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Mycoremediation with *Agaricus bisporus* and *Pleurotus ostreatus* growth substrates versus phytoremediation with *Festuca rubra* and *Brassica* sp. for the recovery of a Pb and γ-HCH contaminated soil

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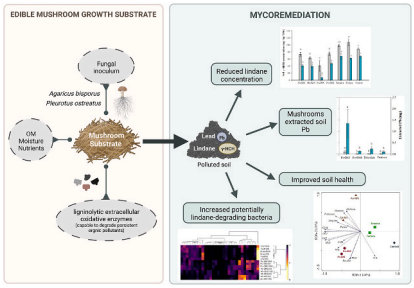
HIGHLIGHTS

- The selection of the most suitable remediation strategy is a daunting challenge.
- Mycoremediation with mushroom growth substrates was compared to phytoremediation.
- The greatest decrease of soil γ-HCH was observed under mycoremediation treatments.
- Fungal treatments led to a significant increase of soil microbial parameters.
- *P. ostreatus* growth substrates demonstrated a great potential for mycoremediation.

GRAPHICAL ABSTRACT



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ABSTRACT

Mycoremediation with mushroom growth substrates can be used for the recovery of mixed contaminated soils due to the benefits derived from the physicochemical characteristics of the substrates, the activity of extracellular enzymes secreted by the fungi, and the presence of the fungal mycelia. The objective of this work was to assess the potential of *Agaricus bisporus* and *Pleurotus ostreatus* growth substrates (inoculated mushroom substrates vs. spent mushroom substrates) for the mycoremediation of soils co-contaminated with lead and lindane (γ-HCH). We compared the efficiency of these mycoremediation strategies with the phytoremediation with *Brassica* spp. Or *Festuca rubra* plants, in terms of both reduction in contaminant levels and enhancement of soil health. An enhanced soil health was achieved as a result of the application of mycoremediation treatments, compared to phytoremediation and control (untreated) treatments. The application of *P. ostreatus* inoculated substrate led to the most significant reduction in γ-HCH concentration (up to 88.9% compared to corresponding controls). In the presence of inoculated mushroom substrate, *P. ostreatus* fruiting bodies extracted more Pb than *Brassica* spp. Or *F. rubra* plants. Mycoremediation with *P. ostreatus* growth substrates appears a promising strategy for the recovery of the health of soils co-contaminated with Pb and γ-HCH.

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1. Introduction

Anthropogenic activities have generated a worldwide problem of soils co-contaminated with organic and inorganic compounds (Polti et al., 2014). This type of contamination, known as mixed contamination, often hampers remediation initiatives owing to the complexities associated with the simultaneous presence of chemicals with different properties (Cameselle and Gouveia, 2019). Lead (Pb) and lindane (γ-hexachlorocyclohexane, γ-HCH) are widespread contaminants that can cause adverse effects on human and environmental health (García-Delgado et al., 2015b; Rigas et al., 2009; Sehube et al., 2017).

The growing demand for edible mushrooms has stimulated their production (Grimm and Wösten, 2018). Actually, the global production of mushrooms and truffles increased from 6.90 to 10.24 million metric tons per year from 2008 to 2017 (Ho et al., 2020). On average, each kilogram (kg) of mushroom generates 5 kg of spent growth substrate waste material (Phan and Sabaratnam, 2012), the so-called "spent mushroom substrate" (SMS), which must be managed in an environmentally sound manner (García-Delgado et al., 2013).

Some of the most commonly consumed mushrooms are *Agaricus bisporus* (champignon) and *Pleurotus ostreatus* (oyster mushroom, a white-rot fungus) (Yang et al., 2013). White-rot fungi (WRF) can efficiently degrade lignin due to the production of ligninolytic extracellular oxidative enzymes (Langer et al., 2021). *Agaricus bisporus*, a litter-degrading fungus (Ten Have and Teunissen, 2001), can also degrade the lignin present in lignocellulose-containing materials by secreting manganese peroxidase and laccase enzymes (Ten Have et al., 2003). Indeed, many authors have reported the capacity of *P. ostreatus* and *A. bisporus* to produce ligninolytic enzymes (Baldrian et al., 2005; Bonnen et al., 1994; Fernández-Fueyo et al., 2014; Giardina et al., 2007; Lankinen et al., 2001; Piscitelli et al., 2005; Pozdnyakova et al., 2018; Ten Have et al., 2003). Due to the structural similarity between lignin and some organic contaminants, white-rot fungi (and also *A. bisporus*) are commonly used for the degradation of persistent organic contaminants (Reddy, 1995), such as polycyclic aromatic hydrocarbons, phenolic compounds (Phan and Sabaratnam, 2012), and organochlorine pesticides (Papadopoulou et al., 2006), among others. Likewise, due to the presence of high amounts of lignin-degrading enzymes in SMS, several authors have underlined the potential of *A. bisporus* and *P. ostreatus* SMS to remediate contaminated soils (El Enshasy et al., 2019; García-Delgado et al., 2015a).

Regarding inorganic contaminants, Raj et al. (2011) highlighted the capacity of some fungi to accumulate high concentrations of metals (e.g., Pb) in their fruiting bodies. Furthermore, Kulshreshtha (2018) and García-Delgado et al. (2013) reported that SMS can act as metal adsorbent due to the presence of fungal mycelia. Nevertheless, there is still insufficient knowledge on the suitability of SMS to reduce bioavailable metal concentrations in soil. In any case, mycoremediation is widely recognized as a bioremediation strategy of great potential, particularly for soils contaminated with recalcitrant organic compounds (Kulshreshtha et al., 2014; Singh, 2006). Importantly, the use of SMS for mycoremediation purposes (an example of waste valorisation) is in accordance with the circular economy paradigm.

On the other hand, phytoremediation with metal tolerant plants has proven potential for the recovery of metal contaminated soils (Burges et al., 2016; Epelde et al., 2014a,b). Within the metal phytoremediation field, there are two main strategies: *phytoextraction*, focused on the reduction of total metal concentrations with (hyper)accumulators (Garbisu and Alkorta, 2001; Hernández-Allica et al., 2008), and *phytostabilization*, focused on the reduction of bioavailable metal concentrations with excluders (Alkorta et al., 2010). The success of phytoremediation depends, to a considerable extent, on the selection of the most appropriate plant species (Kumar et al., 1995). Plants from the *Brassicaceae* family (e.g., Indian mustard - *Brassica juncea,* canola - *B. napus*) have frequently been used for phytoextraction because of their high metal tolerance and metal accumulation in shoots, as well as their fast growth and high biomass production (Singh and Fulekar, 2012). In particular, *B. juncea* plants have a remarkable capacity to accumulate Pb in their aboveground tissues (Kumar et al., 1995). Interestingly, in a study on EDTA-enhanced phytoextraction, Turan and Esringü (2007) found that *B. napus* plants were more effective at taking up metals (Cu, Cd, Pb, Zn) than *B. juncea* plants. *Festuca rubra* plants can tolerate high concentrations of metals through exclusion strategies (metal excluders), which justifies their acknowledged potential for phytostabilization (Galende et al., 2014).

Here, it is important to emphasize that soil remediation strategies must not only reduce the total or bioavailable concentrations of contaminants, but must also result in a recovery of soil health (Epelde et al., 2009). Due to their key role in soil ecosystem functioning, microorganisms have great value as indicators of soil health and, in particular, as bioindicators of the effectiveness of remediation processes in terms of soil health recovery (Burges et al., 2016; Epelde et al., 2014a,b; Gómez-Sagasti et al., 2018).

As mentioned above, many authors have reported the potential of mycoremediation and phytoremediation for the recovery of mixed contaminated soils, but there is still a lack of studies comparing the efficiency of these two biological technologies when applied to the same mixed contaminated soil. Then, in this work, we compared the remediation potential of two mycoremediation strategies using *Agaricus bisporus* and *Pleurotus ostreatus* growth substrates (inoculated mushroom substrates vs. spent mushroom substrates) with that of two phytoremediation strategies using *Brassica* spp. (phytoextraction) and *Festuca rubra* (phytostabilization), in terms of both the reduction in contaminant levels and the enhancement of soil health of a soil co-contaminated with Pb (1930 mg Pb kg^{-1} vs. 350 mg Pb kg^{-1} dry weight – DW – soil) and lindane (100 mg·γ-HCH kg^{-1} DW soil).

2. Materials and methods

2.1. Soil collection and characterization

Soil was taken from two different areas of an outdoor shooting range located in Oiartzun (Spain). In each area, 100 kg of soil were collected at a 0–30 cm depth, and then thoroughly homogenized and sieved (<6 mm). Total Pb concentrations and soil physicochemical properties were determined as described in Section 2.3. One of the shooting range areas had an average total Pb concentration of 347 ± 29 mg kg^{-1} DW soil (hereafter referred to as 350-Pb soil), while the other showed a mean value of 1930 ± 91 mg Pb·kg^{-1} DW soil (hereafter referred to as 1930-Pb soil). Despite these average values, it must be emphasized that most, if not all, shooting ranges are characterized by an extremely high heterogeneity in terms of soil Pb concentrations. However, the values of the other studied physicochemical properties, determined as in section 2.3, were similar in both soils: (1) 350-Pb soil: pH = 5.3, total nitrogen = 0.2%, organic matter = 3.9%, cation exchange capacity = 10.4 meq·100 g^{-1}; and (2) 1930-Pb soil: pH = 5.3, total nitrogen = 0.2%, organic matter = 3.4%, cation exchange capacity = 8.5 meq·100 g^{-1}.

The collected soil was artificially contaminated with γ-hexachlorocyclohexane (γ-HCH) and homogenized using a cement mixer. Briefly, γ-HCH was applied in an *n*-hexane/acetone (50/50 v/v) solution to reach a final concentration of 100 mg γ-HCH·kg^{-1} DW soil. Subsequently, the soil was kept at room temperature for 4 weeks in order to allow for contaminant stabilization.

