861. https://doi.org/10.3390/ijerph14080861

Lixandru, G., 2005. The use of sewage sludge as a fertilizer in agriculture, Pedogenic Factors and Processes in the Temperate Zone. Al Cuza Univ.

Lü, H., Chen, X.-H., Mo, C.-H., Huang, Y.-H., He, M.-Y., Li, Y.-W., Feng, N.-X., Katsoyiannis, A., Cai, Q.-Y., 2021. Occurrence and dissipation mechanism of organic pollutants during the composting of sewage sludge: A critical review. Bioresour. Technol. 328, 124847. https://doi.org/10.1016/j.biortech.2021.124847

Lu, Y., Zheng, G., Zhou, W., Wang, J., Zhou, L., 2019. Bioleaching conditioning increased the bioavailability of polycyclic aromatic hydrocarbons to promote their removal during co-composting of industrial and

municipal sewage sludges. Sci. Total Environ. 665, 1073–1082. https://doi.org/10.1016/j.scitotenv.2019.02.174

Lucid, J.D., Fenton, O., Grant, J., Healy, M.G., 2014. Effect of Rainfall Time Interval on Runoff Losses of Biosolids and Meat and Bone Meal when Applied to a Grassland Soil. Water. Air. Soil Pollut. 225, 2042. https://doi.org/10.1007/s11270-014-2042-6

Luo, N., Gao, Y., Chen, X., Wang, M., Niu, X., Li, G., An, T., 2023. A critical review of environmental exposure, metabolic transformation, and the human health risks of synthetic musks. Crit. Rev. Environ. Sci. Technol. 0, 1–18. https://doi.org/10.1080/10643389.2023.2217068

LüZe, XiaoMin, H., Jing, A., Wei, W., 2017. Joint effects of galaxolide and cadmium on soil microbial community function and abundance. J. Agro-Environ. Sci. 36, 66–75.

Lv, N., Wang, B., Wang, H., Xiao, T., Dong, B., Xu, Z., 2024. The occurrence characteristics, removal efficiency, and risk assessment of polycyclic aromatic hydrocarbons in sewage sludges from across China. Chemosphere 351, 141033. https://doi.org/10.1016/j.chemosphere.2023.141033

Lv, Y., Yuan, T., Hu, J., Wang, W., 2010. Seasonal occurrence and behavior of synthetic musks (SMs) during wastewater treatment process in Shanghai, China. Sci. Total Environ. 408, 4170–4176. https://doi.org/10.1016/j.scitotenv.2010.05.003

Macherius, A., Eggen, T., Lorenz, W.G., Reemtsma, T., Winkler, U., Moeder, M., 2012. Uptake of Galaxolide, Tonalide, and Triclosan by Carrot, Barley, and Meadow Fescue Plants. J. Agric. Food Chem. 60, 7785–7791. https://doi.org/10.1021/jf301917q

Mailler, R., Gasperi, J., Chebbo, G., Rocher, V., 2014. Priority and emerging pollutants in sewage sludge and fate during sludge treatment. Waste Manag. 34, 1217–1226. https://doi.org/10.1016/j.wasman.2014.03.028

McErlean, C., Marchant, R., Banat, I.M., 2006. An Evaluation of Soil Colonisation Potential of Selected Fungi and their Production of Ligninolytic Enzymes for Use in Soil Bioremediation Applications. Antonie Van Leeuwenhoek 90, 147–158. https://doi.org/10.1007/s10482-006-9069-7

Meng, X., Liu, B., Zhang, H., Wu, J., Yuan, X., Cui, Z., 2019. Co-composting of the biogas residues and spent mushroom substrate: Physicochemical properties and maturity assessment. Bioresour. Technol. 276, 281–287. https://doi.org/10.1016/j.biortech.2018.12.097

Muñoz, M., Garrido, M.A., Gomez-Rico, M.F., Font, R., 2018. PCDD/F determination in sewage sludge composting. Influence of aeration and the presence of PCP. Sci. Total Environ. 616–617, 763–773. https://doi.org/10.1016/j.scitotenv.2017.10.249

Nam, T.-H., Jeon, H.-J., Mo, H., Cho, K., Ok, Y.-S., Lee, S.-E., 2015. Determination of biomarkers for polycyclic aromatic hydrocarbons (PAHs) toxicity to earthworm (Eisenia fetida). Environ. Geochem. Health 37, 943–951. https://doi.org/10.1007/s10653-015-9706-z