2.2. Experimental design

For the mycoremediation treatments, *A. bisporus* and *P. ostreatus* inoculated mushroom substrates and spent mushroom substrates were kindly supplied by the Mushroom Technological Research Centre located in Autol (Spain). The term "inoculated mushroom substrate" (IMS) here refers to the growth substrate used for mushroom production immediately after being inoculated with sterilized wheat spawn. Then,

no growth of mushroom mycelium was initially present in the IMS. The term "spent mushroom substrate" (SMS) refers to the spent substrate that is often managed as a waste after being used for mushroom production. *Agaricus bisporus* growth substrate is composed of a mixture of wheat straw, chicken manure, urea, and gypsum, subjected to an accelerated composting process. *Pleurotus ostreatus* growth substrate is composed of moistened wheat straw subjected to a pasteurisation process. Both mushroom growth substrates were prepared at the Mushroom Technological Research Centre (Autol, Spain) following Gil (2011). For the determination of IMS and SMS physicochemical properties, 1 kg of mushroom substrate was dried at 30 °C for 72 h, milled (CT 293 Cyclotec™), and analysed as described in Section 2.3. The physicochemical properties of both mushroom growth substrates (IMS, SMS) are shown in Supplementary Table 1.

Both substrates (IMS, SMS) were manually mixed with the Pb and γ-HCH contaminated soils, according to a 1:4 w/w (substrate:soil) ratio. The mixtures were then placed in 12 L plastic trays in a phytotron under controlled conditions (Table 1) and watered twice a week. *Agaricus bisporus* needed 5 weeks to colonise the entire growth substrate (incubation phase), while *P. ostreatus* took only 2 weeks. The fructification phase (i.e., formation of fruiting bodies) ended with the third *A. bisporus* harvest and with the second *P. ostreatus* harvest. Soil fully colonised by fungal mycelium was sampled at the end of the fructification phase which lasted 5 weeks for *A. bisporus* and 6 weeks for *P. ostreatus*. Then, the whole experiment (inoculation phase *plus* fructification phase) took 10 weeks for *A. bisporus* treatments and 8 weeks for *P. ostreatus* treatments. Soil samples were collected at the end of the incubation and fructification phase.

For the phytoremediation treatments, Pb and γ-HCH contaminated soils were manually mixed with a commercial compost (COMPOSANA®, COMPO Group), following a 1:5 w/w (compost:soil) ratio, and then placed in 4 kg plastic pots. According to the manufacturer, the physicochemical properties of the commercial compost were: pH = 6.0, a total nitrogen content = 1.07%, and an organic matter content = 67%. Subsequently, a total of 30 seeds per pot were sown using a mixture of *Brassica* species (*B. juncea*, *B. napus* subsp. *Cebra,* and *B. napus* subsp. *expower*) or *Festuca rubra* for phytoextraction and phytostabilization purposes, respectively. The seeds were kindly supplied by the University of the Basque Country, Spain. In *Brassica* treatments, after seed germination, only 6 homogeneous plants (2 of each species) were left in the pot, while the others were manually removed. Plants were grown for 12 weeks in a phytotron under controlled conditions (Table 1) and bottom watered twice a week. Plant shoots (shoots from the three *Brassica* species were mixed) and soils were sampled at the end of the 12-week period.

Control (untreated) soils were also included in the experiment under the same controlled conditions. Thus, a total of 14 treatments were established in this study (Table 2), with three biological replicates for each treatment. Some photographs of the experiment are shown in Supplementary Fig. 1.

Table 1
Growth conditions.

Species	Light	Temperature°C	Relative moisture (%)	Time (weeks)
Control	Direct light (12 h)	22–24	70–80	12
Agaricus bisporus				
Incubation phase	Darkness	21–24	70–80	5
Fructification phase	Darkness	17	80	5
Pleurotus ostreatus				
Incubation phase	Darkness	22–24	70–80	2
Fructification phase	Indirect light (12 h)	22–24	80	6
***Brassica* spp.**	Direct light (12 h)	22–24	70–80	12
Festuca rubra	Direct light (12 h)	22–24	70–80	12

2.3. Soil physicochemical parameters

For the quantification of total γ-HCH and Pb concentrations at the end of the experiment, soil samples were collected, dried at 30 °C for 48 h, and then sieved to <5 mm. Soil γ-HCH concentration was measured following Anza et al. (2018). Briefly, γ-HCH was extracted from 1.5 g of fresh weight (FW) soil in 5 mL hexane/acetone (50:50 v/v) by sonicating samples for 15 min. Then, samples were centrifuged for 5 min at 1000 rpm, and 1 mL supernatant was filtered through a 0.22 μm PTFE syringe filters. γ-HCH was determined by gas chromatography (Agilent 6890 N) using Gas Chromatography - μElectron Capture Detector (GC-μECD) with the following parameters: HP-ULTRA 2.5% Phenyl Methyl Siloxane column (25 m × 0.20 mm x 0.33 μm); injection volume was 2 μL in split mode; injector temperature was 250 °C, ramp temperature was 150 °C (2 min) - 20 °C min^{-1} until 300 °C (kept for 3 min); and the flow of carrier gas (helium) was 3 mL min^{-1}. Total Pb concentration was determined following Zhao et al. (1994). Briefly, 0.2 g of dry weight (DW) soil was added to digestion tubes (in duplicate) containing 10 mL of a mixed acid solution (HNO_3 85% + $HClO_4$ 15%). Digestion was performed in a TEKATOR digestion block according to the following temperature program: 60 °C for 1 h, 100 °C for 1 h, 120 °C for 1 h, 130 °C for 1 h, 140 °C for 1 h, 150 °C for 1 h, 170 °C for 1 h, and 80 °C for 4 h. Then, 25 mL HNO_3 1% was added to the digestion tubes and stirred for 30 min. After a 1-h decantation period, Pb concentration was determined using inductively coupled plasma atomic emission spectrometry (ICP-OES, Varian). Extractable Pb was determined according to Houba et al. (2000) and García-Delgado et al. (2015b) using 0.01 M $CaCl_2$ as extractant. Extractable Pb concentrations were determined using ICP-OES spectrophotometry (ICP-OES, Varian). In soils subjected to mycoremediation treatments, extractable Pb was quantified twice: after the incubation phase and after the fructification phase. In soils subjected to phytoremediation treatments, extractable Pb was determined at plant harvest. Substrate:soil and compost:soil ratios (1:4 w/w substrate:soil and 1:5 w/w compost:soil) were taken into account as dilution factors when data on contaminant concentrations were determined.

Soil pH was quantified in water at 1:2.5 w/v. Total carbon (C) and total nitrogen (N) were analysed by combustion with a TruSpec CHN analyser (LECO Corporation, Michigan, USA) according to ISO, 1995 and ISO, 1998. Olsen phosphorus (P) was determined according to Watanabe and Olsen (1965). Cation exchange capacity (CEC) and extractable potassium (K^+) were measured according to official methods of soil analysis by the Spanish Ministry of Agriculture, Fisheries and Food (MAPA, 1994). Soil organic matter content (SOM) was determined

Table 2
Experimental design (n = 3). IMS: inoculated mushroom substrate; SMS: spent mushroom substrate.

Treatment	Pb ($mg \cdot kg^{-1}$)	Strategy
Control	350	Untreated
Control	1930	Untreated
A. bisporus IMS	350	Mycoremediation
A. bisporus IMS	1930	Mycoremediation
A. bisporus SMS	350	Mycoremediation
A. bisporus SMS	1930	Mycoremediation
P. ostreatus IMS	350	Mycoremediation
P. ostreatus IMS	1930	Mycoremediation
P. ostreatus SMS	350	Mycoremediation
P. ostreatus SMS	1930	Mycoremediation
Brassica spp.	350	Phytoextraction
Brassica spp.	1930	Phytoextraction
Festuca rubra	350	Phytostabilization
Festuca rubra	1930	Phytostabilization

according to Nelson and Sommers (1996).

The validation of the abovementioned soil analytical procedures was based on the use of sixteen soil samples from the International Soil-Analytical Exchange Programme (ISE), WEPAL-QUASIMEME (Wageningen University, The Netherlands) as certified reference materials. In each analysis, certified reference samples were used as accuracy and repeatability controls.

2.4. Soil microbial parameters

For the determination of microbial parameters, soil samples were stored at 4 °C for a maximum of two months until analysis. Samples for molecular analyses were stored at −20 °C. Samples were thoroughly homogenized prior to each determination.

To assess the effect of treatments on soil microbial activity, β-D-glucosidase (GLU, EC 3.2.1.21), glucosaminidase (GLM, EC 3.2.1.30), phosphatase (PHO, EC 3.1.3.2), sulphatase (SUL, EC 3.1.6.1), L-leucine-aminopeptidase (LEU, EC 3.4.11.1), and L-alanine-aminopeptidase (LAP, EC 3.4.11.2) enzyme activities were measured according to ISO/TS 22939, 2010. Soil respiration was determined by measuring CO_2 evolution in hermetic flasks incubated at 30 °C for 72 h, according to ISO, 2002.

For the determination of soil microbial biomass, microbial biomass carbon and total prokaryotic abundance were measured (see below). In order to assess *A. bisporus* and *P. ostreatus* growth, the abundance of Basidiomycota was also determined. Microbial biomass carbon (C_{MB}) was measured according to the fumigation-extraction method described in Vance et al. (1987). Briefly, four technical replicates of 2.5 mg FW soil were weighed for each treatment. Two of these replicates were fumigated for 24 h with 0.5 mL of amylene-stabilized $CHCl_3$ under vacuum conditions. Then, the four replicates were extracted in 6 mL K_2SO_4 (0.5 M) by keeping samples in agitation for 30 min at 20 °C. Two millilitres of the soil extract was mixed with 3.5 mL of chromium reagent [chromium VI oxide (0.18% w/v) and sulphuric acid (65% v/v)] and incubated at 150 °C for 90 min. Organic carbon concentration was determined colorimetrically at 445 nm using an UV-VIS Spectrophotometer UV-1800 (Shimadzu). Microbial biomass carbon (C_{MB}) was calculated as the difference between organic carbon concentration of the fumigated and non-fumigated extracts (Wu et al., 1990). To quantify total prokaryotic and Basidiomycota abundances, 250 mg of fresh weight soil were washed twice in 120 mM K_2PO_4 (pH 7.8) to wash away extracellular DNA. Then, DNA was extracted using the PowerSoil™ Isolation Pro Kit (QIAGEN). Extracted DNA concentrations were measured using NanoDrop One (Thermo Fisher). Real-time qPCR (qPCR) was carried out to assess both total prokaryotic and Basiodiomycota abundances. For Basidiomycota, the following primers were used: ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS4F (CAGGAGACTTGTACACGGTCCAG) (Xu et al., 2014). For total prokaryotes, 16 S rRNA gene fragments were quantified using Ba519f (CAGCMGCCGCGGTAANWC) and Ba907r (CCGTCAATTCMTTTRAGTT) primers following Epelde et al. (2014a,b). Each measurement was carried out in triplicate.