Ning, X.-A., Lin, M.-Q., Shen, L.-Z., Zhang, J.-H., Wang, J.-Y., Wang, Y.-J., Yang, Z.-Y., Liu, J.-Y., 2014. Levels, composition profiles and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in sludge from ten textile dyeing plants. Environ. Res. 132, 112–118. https://doi.org/10.1016/j.envres.2014.03.041

Novotný, Č., Erbanová, P., Cajthaml, T., Rothschild, N., Dosoretz, C., Šašek, V., 2000. Irpex lacteus, a white rot fungus applicable to water and soil bioremediation. Appl. Microbiol. Biotechnol. 54, 850–853. https://doi.org/10.1007/s002530000432

Oleszczuk, P., 2009. Application of three methods used for the evaluation of polycyclic aromatic hydrocarbons (PAHs) bioaccessibility for sewage sludge composting. Bioresour. Technol. 100, 413–420. https://doi.org/10.1016/j.biortech.2008.05.039

Oleszczuk, P., 2008. Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. Ecotoxicol. Environ. Saf. 69, 496–505. https://doi.org/10.1016/j.ecoenv.2007.04.006

Oleszczuk, P., Baran, S., 2003. Degradation of Individual Polycyclic Aromatic Hydrocarbons (PAHs) in Soil Polluted With Aircraft Fuel. Pol. J. Environ. Stud. 12.

Olofsson, U., Brorström-Lundén, E., Kylin, H., Haglund, P., 2013. Comprehensive mass flow analysis of Swedish sludge contaminants. Chemosphere 90, 28–35. https://doi.org/10.1016/j.chemosphere.2012.07.002

Osemwengie, L.I., Steinberg, S., 2001. On-site solid-phase extraction and laboratory analysis of ultra-trace synthetic musks in municipal sewage effluent using gas chromatography–mass spectrometry in the full-scan mode. J. Chromatogr. A 932, 107–118. https://doi.org/10.1016/S0021-9673(01)01216-X

Ozaki, N., Nakazato, A., Nakashima, K., Kindaichi, T., Ohashi, A., 2017. Loading and removal of PAHs, fragrance compounds, triclosan and toxicity by composting process from sewage sludge. Sci. Total Environ. 605–606, 860–866. https://doi.org/10.1016/j.scitotenv.2017.06.165

Ozcan, S., Tor, A., Aydin, M.E., 2013. Investigation on the Levels of Heavy Metals, Polycyclic Aromatic Hydrocarbons, and Polychlorinated Biphenyls in Sewage Sludge Samples and Ecotoxicological Testing. CLEAN – Soil Air Water 41, 411–418. https://doi.org/10.1002/clen.201100187

Panneerselvam, P., Mitra, D., Mohanty, B., Rittick, M., Shadangi, S., 2022. Soil pollution and their impact on soil microorganisms. Environ. Pollut. 70–85.

Paraíba, L.C., Queiroz, S.C.N., Maia, A. de H.N., Ferracini, V.L., 2010. Bioconcentration factor estimates of polycyclic aromatic hydrocarbons in grains of corn plants cultivated in soils treated with sewage sludge. Sci. Total Environ. 408, 3270–3276. https://doi.org/10.1016/j.scitotenv.2010.04.026

Parolini, M., Magni, S., Traversi, I., Villa, S., Finizio, A., Binelli, A., 2015. Environmentally relevant concentrations of galaxolide (HHCB) and tonalide (AHTN) induced oxidative and genetic damage in Dreissena polymorpha. J. Hazard. Mater. 285, 1–10. https://doi.org/10.1016/j.jhazmat.2014.11.037

Patel, A.B., Shaikh, S., Jain, K.R., Desai, C., Madamwar, D., 2020. Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. Front. Microbiol. 11.