In order to unravel the effects of treatments on prokaryotic community composition, a dual indexing approach was used to prepare amplicon libraries with primers targeting the V4 region of the 16 S rRNA gene (Lanzén et al., 2016; Urra et al., 2019). Adapter-linked 519 F (CAGCMGCCGCGGTAA) and 806 R (GGACTACHVGGGTWTCTAAT) primers were used in the first amplification step, and barcoded primers were used in the second amplification step (10 cycles) (Lanzén et al., 2016). AMPure XP was used to clean amplicon libraries (Beckman Coulter Genomics). Sequencing was carried out using an Illumina MiSeq desktop sequencer with V2 RUN kit (500 cycles) at SGIker-University of Basque Country.

To our knowledge, there are currently no interlaboratory comparison programmes or certified reference materials for the determination of soil enzyme activities, respiration and C_{MB}. Therefore, in order to validate the determination of the abovementioned enzyme activities, two calibration curves were made using 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC) substrates as controls with 4 technical replicates for each sample. In the same way, soil respiration and C_{MB} were determined twice on two consecutive days. The results presented here correspond to the average values of the technical replicates of each treatment.

2.5. Mushroom and plant parameters

Immediately after harvesting, plant shoots and mushrooms were washed thoroughly with deionized water and then oven dried at 70 °C for 48 h. Dry weights were recorded and plant and mushroom samples were milled (CT 293 Cyclotec™) prior to laboratory analyses. For the determination of the amount of Pb extracted from soil by plants or mushrooms, 0.5 g of milled plant or mushroom tissue were digested with a mixture of $HNO_3/HClO_4$ (Zhao et al., 1994), and then Pb concentrations were determined using an ICP-OES spectrophotometer (ICP-OES, Varian).

Bioconcentration factors (BF = ratio between metal concentration in aboveground plant tissues or mushroom tissues and initial metal concentration in soil, i.e. 347 or 1930 mg kg^{-1} DW soil) were calculated. Plant and mushroom Extraction efficiency (PE) was calculated as follows, according to Lampis et al. (2009):

$$PE = C_{tissue} \text{ x } DW_{tissue} \text{ / } C_{soil} \text{ x } DW_{soil}$$

where C_{tissue} = Pb concentration in aboveground plant or mushroom tissue, DW_{tissue} = dry weight of aboveground plant or mushroom biomass, C_{soil} = initial Pb concentration in soil, and DW_{soil} = soil dry weight.

2.6. Data treatment

Quality control of sequenced samples was carried out using FASTQC software (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). PCR primers were trimmed using cutadapt (Martin, 2011). FASTQ files were analysed via QIIME2 (Bolyen et al., 2019). Low-quality reads were filtered by qiime quality-filter q-score-joined on default options. Denoising process was performed by Deblur (qiime deblur denoise-16 S) (Amir et al., 2017) and resulting reads were classified using qiime feature-classifier classify-sklearn and silva-132-99-nb-classifier.qza as the reference model to obtain the taxonomy of the amplicon sequence variants (ASVs). The R library Rcmdr (Field et al., 2012) and vegan package (Oksanen et al., 2015) were used for the visualization of 16 S rRNA amplicon sequencing data and the calculation of biodiversity indices. The effect of treatments on the relative abundance values of the 30 most abundant prokaryotic families was assessed using Orange data mining toolbox (Demsar et al., 2013).

The effect of treatments on soil parameters was assessed using a one-way ANOVA with SPSS Statistics 18.0.0 Software (SPSS, Chicago, IL, USA). When the effect of treatments was significant ($p < 0.05$), differences were tested using Duncan's test. Redundancy analyses (RDA) were used to explore the effect of treatments on soil parameters using CANOCO 5.0 (ter Braak and Ś; milauer, 2012).

3. Results

3.1. Contaminant concentrations

Mycoremediation treatments showed the most significant reduction in soil γ-HCH concentrations, compared to control (untreated) and phytoremediation treatments (Fig. 1A). The most effective treatment for γ-HCH removal was the application of *P. ostreatus* IMS, which reduced γ-HCH concentration by 88.9% in 1930-Pb soil and 54.4% in 350-Pb soil, respectively, compared to their corresponding controls. Phytoremediation treatments did not significantly reduced γ-HCH concentrations, compared to untreated control.

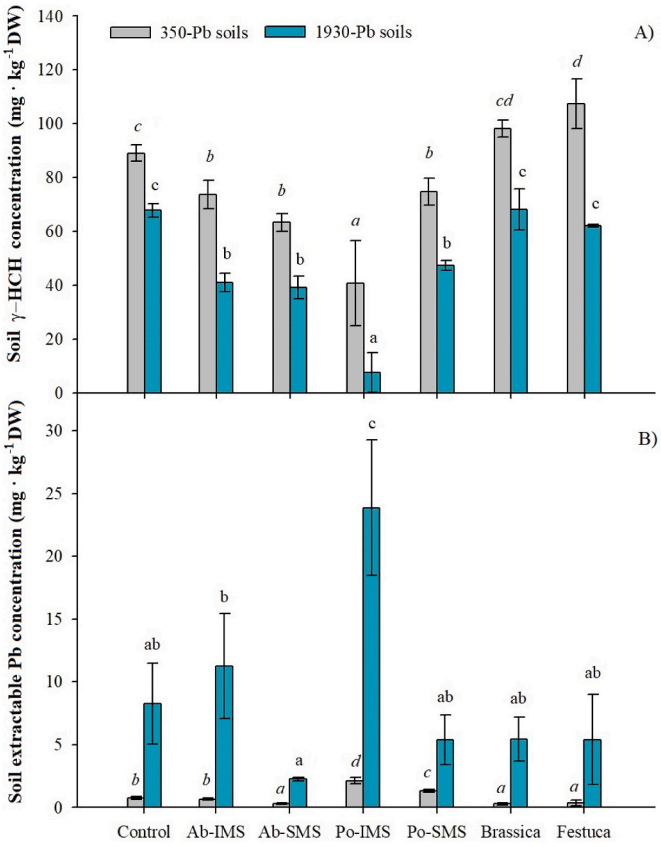

Fig. 1. Effect of treatments on soil (A) γ-HCH and (B) extractable Pb concentrations. Mean values (n = 3) ± standard deviations. For statistical analysis, 350-Pb and 1930-Pb soils were analysed separately. Treatments with different letters are significantly ($p < 0.05$) different according to Duncan's test. Ab-IMS: *A. bisporus* inoculated mushroom substrate; Ab-SMS: *A. bisporus* spent mushroom substrate; Po-IMS: *P. ostreatus* inoculated mushroom substrate; Po-SMS: *P. ostreatus* spent mushroom substrate.

In both soils (350-Pb and 1930-Pb), mycoremediation with *P. ostreatus* IMS significantly increased extractable Pb concentrations, compared to controls (Fig. 1B, Supplementary Table 2). In 350-Pb soil, the application of *P. ostreatus* SMS also led to a significant increase of extractable Pb concentrations, compared to phytoremediation and control treatments. On the contrary, in 350-Pb soil, a significant reduction of extractable Pb concentration was observed under both phytoremediation treatments (*Brassica* spp. And *F. rubra*), compared to untreated control. In 1930-Pb soil, the lowest extractable Pb concentration (2.25 mg Pb kg^{-1} DW soil) was detected in soils treated with *A. bisporus* SMS (Fig. 1B, Supplementary Table 2). Nevertheless, no statistically significant differences were detected between such treatment and *Brassica* spp. (5.42 mg Pb kg^{-1}), *F. rubra* (5.40 mg Pb kg^{-1}), *P. ostreatus* SMS (5.38 mg Pb kg^{-1}), and control (8.24 mg Pb kg^{-1}) treatments, in terms of extractable Pb concentrations. Extractable Pb concentrations in soil were higher after the incubation vs. the fructification phase (Supplementary Table 2). In 350-Pb soils, total Pb concentrations were lower under mycoremediation and phytoremediation treatments, compared to control (Supplementary Table 2).

3.2. Physicochemical and microbial indicators of soil health

The application of the studied treatments led to an overall increase of soil physicochemical parameter values (Table 3). More concretely, mycoremediation treatments with *A. bisporus* IMS and SMS showed the highest values of total N, Olsen P, extractable K^+ and pH (Table 3). Less relevant changes in soil physicochemical parameters were observed as a result of the application of *P. ostreatus* IMS and SMS. In turn, phytoremediation treatments increased CEC, total C and SOM values (Table 3).

Fig. 2 shows the effect of treatments on soil microbial properties (numerical values are shown in Supplementary Tables 3, 4 and 5). Values of soil microbial parameters were generally lower in phytoremediation vs. mycoremediation treatments, although in both cases exceeded control soil values. Mycoremediation treatments led to an increase of microbial activity, biomass and diversity in both 350-Pb (Fig. 2A) and 1930-Pb (Fig. 2B) soils, compared to phytoremediation and control treatments.