Pedersen, J.A., Soliman, M., Suffet, I.H., 2005. Human pharmaceuticals, hormones, and personal care product ingredients in runoff from agricultural fields irrigated with treated wastewater. J. Agric. Food Chem. 53, 1625–1632. https://doi.org/10.1021/jf049228m

Peltre, C., Nyord, T., Bruun, S., Jensen, L.S., Magid, J., 2015. Repeated soil application of organic waste amendments reduces draught force and fuel consumption for soil tillage. Agric. Ecosyst. Environ. 211, 94–101. https://doi.org/10.1016/j.agee.2015.06.004

Peng, F.-J., Kiggen, F., Pan, C.-G., Bracewell, S.A., Ying, G.-G., Salvito, D., Selck, H., Van den Brink, P.J., 2019. Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicol. Environ. Saf. 169, 902–910. https://doi.org/10.1016/j.ecoenv.2018.11.092

Phillips, P.J., Chalmers, A.T., Gray, J.L., Kolpin, D.W., Foreman, W.T., Wall, G.R., 2012. Combined Sewer Overflows: An Environmental Source of Hormones and Wastewater Micropollutants. Environ. Sci. Technol. 46, 5336–5343. https://doi.org/10.1021/es3001294

Pinkas, A., Gonçalves, C.L., Aschner, M., 2017. Neurotoxicity of fragrance compounds: A review. Environ. Res. 158, 342–349. https://doi.org/10.1016/j.envres.2017.06.035

Poluszyńska, J., Jarosz-Krzemińska, E., Helios-Rybicka, E., 2017. Studying the Effects of Two Various Methods of Composting on the Degradation Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in Sewage Sludge. Water. Air. Soil Pollut. 228, 305. https://doi.org/10.1007/s11270-017-3481-7

Poulsen, T.G., Bester, K., 2010. Organic Micropollutant Degradation in Sewage Sludge during Composting under Thermophilic Conditions. Environ. Sci. Technol. 44, 5086–5091. https://doi.org/10.1021/es9038243

Prakash, V., Saxena, S., Sharma, A., Singh, S., Singh, S.K., 2015. Treatment of oil sludge contamination by composting. J. Bioremediation Biodegredation 6, 1–6.

Pulkrabová, J., Černý, J., Száková, J., Švarcová, A., Gramblička, T., Hajšlová, J., Balík, J., Tlustoš, P., 2019. Is the long-term application of sewage sludge turning soil into a sink for organic pollutants?: evidence from field studies in the Czech Republic. J. Soils Sediments 19, 2445–2458. https://doi.org/10.1007/s11368-019-02265-y

Qadir, M., Mateo-Sagasta, J., Jiménez, B., Siebe, C., Siemens, J., Hanjra, M.A., 2015. Environmental Risks and Cost-Effective Risk Management in Wastewater Use Systems, in: Drechsel, P., Qadir, M., Wichelns, D.

(Eds.), Wastewater: Economic Asset in an Urbanizing World. Springer Netherlands, Dordrecht, pp. 55–72. https://doi.org/10.1007/978-94-017-9545-6_4

Ramdani, N., Hamou, A., Lousdad, A., Al-Douri, Y., 2015. Physicochemical characterization of sewage sludge and green waste for agricultural utilization. Environ. Technol. 36, 1594–1604. https://doi.org/10.1080/09593330.2014.998716

Ramos, S., Homem, V., Santos, L., 2021a. Modified dispersive solid-phase extraction and cleanup followed by GC-MS/MS analysis to quantify ultraviolet filters and synthetic musk compounds in soil samples. J. Sep. Sci. 44, 3107–3116. https://doi.org/10.1002/jssc.202100281

Ramos, S., Homem, V., Santos, L., 2021b. Uptake and translocation of UV-filters and synthetic musk compounds into edible parts of tomato grown in amended soils. Sci. Total Environ. 792, 148482. https://doi.org/10.1016/j.scitotenv.2021.148482

Reiner, J.L., Berset, J.D., Kannan, K., 2007. Mass Flow of Polycyclic Musks in Two Wastewater Treatment Plants. Arch. Environ. Contam. Toxicol. 52, 451–457. https://doi.org/10.1007/s00244-006-0203-3

Rivier, P.-A., Havranek, I., Coutris, C., Norli, H.R., Joner, E.J., 2019. Transfer of organic pollutants from sewage sludge to earthworms and barley under field conditions. Chemosphere 222, 954–960. https://doi.org/10.1016/j.chemosphere.2019.02.010

Rodarte-Morales, A.I., Feijoo, G., Moreira, M.T., Lema, J.M., 2011. Degradation of selected pharmaceutical and personal care products (PPCPs) by white-rot fungi. World J. Microbiol. Biotechnol. 27, 1839–1846. https://doi.org/10.1007/s11274-010-0642-x

Sadef, Y., Poulsen, T.G., Habib, K., Iqbal, T., Nizami, A.S., 2016. Uncertainty in degradation rates for organic micro-pollutants during full-scale sewage sludge composting. Waste Manag. 56, 396–402. https://doi.org/10.1016/j.wasman.2016.06.018

Sánchez-Monedero, M.A., Roig, A., Paredes, C., Bernal, M.P., 2001. Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. Bioresour. Technol. 78, 301–308. https://doi.org/10.1016/S0960-8524(01)00031-1

Saravanan, A., Thamarai, P., Deivayanai, V.C., Karishma, S., Shaji, A., Yaashikaa, P.R., 2024. Current strategies on bioremediation of personal care products and detergents: Sustainability and life cycle assessment. Chemosphere 354, 141698. https://doi.org/10.1016/j.chemosphere.2024.141698

Sayara, T., Borràs, E., Caminal, G., Sarrà, M., Sánchez, A., 2011. Bioremediation of PAHs-contaminated soil through composting: Influence of bioaugmentation and biostimulation on contaminant biodegradation. Int. Biodeterior. Biodegrad. 65, 859–865. https://doi.org/10.1016/j.ibiod.2011.05.006

Segura, A., Ramos, J.L., 2013. Plant–bacteria interactions in the removal of pollutants. Curr. Opin. Biotechnol., Energy biotechnology • Environmental biotechnology 24, 467–473. https://doi.org/10.1016/j.copbio.2012.09.011

Semple, K.T., Morriss, A.W.J., Paton, G.I., 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. Eur. J. Soil Sci. 54, 809–818. https://doi.org/10.1046/j.1351-0754.2003.0564.x

Sharma, H., Jain, V.K., Khan, Z.H., 2007. Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in the urban environment of Delhi. Chemosphere 66, 302–310. https://doi.org/10.1016/j.chemosphere.2006.05.003

Siebielska, I., 2014. Comparison of changes in selected polycyclic aromatic hydrocarbons concentrations during the composting and anaerobic digestion processes of municipal waste and sewage sludge mixtures. Water Sci. Technol. 70, 1617–1624. https://doi.org/10.2166/wst.2014.417

Smyth, S.A., Lishman, L., McBean, E., Kleywegt, S., Yang, J.-J., Svoboda, M., Lee, H.-B., Seto, P., 2007. Fate of Polycyclic and Nitro Musks during Aerobic and Anaerobic Sludge Digestion. https://www.researchgate.net/profile/Shirley-Anne-Smyth/publication/228495803_Fate_of_Polycyclic_and_Nitro_Musks_during_Aerobic_and_Anaerobic_Sludge_Digestion/links/5469546a0cf2f5eb1804ed22/Fate-of-Polycyclic-and-Nitro-Musks-during-Aerobic-and-Anaerobic-Sludge-Digestion.pdf

Srogi, K., 2007. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ. Chem. Lett. 5, 169–195. https://doi.org/10.1007/s10311-007-0095-0

Stella, T., Covino, S., Čvančarová, M., Filipová, A., Petruccioli, M., D'Annibale, A., Cajthaml, T., 2017. Bioremediation of long-term PCB-contaminated soil by white-rot fungi. J. Hazard. Mater. 324, 701–710. https://doi.org/10.1016/j.jhazmat.2016.11.044

Suciu, N.A., Lamastra, L., Trevisan, M., 2015a. PAHs content of sewage sludge in Europe and its use as soil fertilizer. Waste Manag. 41, 119–127. https://doi.org/10.1016/j.wasman.2015.03.018

Sun, P., Casteel, K., Dai, H., Wehmeyer, K.R., Kiel, B., Federle, T., 2014. Distributions of polycyclic musk fragrance in wastewater treatment plant (WWTP) effluents and sludges in the United States. Sci. Total Environ. 493, 1073–1078. https://doi.org/10.1016/j.scitotenv.2014.04.038