In 350-Pb soil, values of microbial parameters increased towards the

Table 3
Effect of treatments on physicochemical properties of 350-Pb and 1930-Pb soils. Mean values (n = 3) ± SD. Letters show significant differences ($p < 0.05$) among treatments based on one-way ANOVA and Duncan's test. SOM: soil organic matter; CEC: cation exchange capacity; Ab-IMS: *A. bisporus* inoculated mushroom substrate; Ab-SMS: *A. bisporus* spent mushroom substrate; Po-IMS: *P. ostreatus* inoculated mushroom substrate; Po-SMS: *P. ostreatus* spent mushroom substrate; ns: non-significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

	350-Pb						
Treatment	Total C	Total N	Olsen P	K^+	pH	SOM	CEC
	%	%	mg kg^{-1}	mg kg^{-1}		%	mEq 100 g^{-1}
Control	3.35 ± 0.07^{d}	0.29 ± 0.01^{d}	8.27 ± 4.39^{de}	156 ± 63.8^{c}	5.16 ± 0.11^{d}	5.20 ± 0.25^{c}	10.4 ± 0.22^{d}
Ab-IMS	12.6 ± 4.88^{ab}	0.99 ± 0.08^{a}	183 ± 13.5^{a}	3483 ± 427^{a}	6.31 ± 0.20^{b}	15.9 ± 6.12^{ab}	33.9 ± 2.90^{a}
Ab-SMS	8.47 ± 1.32^{bc}	0.86 ± 0.14^{b}	125 ± 16.2^{b}	3979 ± 672^{a}	6.75 ± 0.11^{a}	12.1 ± 1.39^{b}	29.8 ± 3.47^{a}
Po-IMS	7.57 ± 1.68^{cd}	0.37 ± 0.04^{d}	4.88 ± 1.69^{e}	922 ± 30.5^{b}	5.83 ± 0.01^{c}	12.1 ± 3.29^{b}	15.3 ± 0.58^{c}
Po-SMS	12.2 ± 2.27^{ab}	0.39 ± 0.02^{cd}	8.51 ± 3.98^{de}	878 ± 158^{b}	5.90 ± 0.27^{bc}	18.4 ± 2.38^{a}	19.2 ± 0.42^{b}
Brassica	14.7 ± 1.68^{a}	0.50 ± 0.02^{c}	23.7 ± 1.90^{cd}	67.7 ± 11.6^{c}	5.90 ± 0.41^{bc}	15.1 ± 0.54^{ab}	30.2 ± 1.61^{a}
Festuca	12.9 ± 2.25^{ab}	0.51 ± 0.03^{c}	33.1 ± 6.13^{c}	123 ± 47.6^{c}	5.79 ± 0.30^{c}	14.2 ± 2.14^{ab}	30.7 ± 3.12^{a}
p	***	***	***	***	***	**	***
	1930-Pb						
Treatment	**Total C**	**Total N**	**Olsen P**	**K^+**	pH	**SOM**	**CEC**
	%	%	mg kg^{-1}	mg kg^{-1}		%	mEq 100 g^{-1}
Control	2.70 ± 0.11^{d}	0.22 ± 0.01^{d}	5.29 ± 1.15^{d}	85.0 ± 8.72^{d}	5.63 ± 0.06^{d}	4.50 ± 0.15^{c}	8.49 ± 0.30^{e}
Ab-IMS	111 ± 1.06^{a}	0.67 ± 0.06^{a}	151 ± 9.74^{a}	2711 ± 193^{b}	6.39 ± 0.28^{bc}	16.6 ± 4.64^{a}	23.3 ± 1.03^{b}
Ab-SMS	6.96 ± 1.20^{c}	0.62 ± 0.04^{a}	123 ± 9.92^{b}	3098 ± 395^{a}	7.06 ± 0.04^{a}	9.20 ± 0.93^{b}	23.3 ± 1.33^{b}
Po-IMS	8.19 ± 2.42^{bc}	0.24 ± 0.05^{cd}	8.09 ± 4.48^{d}	794 ± 151^{c}	5.82 ± 0.46^{d}	11.6 ± 1.25^{b}	12.8 ± 0.62^{d}
Po-SMS	9.58 ± 0.03^{abc}	0.30 ± 0.02^{c}	11.6 ± 10.1^{cd}	744 ± 73.7^{c}	6.03 ± 0.34^{cd}	13.0 ± 3.42^{ab}	15.8 ± 0.56^{c}
Brassica	10.1 ± 1.88^{ab}	0.40 ± 0.01^{b}	22.5 ± 2.78^{c}	55.0 ± 14.0^{d}	6.60 ± 0.38^{ab}	12.5 ± 2.65^{ab}	24.7 ± 0.56^{ab}
Festuca	10.1 ± 1.88^{ab}	0.38 ± 0.00^{b}	24.6 ± 6.00^{c}	76.3 ± 11.8^{d}	5.86 ± 0.11^{d}	12.4 ± 1.64^{ab}	25.1 ± 0.65^{a}
p	***	***	***	***	***	**	***

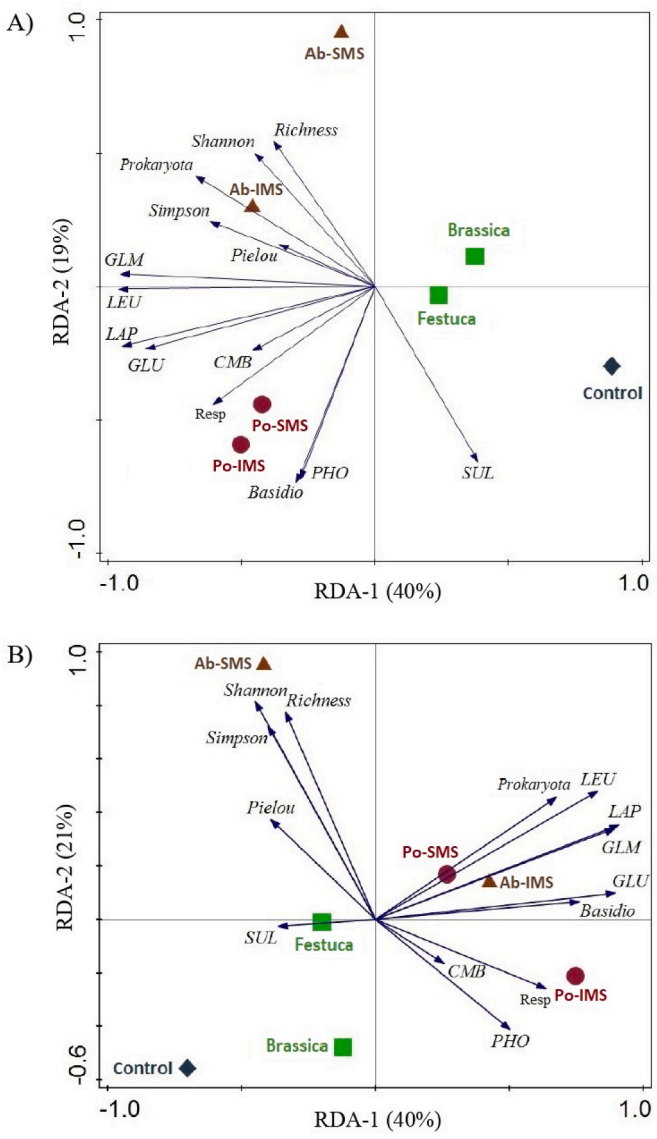

Fig. 2. Biplot of the redundancy analysis of the effect of treatments on soil microbial parameters in (A) 350-Pb and (B) 1930-Pb soils. The analysis was performed with the treatments as explanatory variables (symbols) and microbial parameters as response variables (arrows). Resp: respiration; Prokaryota: prokaryotic abundance; Basidio: Basidiomycota abundance; GLU: β-D glucosidase; GLM: glucosaminidase; SUL: sulphatase; PHO: phosphatase; LAP: L-alanine-aminopeptidase; LEU: L-leucine-aminopeptidase; CMB: microbial biomass carbon.

left side of RDA-1 (this axis explained 40% of the variance) where mycoremediation treatments were located. Compared to control soil, treatments with *P. ostreatus* growth substrates (IMS and SMS) showed the greatest enhancement of basal respiration (1.63 times higher than control in the presence of IMS), enzyme activities (GLU: 5.67 times higher in the presence of IMS; GLM: 11.73 and 9.30 times higher in the presence of IMS and SMS, respectively; PHO: 3.04 and 2.60 times higher in the presence of IMS and SMS, respectively; and LAP: 5.94 and 5.69 times higher in the presence of IMS and SMS, respectively) and microbial biomass carbon. The highest values of microbial diversity indexes corresponded to the *A. bisporus* SMS treatment (this treatment showed lower microbial parameter values than the other mycoremediation treatments). In 350-Pb soil, the highest values of total prokaryotic abundance and microbial diversity (Richness, Shannon, Simpson and Pielou indexes) were detected in the presence of *A. bisporus* IMS and SMS. The abundance of Basidiomycota was significantly higher in *P. ostreatus* treatments (8.19 and 4.13 times greater than control for SMS and IMS, respectively), compared to *A. bisporus*, phytoremediation and control treatments (Supplementary Table 4). The abundance of Basidiomycota was higher in control and phytoremediation treatments than in the presence of *A. bisporus* SMS substrate.

In 1930-Pb soil, microbial diversity indexes presented the most significant increase in the presence of *A. bisporus* SMS (Shannon: 1.22 times higher; Simpson: 1.02 times higher; Pielou: 1.03 times higher; and Richness: 2.05 times higher than control), while microbial activity (respiration, GLU, GLM, PHO, LEU, LAP) and biomass (C_{MB}, total prokaryotic abundance) parameters showed the greatest values in the presence of *P. ostreatus* IMS and SMS (Supplementary Tables 3 and 4). Similar to what was observed in 350-Pb soil, Basidiomycota abundance showed higher values in the presence of both *P. ostreatus* substrates, compared to *A. bisporus,* phytoremediation and control treatments (Supplementary Table 4).

The heatmap shown in Fig. 3 reflects the effect of treatments on the 30 most abundant prokaryotic families. Mycoremediation treatments led to a significantly higher abundance of *Paenibacillaceae, Flavobacteriaceae, Burkholderiaceae, Rhizobiaceae, Sphingobacteriaceae,* and *Rhodanobacteraceae*, compared to control and phytoremediation treatments (Fig. 3). Under mycoremediation, prokaryotic families were grouped first by fungal species (*A. bisporus* and *P. ostreatus*) and then by type of growth substrate (IMS and SMS) (Fig. 3). A higher abundance of *Xanthobacteraceae*, *Rhizobiaceae*, *Burkholderiaceae*, *Isosphaeraceae*, and *Streptomycetaceae* was observed in *P. ostreatus* treatments compared to *A. bisporus* treatments, while *Pirellulaceae* showed higher abundances in *A. bisporus* vs. *P. ostreatus* treatments. The application of SMS led to a significantly higher abundance of *Microscillacceae*, compared to IMS, while the latter increased the abundance of *Paenibacillaceae*. Total Pb concentrations in soil did not significantly affect prokaryotic composition. *Xanthobacteraceae*, *Chthoniobacteraceae, Isosphaeraceae*, and *Streptomycetaceae* were more abundant under phytoremediation and control treatments. The most significant differences in terms of prokaryotic composition were observed for mycoremediation treatments, compared to phytoremediation and control treatments (in general, no significant differences were observed between phytoremediation and control treatments). Numerical values and significant differences between treatments on the 30 most abundant prokaryotic families are shown in Supplementary Table 6.

3.3. Mushroom and plant parameters

The application of *P. ostreatus* IMS led to highest values of mushroom biomass (Fig. 4A). In contrast, only a few *A. bisporus* fruiting bodies managed to grow under our experimental conditions (Supplementary Table 7). As a matter of fact, *A. bisporus* champignons were only harvested from one of the three replicates in the presence of IMS (and no *A. bisporus* mushrooms grew in the presence of SMS). Due to the very limited *A. bisporus* biomass production obtained under our experimental conditions, its possible application to extract metals (mycoextraction) from soil under real field conditions was discarded. Then, this treatment was not taken into account when statistically analysing plant vs. mushroom parameters. The highest amount of Pb extracted from soil was observed in the presence of *P. ostreatus* IMS (Fig. 4B). No significant differences were observed among the other treatments regarding the amount of Pb extracted from soil (Fig. 4B, Supplementary Table 7).

In 350-Pb soil, a significant increase of BF values was observed in *P. ostreatus* treatments compared to phytoremediation treatments (Fig. 4C). In 1930-Pb soil, a significantly lower value of BF was detected in *F. rubra* treatment compared to *Brassica* spp. And *P. ostreatus* treatments (Fig. 4C). Finally, in 350-Pb soil, the application of *P. ostreatus* IMS led to the highest values of extraction efficiency (Fig. 4D).

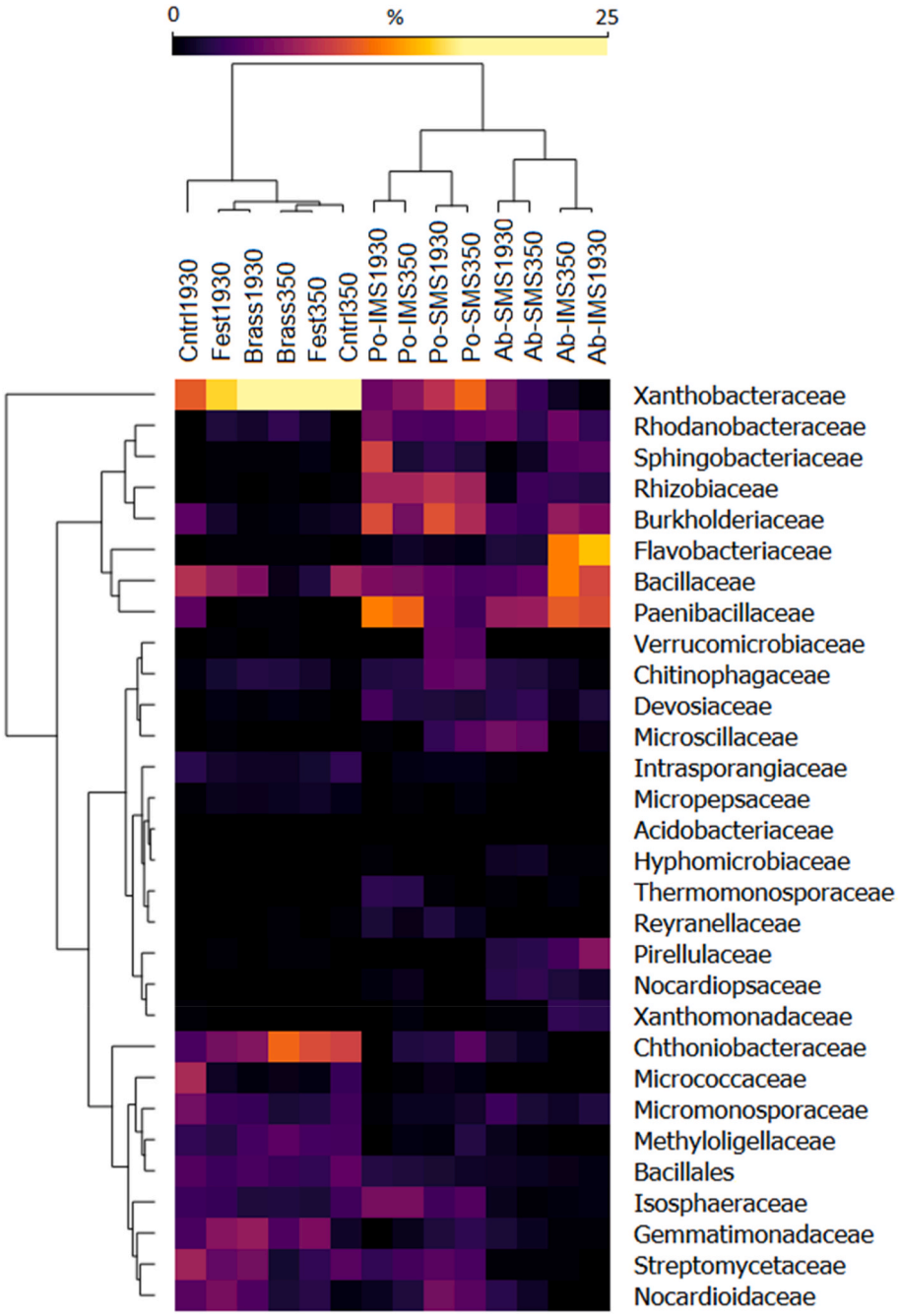

Fig. 3. Heatmap of the relative abundances (%) of the 30 most abundant prokaryotic families. 1930: 1930-Pb soils; 350: 350-Pb soils; Ab-IMS: *A. bisporus* inoculated mushroom substrate; Ab-SMS: *A. bisporus* spent mushroom substrate; Po-IMS: *P. ostreatus* inoculated mushroom substrate; Po-SMS: *P. ostreatus* spent mushroom substrate; Fest: *Festuca rubra* treatments; Brass: *Brassica* treatments; Cntrl: untreated control.

4. Discussion

In this study, we evaluated the effectiveness of mycoremediation treatments with *A. bisporus* and *P. ostreatus* IMS and SMS, in comparison with phytoremediation with *Brassica* spp. And *F. rubra*, for the remediation of a mixed-contaminated (γ-HCH and Pb) soil. Papadopoulou et al. (2006) pointed out that fungal biodegradation of γ-HCH can be accomplished thanks to the action of non-specific extracellular oxidative enzymes naturally produced by white-rot fungi to decompose wood (i.e., manganese peroxidases, laccases, etc.), which can explain the reduction in γ-HCH concentration observed here in the presence of *P. ostreatus*. The highest abundances of Basidiomycota were found in *P. ostreatus* treatments, which could be linked to a higher production of these extracellular enzymes. The bioremediation potential of fungi may also rely on their growth through hyphal extension, which allows them to penetrate across different matrices and can act as dispersion vectors for contaminant-degrading bacteria (Sadañoski et al., 2020). García--Delgado et al. (2015b) observed that the application of *A. bisporus* SMS resulted in a bioaugmentation effect by the fungus itself, as well as with other microbial species capable of degrading organic contaminants. In our study, mycoremediation treatments could have led to the introduction in the soil of bacterial species with the capacity to degrade γ-HCH (Jaiswal et al., 2022; Lal et al., 2010; Morya et al., 2019; Wang et al., 2022), thereby stimulating its removal.

The treatment showing the greatest reduction in soil extractable Pb concentrations was the application of *A. bisporus* SMS. Soils subjected to

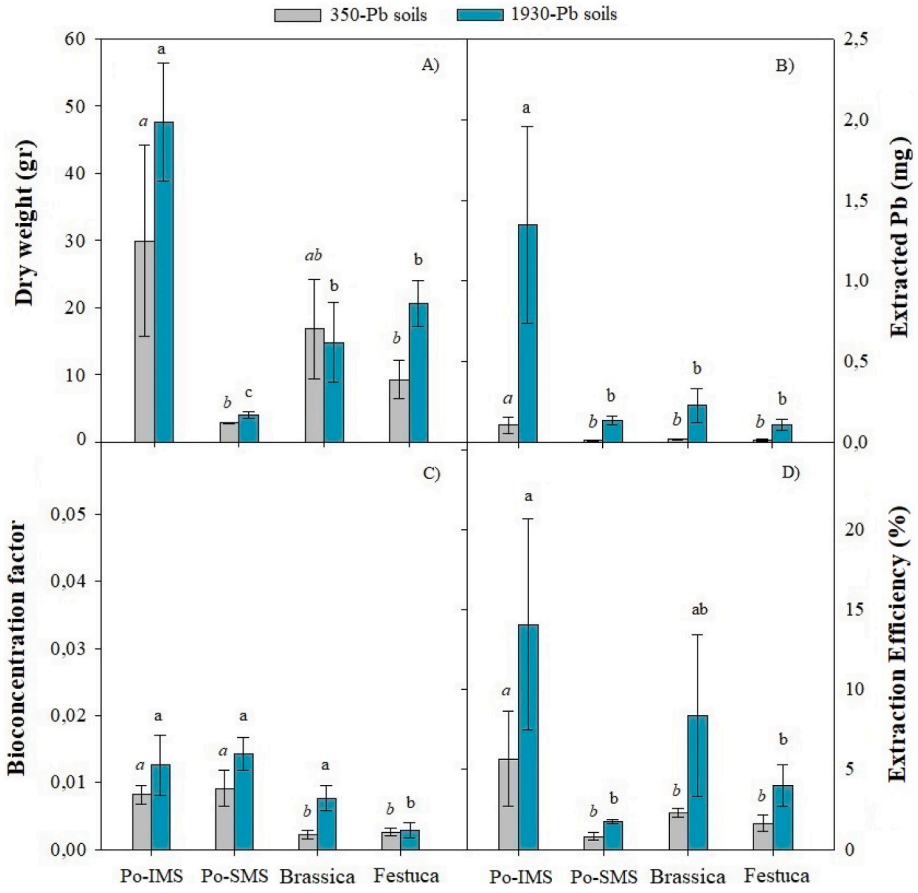

Fig. 4. Effect of treatments on (A) harvested biomass, (B) amount of Pb extracted, (C) bioconcentration factor (BF), and (D) extraction efficiency (PE). Mean values (n = 3) ± standard deviations. For statistical analysis, 350-Pb and 1930-Pb soils were analysed separately. Treatments with different letters are significantly ($p < 0.05$) different according to Duncan's test. *Agaricus bisporus* treatments were not taken into account due to the lack of mushroom growth.