Sun, S.-J., Zhao, Z.-B., Li, B., Ma, L.-X., Fu, D.-L., Sun, X.-Z., Thapa, S., Shen, J.-M., Qi, H., Wu, Y.-N., 2019. Occurrence, composition profiles and risk assessment of polycyclic aromatic hydrocarbons in municipal sewage sludge in China. Environ. Pollut. 245, 764–770. https://doi.org/10.1016/j.envpol.2018.11.067

Taha, M., Shahsavari, E., Aburto-Medina, A., Foda, M.F., Clarke, B., Roddick, F., Ball, A.S., 2018. Bioremediation of biosolids with *Phanerochaete chrysosporium* culture filtrates enhances the degradation of polycyclic aromatic hydrocarbons (PAHs). Appl. Soil Ecol. 124, 163–170. https://doi.org/10.1016/j.apsoil.2017.11.002

Tavera Busso, I., Tames, F., Silva, J.A., Ramos, S., Homem, V., Ratola, N., Carreras, H., 2018. Biomonitoring levels and trends of PAHs and synthetic musks associated with land use in urban environments. Sci. Total Environ. 618, 93–100. https://doi.org/10.1016/j.scitotenv.2017.10.295

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38, 4075–4084. https://doi.org/10.1016/j.watres.2004.07.015

Thacharodi, A., Hassan, S., Singh, T., Mandal, R., Chinnadurai, J., Khan, H.A., Hussain, M.A., Brindhadevi, K., Pugazhendhi, A., 2023. Bioremediation of polycyclic aromatic hydrocarbons: An updated

microbiological review. Chemosphere 328, 138498. https://doi.org/10.1016/j.chemosphere.2023.138498

Tomasi Morgano, M., Leibold, H., Richter, F., Stapf, D., Seifert, H., 2018. Screw pyrolysis technology for sewage sludge treatment. Waste Manag. 73, 487–495. https://doi.org/10.1016/j.wasman.2017.05.049

Tomczyk, B., Siatecka, A., Jędruchniewicz, K., Sochacka, A., Bogusz, A., Oleszczuk, P., 2020. Polycyclic aromatic hydrocarbons (PAHs) persistence, bioavailability and toxicity in sewage sludge- or sewage sludge-derived biochar-amended soil. Sci. Total Environ. 747, 141123. https://doi.org/10.1016/j.scitotenv.2020.141123

Vallecillos, L., Borrull, F., Pocurull, E., 2012. Determination of musk fragrances in sewage sludge by pressurized liquid extraction coupled to automated ionic liquid-based headspace single-drop microextraction followed by GC-MS/MS. J. Sep. Sci. 35, 2735–2742. https://doi.org/10.1002/jssc.201200326

Vallecillos, L., Sadef, Y., Borrull, F., Pocurull, E., Bester, K., 2017. Degradation of synthetic fragrances by laccase-mediated system. J. Hazard. Mater. 334, 233–243. https://doi.org/10.1016/j.jhazmat.2017.04.003

Vecchiato, M., Bonato, T., Barbante, C., Gambaro, A., Piazza, R., 2021. Organic pollutants in protected plain areas: The occurrence of PAHs, musks, UV-filters, flame retardants and hydrocarbons in woodland soils. Sci. Total Environ. 796, 149003. https://doi.org/10.1016/j.scitotenv.2021.149003

Verlicchi, P., Zambello, E., 2015. Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil — A critical review. Sci. Total Environ. 538, 750–767. https://doi.org/10.1016/j.scitotenv.2015.08.108

Wang, C., Sun, H., Li, J., Li, Y., Zhang, Q., 2009. Enzyme activities during degradation of polycyclic aromatic hydrocarbons by white rot fungus Phanerochaete chrysosporium in soils. Chemosphere 77, 733–738. https://doi.org/10.1016/j.chemosphere.2009.08.028

Wang, M., Peng, C., Chen, W., Markert, B., 2013. Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicol. Environ. Saf. 97, 242–247. https://doi.org/10.1016/j.ecoenv.2013.07.032

Wang, X., Zheng, G., Chen, T., Shi, X., Wang, Y., Nie, E., Liu, J., 2019. Effect of phosphate amendments on improving the fertilizer efficiency and reducing the mobility of heavy metals during sewage sludge composting. J. Environ. Manage. 235, 124–132. https://doi.org/10.1016/j.jenvman.2019.01.048