this treatment showed the highest pH values which could explain the abovementioned reduction in extractable Pb (Kapahi and Sachdeva, 2017). Likewise, soil pH is known to regulate the ionization of pH-dependent ion-exchange sites on organic matter and metal oxide clay minerals, thus affecting metal availability (Bradham et al., 2006). García-Delgado et al. (2013) pointed out that the presence of inorganic compounds, such as clays, Fe and Mn oxides, $CaCO_3$, and $CaSO_4$, present in *A. bisporus* growth substrates can induce metal adsorption and precipitation. These authors observed a high presence of carboxyl acidic groups in *A. bisporus* SMS, which can increase their CEC and induce the formation of metal complexes. Salman et al. (2015) pointed out that ion-exchange mechanisms strongly affect soil Pb availability and, hence, Pb removal. In our study, in agreement with Liu et al. (2018), *P. ostreatus* spent mushroom substrate showed the lowest CEC values, which could result in higher Pb availability. In any case, it is very hard to determine the effect of individual soil properties on metal bioavailability (Bradham et al., 2006). Frutos et al. (2016) proposed that electrostatic attraction and complexation by cellulose can enhance Pb adsorption by SMS. *Agaricus bisporus* is a secondary decomposer that grows better on composted material with higher levels of cellulose. In turn, *P. ostreatus* is a primary decomposer which requires materials with a higher lignin content (Zied et al., 2020). Therefore, *A. bisporus* growth substrates normally have higher cellulose contents than *P. ostreatus* growth substrates, a fact which may have also influenced our data on extractable Pb levels.

Extractable Pb concentrations were higher after the incubation vs. the fructification phase, in agreement with García-Delgado et al. (2015b) who observed an initial increase of Pb bioavailability when SMS were applied (this increment decreased over time). We speculate that the combination of *P. ostreatus* treatments with metal (hyper)accumulator plants could enhance metal uptake and accumulation, since the application of *P. ostreatus* substrates produced the most significant increase of extractable Pb concentrations from a very early stage.

Regarding soil physicochemical parameters, the presence of *A. bisporus* substrates led to a significant increase of pH, total N, Olsen P and extractable K^+. *Agaricus bisporus* growth substrates showed higher values of these parameters compared to *P. ostreatus* growth substrates. The lower N, P and K^+ content found under phytoremediation treatments could be due to plant nutrient uptake (Hidalgo et al., 2022). Values of CEC, total C and SOM significantly increased in mycoremediation and phytoremediation treatments, compared to control treatment, probably due to the fact that the addition of fungal growth substrates and commercial compost increased total C and SOM values, which then affected CEC values (Noguchi et al., 2005).

Regarding soil microbial parameters, significantly higher values were observed in the presence of fungal growth substrates: values of microbial activity (enzyme activities, respiration), biomass (C_{MB}, total prokaryotic abundance) and diversity (Shannon, Simpson, Pielou, Richness) increased under mycoremediation treatments. Microbial properties are ecologically-relevant, sensitive indicators of soil health (Lehmann et al., 2020) and, hence, they are often used as bioindicators of the effectiveness of remediation treatments (Barrutia et al., 2011; Burges et al., 2016; Epelde et al., 2014a,b). The abovementioned increase in microbial parameter values is probably responsible for the higher γ-HCH degradation rates observed under mycoremediation treatments.

The presence of *P. ostreatus* IMS resulted in the highest Pb extraction from soil, most likely owing to the higher values of extractable Pb and

the higher amount of aboveground biomass detected under such treatment, compared to phytoremediation treatments. *Pleurotus* spp. Can thrive under a wider range of environmental conditions (Zied et al., 2020), compared to other fungal species such as *Agaricus* spp., which could explain the lack of *A. bisporus* growth observed in our study.

Bioconcentration factor values greater than 1 point out to the suitability of the studied organism for metal accumulation (Zhang et al., 2002). Nevertheless, metal accumulation in aerial parts depends not only on the potential of the studied organism to uptake and translocate metals to those parts, but also on the nature of the particular metal under consideration and the specific soil conditions. Here, BF values were very low (BF < 0.05) for all the treatments, including *Brassica* spp. Treatment. *Brassica* spp. Are known for their potential to accumulate metals in their aerial parts (Van Ginneken et al., 2007). In our study, the low values of soil bioavailable (extractable) Pb most likely hindered Pb phytoextraction by *Brassica* spp. (Neugschwandtner et al., 2008). The highest BF values were observed in *P. ostreatus* treatments, which also showed the greatest extractable Pb concentrations.

Above certain concentrations, metals are toxic for white-rot fungi, thus negatively affecting their growth and production of extracellular enzymes (Baldrian and Gabriel, 2003; Bhattacharya et al., 2014), and hence their capacity to degrade organic contaminants. However, the impact of metals on the production of ligninolytic enzymes by white-rot fungi varies depending on the specific metal, the fungal species, and the environmental conditions (Ceci et al., 2019). Here, γ-HCH degradation was significantly higher in 1930-Pb vs. 350-Pb soil. Previous studies have reported an increase of *P. ostreatus* (Baldrian et al., 2005) and *A. bisporus* (García-Delgado et al., 2013) lacasse activity in the presence of metals, including Pb. Furthermore, Pb extraction by plants and mushrooms was significantly greater in 1930-Pb vs. 350-Pb soil, possibly due to the higher values of extractable Pb found in the former.

5. Conclusions

The selection of the most appropriate remediation strategy for mixed contaminated soils is a daunting challenge since, apart from the specific characteristics of the soil and the different contaminants, one must take into consideration potential interactions among the contaminants, and among these and the soil matrix. Besides, the selected remediation strategy must be cost-effective and respectful with the integrity of the soil physical and biological matrix. Here, we have shown the great potential of mycoremediation with *P. ostreatus* growth substrates (particularly, the IMS) for the biological remediation of a soil co-contaminated with γ-HCH and Pb, in terms of both reduction in contaminant levels and enhancement of soil health. The combination of *P. ostreatus* growth substrates with plants suitable for metal phytoextraction (accumulators, hyperaccumulators) could facilitate the simultaneous removal of metals from soil and the degradation of organic contaminants, thus helping to overcome the limitations of each individual strategy.

Credit author contributions statement

June Hidalgo: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Lur Epelde**: Conceptualization, Methodology, Writing – review & editing. **Mikel Anza**: Validation, Formal analysis, Investigation, Data curation. **José M. Becerril**: Resources, Writing – review & editing, Funding acquisition. **Carlos Garbisu**: Conceptualization, Methodology, Writing – review & editing, Supervision, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.138538.

References

Alkorta, I., Becerril, J.M., Garbisu, C., 2010. Phytostabilization of metal contaminated soils. Rev. Environ. Health 25, 135–146. https://doi.org/10.1515/REVEH.2010.25.2.135.

Amir, A., McDonald, D., Navas-Molina, J.A., Kopylova, E., Morton, J.T., Zech Xu, Z., Kightley, E.P., Thompson, L.R., Hyde, E.R., Gonzalez, A., Knight, R., 2017. Deblur rapidly resolves single-nucleotide community sequence patterns. mSystems 2. https://doi.org/10.1128/MSYSTEMS.00191-16/FORMAT/EPUB.

Anza, M., Salazar, O., Epelde, L., Garbisu, C., 2018. Data on the selection of biostimulating agents for the bioremediation of soil simultaneously contaminated with lindane and zinc. Data Brief 20, 1371–1377. https://doi.org/10.1016/j.dib.2018.08.203.

Baldrian, P., Gabriel, J., 2003. Lignocellulose degradation by *Pleurotus ostreatus* in the presence of cadmium. FEMS Microbiol. Lett. 220, 235–240. https://doi.org/10.1016/S0378-1097(03)00102-2.

Baldrian, P., Valášková, V., Merhautová, V., Gabriel, J., 2005. Degradation of lignocellulose by *Pleurotus ostreatus* in the presence of copper, manganese, lead and zinc. Res. Microbiol. 156, 670–676. https://doi.org/10.1016/j.resmic.2005.03.007.

Barrutia, O., Garbisu, C., Epelde, L., Sampedro, M.C., Goicolea, M.A., Becerril, J.M., 2011. Plant tolerance to diesel minimizes its impact on soil microbial characteristics during rhizoremediation of diesel-contaminated soils. Sci. Total Environ. 409, 4087–4093. https://doi.org/10.1016/j.scitotenv.2011.06.025.

Bhattacharya, S., Das, A., Prashanthi, K., Palaniswamy, M., Angayarkanni, J., 2014. Mycoremediation of benzo[a]pyrene by *Pleurotus ostreatus* in the presence of heavy metals and mediators. 3 Biotech 4, 205–211. https://doi.org/10.1007/s13205-013-0148-y.

Bolyen, E., Rideout, J.R., Dillon, M.R., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Bonnen, A.M., Anton, L.H., Orth, A.B., 1994. Ligning-degrading enzymes of the commercial button mushroom, *Agaricus bisporus*. Appl. Environ. Microbiol. 60, 960–965. https://doi.org/10.1128/aem.60.3.960-965.1994.

Bradham, K.D., Dayton, E.A., Basta, N.T., Schroder, J., Payton, M., Lanno, R.P., 2006. Effect of soil properties on lead bioavailability and toxicity to earthworms. Environ. Toxicol. Chem. 25, 769–775. https://doi.org/10.1897/04-552R.1.

Burges, A., Epelde, L., Benito, G., Artetxe, U., Becerril, J.M., Garbisu, C., 2016. Enhancement of ecosystem services during endophyte-assisted aided phytostabilization of metal contaminated mine soil. Sci. Total Environ. 562, 480–492. https://doi.org/10.1016/j.scitotenv.2016.04.080.

Cameselle, C., Gouveia, S., 2019. Phytoremediation of mixed contaminated soil enhanced with electric current. J. Hazard Mater. 361, 95–102. https://doi.org/10.1016/j.jhazmat.2018.08.062.

Ceci, A., Pinzari, F., Russo, F., Persiani, A.M., Gadd, G.M., 2019. Roles of saprotrophic fungi in biodegradation or transformation of organic and inorganic pollutants in co-contaminated sites. Appl. Microbiol. Biotechnol. 103, 53–68. https://doi.org/10.1007/s00253-018-9451-1.

Demsar, J., Curk, T., Erjavec, A., Gorup, C., Hocevar, T., Milutinovic, M., Mozina, M., Polajnar, M., Toplak, M., Staric, A., Stajdohar, M., Umek, L., Zagar, L., Zbontar, J., Zitnik, M., Zupan, B., 2013. Orange: data mining toolbox in Python. J. Mach. Learn. Res. 2349–2353.

El Enshasy, H., Agouillal, F., Mat, Z., Malek, R.A., Hanapi, S.Z., Leng, O.M., Dailin, D.J., Sukmawati, D., 2019. *Pleurotus Ostreatus*: A Biofactory for Lignin-Degrading Enzymes of Diverse Industrial Applications. https://doi.org/10.1007/978-3-030-25506-0_5.

Epelde, L., Becerril, J.M., Alkorta, I., Garbisu, C., 2014a. Adaptive long-term monitoring of soil health in metal phytostabilization: ecological attributes and ecosystem

services based on soil microbial parameters. Int. J. Phytoremediation 16, 971–981. https://doi.org/10.1080/15226514.2013.810578.
Epelde, L., Becerril, J.M., Mijangos, I., Garbisu, C., 2009. Evaluation of the efficiency of a phytostabilization process with biological indicators of soil health. J. Environ. Qual. 38, 2041–2049. https://doi.org/10.2134/jeq2009.0006.
Epelde, L., Burges, A., Mijangos, I., Garbisu, C., 2014b. Microbial properties and attributes of ecological relevance for soil quality monitoring during a chemical stabilization field study. Appl. Soil Ecol. 75, 1–12. https://doi.org/10.1016/j.apsoil.2013.10.003.
Fernández-Fueyo, E., Castanera, R., Ruiz-Dueñas, F.J., López-Lucendo, M.F., Ramírez, L., Pisabarro, A.S.G., Martínez, A.T., 2014. Ligninolytic peroxidase gene expression by Pleurotus ostreatus: differential regulation in lignocellulose medium and effect of temperature and pH. Fungal Genet. Biol. 72, 150–161. https://doi.org/10.1016/j.fgb.2014.02.003.
Field, A., Miles, J., Fiel, Z., 2012. Discovering Statistics Using R. SAGE Publications, London. https://doi.org/10.7326/M16-0968.
Frutos, I., García-Delgado, C., Gárate, A., Eymar, E., 2016. Biosorption of heavy metals by organic carbon from spent mushroom substrates and their raw materials. Int. J. Environ. Sci. Technol. 13, 2713–2720. https://doi.org/10.1007/s13762-016-1100-6.
Galende, M.A., Becerril, J.M., Barrutia, O., Artetxe, U., Garbisu, C., Hernández, A., 2014. Field assessment of the effectiveness of organic amendments for aided phytostabilization of a Pb-Zn contaminated soil. J. Geochem. Explor. 145, 181–189. https://doi.org/10.1016/j.gexplo.2014.06.006.
Garbisu, C., Alkorta, I., 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour. Technol. 77, 229–236. https://doi.org/10.1016/S0960-8524(00)00108-5.
García-Delgado, C., D'Annibale, A., Pesciaroli, L., Yunta, F., Crognale, S., Petruccioli, M., Eymar, E., 2015a. Implications of polluted soil biostimulation and bioaugmentation with spent mushroom substrate (*Agaricus bisporus*) on the microbial community and polycyclic aromatic hydrocarbons biodegradation. Sci. Total Environ. 508, 20–28. https://doi.org/10.1016/j.scitotenv.2014.11.046.
García-Delgado, C., Jiménez-Ayuso, N., Frutos, I., Gárate, A., Eymar, E., 2013. Cadmium and lead bioavailability and their effects on polycyclic aromatic hydrocarbons biodegradation by spent mushroom substrate. Environ. Sci. Pollut. Res. 20, 8690–8699. https://doi.org/10.1007/s11356-013-1829-0.
García-Delgado, C., Yunta, F., Eymar, E., 2015b. Bioremediation of multi-polluted soil by spent mushroom (*Agaricus bisporus*) substrate: polycyclic aromatic hydrocarbons degradation and Pb availability. J. Hazard Mater. 300, 281–288. https://doi.org/10.1016/j.jhazmat.2015.07.008.
Giardina, P., Autore, F., Faraco, V., Festa, G., Palmieri, G., Piscitelli, A., Sannia, G., 2007. Structural characterizzation of heterodimeric laccases from *Pleurotus ostreatus*. Appl. Microbiol. Biotechnol. 75, 1293–1300. https://doi.org/10.1007/s00253-007-0954-4.
Gil, F.H., 2011. Utilización del SPCH en agricultura. Manual de buenas prácticas agrícolas para su uso como fertilizante.
Gómez-Sagasti, M.T., Hernández, A., Artetxe, U., Garbisu, C., Becerril, J.M., 2018. How valuable are organic amendments as tools for the phytomanagement of degraded soils? The knowns, known unknowns, and unknowns. Front. Sustain. Food Syst. 2, 68. https://doi.org/10.3389/fsufs.2018.00068.
Grimm, D., Wösten, H.A.B., 2018. Mushroom cultivation in the circular economy. Appl. Microbiol. Biotechnol. 102, 7795–7803. https://doi.org/10.1007/s00253-018-9226-8.
Hernández-Allica, J., Becerril, J.M., Garbisu, C., 2008. Assessment of the phytoextraction potential of high biomass crop plants. Environ. Pollut. 152, 32–40. https://doi.org/10.1016/j.envpol.2007.06.002.
Hidalgo, J., Anza, M., Epelde, L., Becerril, J.M., Garbisu, C., 2022. Zero-valent iron nanoparticles and organic amendment assisted rhizoremediation of mixed contaminated soil using *Brassica napus*. Environ. Technol. Innov. 28, 102621 https://doi.org/10.1016/j.eti.2022.102621.
Ho, L.-H., Zulkifli, N.A., Tan, T.-C., 2020. Edible mushroom: nutritional properties, potential nutraceutical values, and its utilisation in food product development. In: Passari, A.K., Sánchez, S. (Eds.), An Introduction to Mushroom. IntechOpen, p. 19. https://doi.org/10.5772/intechopen.91827.
Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., van Vark, W., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Commun. Soil Sci. Plant Anal. 31, 1299–1396. https://doi.org/10.1080/00103620009370514.
ISO, 1995. Soil Quality — Determination of Organic and Total Carbon after Dry Combustion (Elementary Analysis), 10694.
ISO, 1998. Soil Quality — Determination of Total Nitrogen Content by Dry Combustion ("elemental Analysis"), 13878.
ISO, 2002. Soil Quality – Laboratory Methods for Determination of Microbial Soil Respiration, 16072.
ISO/TS 22939, 2010. Soil Quality — Measurement of Enzyme Activity Patterns in Soil Samples Using Fluorogenic Substrates in Micro-well Plates.
Jaiswal, S., Singh, D.K., Shukla, P., 2022. Lindane bioremediation by *Paenibacillus dendritiformis* SJPS-4, its metabolic pathway analysis and functional gene annotation. Environ. Technol. Innov. 27, 102433 https://doi.org/10.1016/j.eti.2022.102433.
Kapahi, M., Sachdeva, S., 2017. Mycoremediation potential of *Pleurotus* species for heavy metals: a review. Bioresour. Bioprocess. 4 https://doi.org/10.1186/s40643-017-0162-8.
Kulshreshtha, S., 2018. Mushroom biomass and spent mushroom substrate as adsorbent to remove pollutants. https://doi.org/10.1007/978-3-319-92162-4_9.
Kulshreshtha, S., Mathur, N., Bhatnagar, P., 2014. Mushroom as a product and their role in mycoremediation. Amb. Express 4, 1–7. https://doi.org/10.1186/s13568-014-0029-8.
Kumar, P.B.A.N., Dushenkov, V., Motto, H., Raskin, I., 1995. Phytoextraction: the use of plants to remove heavy metals from soils. Environ. Sci. Technol. 29, 1232–1238. https://doi.org/10.1021/es00005a014.
Lal, R., Pandey, G., Sharma, P., Kumari, K., Malhotra, S., Pandey, R., Raina, V., Kohler, H.-P.E., Holliger, C., Jackson, C., Oakeshott, J.G., 2010. Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. Microbiol. Mol. Biol. Rev. 74, 58–80. https://doi.org/10.1128/mmbr.00029-09.
Lampis, S., Ferrari, A., Cristina, A., Cunha-Queda, A.C.F., Alvarenga, P., Di Gregorio, S., Vallini, G., 2009. Selenite resistant rhizobacteria stimulate SeO_3^{2-} phytoextraction by *Brassica juncea* in bioaugmented water-filtering artificial beds. Environ. Sci. Pollut. Res. 16, 663–670. https://doi.org/10.1007/s11356-008-0088-y.
Langer, G.J., Bubkamp, J., Terhonen, E., Blumenstein, K., 2021. Chapter 10 –Fungi inhabiting woody tree tissues. In: Asiegbu, F.O., Kovalchuk, A. (Eds.), Forest Microbiology. Academic Press, pp. 175–205. https://doi.org/10.1016/B978-0-12-822542-4.00012-7.
Lankinen, V.P., Bonnen, A.M., Anton, L.H., Wood, D.A., Kalkkinen, N., Hatakka, A., Thurston, C.F., 2001. Characteristics and N-terminal amino acid sequence of manganese peroxidase from solid substrate cultures of *Agaricus bisporus*. Appl. Microbiol. Biotechnol. 55, 170–176.
Lanzén, A., Epelde, L., Blanco, F., Martin, I., Artetxe, U., Garbisu, C., 2016. Multi-targeted metagenetic analysis of the influence of climate and environmental parameters on soil microbial communities along an elevational gradient. Sci. Rep. 6, 1–13. https://doi.org/10.1038/srep28257.
Lehmann, J., Bossio, D.A., Kögel-Knabner, I., Rillig, M.C., 2020. The concept and future prospects of soil health. Nat. Rev. Earth Environ. 1, 544–553. https://doi.org/10.1038/s43017-020-0080-8.
Liu, X., Bai, X., Dong, L., Liang, J., Jin, Y., Wei, Y., Li, Y., Huang, S., Qu, J., 2018. Composting enhances the removal of lead ions in aqueous solution by spent mushroom substrate: biosorption and precipitation. J. Clean. Prod. 200, 1–11. https://doi.org/10.1016/j.jclepro.2018.07.182.
Mapa, 1994. Métodos oficiales de análisis de suelos y aguas para riego. In: Ministerio de Agricultura, Pesca y Alimentación. Métodos Oficiales de Análisis, vol. III.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12.
Morya, R., Kumar, M., Singh, S.S., Thakur, I.S., 2019. Genomic analysis of *Burkholderia* sp. ISTR5 for biofunneling of lignin-derived compounds. Biotechnol. Biofuels 12, 1–14. https://doi.org/10.1186/s13068-019-1606-5.
Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Methods of Soil Analysis, Part 3: Chemical Methods, pp. 961–1010. https://doi.org/10.2136/sssabookser5.3.c34.
Neugschwandtner, R.W., Tlustoš, P., Komárek, M., Száková, J., 2008. Phytoextraction of Pb and Cd from a contaminated agricultural soil using different EDTA application regimes: laboratory versus field scale measures of efficiency. Geoderma 144, 446–454. https://doi.org/10.1016/j.geoderma.2007.11.021.
Noguchi, A., Kageyama, M., Shinmachi, F., Schmidhalter, U., Hasegawa, I., 2005. Potential for using plant xylem sap to evaluate inorganic nutrient availability in soil: II. comparison between the xylem sap method and the extraction method. Soil Sci. Plant Nutr. 51, 343–350. https://doi.org/10.1111/j.1747-0765.2005.tb00039.x.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, J.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2015. Vegan: Community Ecology Package. R Package Version 2.3-1.
Papadopoulou, K., Rigas, F., Doulia, D., 2006. Lindane degradation in soil *by Pleurotus ostreatus*. WSEAS Trans. Environ. Dev. 2, 489–496.
Phan, C.W., Sabaratnam, V., 2012. Potential uses of spent mushroom substrate and its associated lignocellulosic enzymes. Appl. Microbiol. Biotechnol. 96, 863–873. https://doi.org/10.1007/s00253-012-4446-9.
Piscitelli, A., Giardina, P., Mazzoni, C., Sannia, G., 2005. Recombinant expression of *Pleurotus ostreatus* laccases in *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 69, 428–439. https://doi.org/10.1007/s00253-005-0004-z.
Polti, M.A., Aparicio, J.D., Benimeli, C.S., Amoroso, M.J., 2014. Simultaneous bioremediation of Cr(VI) and lindane in soil by actinobacteria. Int. Biodeterior. Biodegrad. 88, 48–55. https://doi.org/10.1016/j.ibiod.2013.12.004.
Pozdnyakova, N., Dubrovskaya, E., Chernyshova, M., Makarov, O., Golubev, S., Balandina, S., Turkovskaya, O., 2018. The degradation of three-ringed polycyclic aromatic hydrocarbons by wood-inhabiting fungus *Pleurotus ostreatus* and soil-inhabiting fungus *Agaricus bisporus*. Fungal Biol 122, 363–372. https://doi.org/10.1016/j.funbio.2018.02.007.
Raj, D.D., Mohan, B., Vidya Shetty, B.M., 2011. Mushrooms in the remediation of heavy metals from soil. Int. J. Environ. Pollut Control Manag. 3, 89–101.
Reddy, C.A., 1995. The potential for white-rot fungi in the treatment of pollutants. Curr. Opin. Biotechnol. 6, 320–328. https://doi.org/10.1016/0958-1669(95)80054-9.
Rigas, F., Papadopoulou, K., Philippoussis, A., Papadopoulou, M., Chatzipavlidis, J., 2009. Bioremediation of lindane contaminated soil by *Pleurotus ostreatus* in non sterile conditions using multilevel factorial design. Water Air Soil Pollut. 197, 121–129. https://doi.org/10.1007/s11270-008-9795-8.
Sadañoski, M.A., Tatarin, A.S., Barchuk, M.L., Gonzalez, M., Pegoraro, C.N., Fonseca, M. I., Levin, L.N., Villalba, L.L., 2020. Evaluation of bioremediation strategies for treating recalcitrant halo-organic pollutants in soil environments. Ecotoxicol. Environ. Saf. 202 https://doi.org/10.1016/j.ecoenv.2020.110929.
Sehube, N., Kelebemang, R., Totolo, O., Laetsang, M., Kamwi, O., Dinake, P., 2017. Lead pollution of shooting range soils. S. Afr. J. Chem. 70, 21–28. https://doi.org/10.17159/0379-4350/2017/v70a4.
Salman, M., Athar, M., Farooq, U., 2015. Biosorption of heavy metals from aqueous solutions using indigenous and modified lignocellulosic materials. Rev. Environ. Sci. Biotechnol. 14, 211–228. https://doi.org/10.1007/s11157-015-9362-x.