Wang, Z., Gao, M., Wang, Zhe, She, Z., Hu, B., Wang, Y., Zhao, C., 2013. Comparison of physicochemical parameters during the forced-aeration composting of sewage sludge and maize straw at different initial C/N ratios. J. Air Waste Manag. Assoc. 63, 1130–1136. https://doi.org/10.1080/10962247.2013.800616

Ward, O., Singh, A., Van Hamme, J., 2003. Accelerated biodegradation of petroleum hydrocarbon waste. J. Ind. Microbiol. Biotechnol. 30, 260–270. https://doi.org/10.1007/s10295-003-0042-4

Wei, H., Song, S., Tian, H., Liu, T., 2014. Effects of phenanthrene on seed germination and some physiological activities of wheat seedling. C. R. Biol. 337, 95–100. https://doi.org/10.1016/j.crvi.2013.11.005

Wluka, A.-K., Huang, Y., Coenen, L., Dsikowitzky, L., Schwarzbauer, J., 2021. Structural diversity of organic contaminants in sewage sludge: a comparison of sewage fingerprints from Germany and China. Discov. Water 1, 4. https://doi.org/10.1007/s43832-021-00004-4

Wong, F., Robson, M., Melymuk, L., Shunthirasingham, C., Alexandrou, N., Shoeib, M., Luk, E., Helm, P., L. Diamond, M., Hung, H., 2019. Urban sources of synthetic musk compounds to the environment. Environ. Sci. Process. Impacts 21, 74–88. https://doi.org/10.1039/C8EM00341F

Wu, H., Lai, C., Zeng, G., Liang, J., Chen, J., Xu, J., Dai, J., Li, X., Liu, J., Chen, M., Lu, L., Hu, L., Wan, J., 2017. The interactions of composting and biochar and their implications for soil amendment and pollution remediation: a review. Crit. Rev. Biotechnol. 37, 754–764. https://doi.org/10.1080/07388551.2016.1232696

Wu, W., Ma, M., Hu, Y., Yu, W., Liu, H., Bao, Z., 2021. The fate and impacts of pharmaceuticals and personal care products and microbes in agricultural soils with long term irrigation with reclaimed water. Agric. Water Manag. 251, 106862. https://doi.org/10.1016/j.agwat.2021.106862

Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Sci. Total Environ. 536, 655–666. https://doi.org/10.1016/j.scitotenv.2015.07.129

Xia, K., Bhandari, A., Das, K., Pillar, G., 2005. Occurrence and Fate of Pharmaceuticals and Personal Care Products (PPCPs) in Biosolids. J. Environ. Qual. 34, 91–104. https://doi.org/10.2134/jeq2005.0091

Yu, Z., Tang, J., Liao, H., Liu, X., Zhou, P., Chen, Z., Rensing, C., Zhou, S., 2018. The distinctive microbial community improves composting efficiency in a full-scale hyperthermophilic composting plant. Bioresour. Technol. 265, 146–154. https://doi.org/10.1016/j.biortech.2018.06.011

Zafra, G., Absalón, Á.E., Anducho-Reyes, M.Á., Fernandez, F.J., Cortés-Espinosa, D.V., 2017. Construction of PAH-degrading mixed microbial consortia by induced selection in soil. Chemosphere 172, 120–126. https://doi.org/10.1016/j.chemosphere.2016.12.038

Zafra, G., Cortés-Espinosa, D.V., 2015. Biodegradation of polycyclic aromatic hydrocarbons by Trichoderma species: a mini review. Environ. Sci. Pollut. Res. 22, 19426–19433. https://doi.org/10.1007/s11356-015-5602-4

Zhang, S., Yao, H., Lu, Y., Yu, X., Wang, J., Sun, S., Liu, M., Li, D., Li, Y.-F., Zhang, D., 2017. Uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by maize from soil irrigated with wastewater. Sci. Rep. 7, 12165. https://doi.org/10.1038/s41598-017-12437-w

Zheng, M., Hu, S., Liu, X., Wang, W., Yin, X., Zheng, L., Wang, L., Lou, Y., 2019. Levels and distribution of synthetic musks in farmland soils from the Three Northeast Provinces of China. Ecotoxicol. Environ. Saf. 172, 303–307. https://doi.org/10.1016/j.ecoenv.2019.01.100