Singh, H., 2006. Mycoremediation: Fungal Bioremediation. Jonh Wiley & Sons, Inc, Hoboken, New Jersey. https://doi.org/10.1002/0470050594.

Singh, A., Fulekar, M.H., 2012. Phytoremediation of heavy metals by *Brassica juncea* in aquatic and terrestrial environment. In: Anjum, N., Ahmad, I., Pereira, M., Duarte, A., Umar, S., Khan, N. (Eds.), The Plant Family Brassicaceae. Environ. Pollut, vol. 21. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-3913-0_6.

Ten Have, R., Teunissen, P.J., 2001. Oxidative mechanisms involved in lignin degradation by white-rot fungi. Chem. Rev. 101, 3397–3414. https://doi.org/10.1021/cr000115l.

Ten Have, R., Wijngaard, H., Ariës-Kronenburg, N.A.E., Straatsma, G., Schaap, P.J., 2003. Lignin degradation by *Agaricus bisporus* accounts for a 30 increase in bioavailable holocellulose during cultivation on compost. J. Agric. Food Chem. 51, 2242–2245. https://doi.org/10.1021/jf021131h.

Turan, M., A Esringü, A., 2007. Phytoremediation based on canola (*Brassica napus* L.) and Indian mustard (*Brassica juncea* L.) planted on spiked soil by aliquot amount of Cd, Cu, Pb, and Zn. Plant Soil Environ. 53, 7–15.

Urra, J., Alkorta, I., Mijangos, I., Epelde, L., Garbisu, C., 2019. Application of sewage sludge to agricultural soil increases the abundance of antibiotic resistance genes without altering the composition of prokaryotic communities. Sci. Total Environ. 647, 1410–1420. https://doi.org/10.1016/j.scitotenv.2018.08.092.

Van Ginneken, L., Meers, E., Guisson, R., Ruttens, A., Elst, K., Tack, F.M.G., Vangronsveld, J., Diels, L., Dejonghe, W., 2007. Phytoremediation for heavy metal-contaminated soils combined with bioenergy production. J. Environ. Eng. Landsc. Manag. 15, 227–236. https://doi.org/10.1080/16486897.2007.9636935.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Wang, J., Tang, K., Hu, X., Wang, H., Gudda, F.O., Odinga, E.S., El-Ramady, H., Ling, W., 2022. Impact of hexachlorocyclohexane addition on the composition and potential functions of the bacterial community in red and purple paddy soil. Environ. Pollut. 297, 118795 https://doi.org/10.1016/j.envpol.2022.118795.

Watanabe, F.S., Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. J. 26, 677–678. https://doi.org/10.2136/sssaj1965.03615995002900060025x.

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction: an automated procedure. Soil Biol. Biochem. 22, 1167–1169. https://doi.org/10.1016/0038-0717(90)90046-3.

Xu, W., Pang, K.L., Luo, Z.H., 2014. High fungal diversity and abundance recovered in the deep-sea sediments of the Pacific ocean. Microb. Ecol. 68, 688–698. https://doi.org/10.1007/s00248-014-0448-8.

Yang, S., Hai, F.I., Nghiem, L.D., Price, W.E., Roddick, F., Moreira, M.T., Magram, S.F., 2013. Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: a critical review. Bioresour. Technol. 141, 97–108. https://doi.org/10.1016/j.biortech.2013.01.173.

Zhang, W., Cai, Y., Tu, C., Ma, L.Q., 2002. Arsenic speciation and distribution in an arsenic hyperaccumulating plant. Sci. Total Environ. 300, 167–177. https://doi.org/10.1016/S0048-9697(02)00165-1.

Zhao, F.J., Mcgrath, S.P., Crosland, A.R., 1994. Comparison of three wet digestion methods for the determination of plant sulphur by inductively coupled plasma atomic emission spectroscopy (ICP- AES). Commun. Soil Sci. Plant Anal. 25, 407–418. https://doi.org/10.1080/00103629409369047.

Zied, D.C., Sánchez, J.E., Noble, R., Pardo-Giménez, A., 2020. Use of spent mushroom substrate in new mushroom crops to promote the transition towards a circular economy. Agronomy 10, 1–20. https://doi.org/10.3390/agronomy10091239.