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Mycoremediation of Soils Polluted with Trichloroethylene: First Evidence of *Pleurotus* Genus Effectiveness

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Abstract: Trichloroethylene (TCE) is a proven carcinogenic chlorinated organic compound widely used as a solvent in industrial cleaning solutions; it is easily found in the soil, air, and water and is a hazardous environmental pollutant. Most studies have attempted to remove TCE from air and water using different anaerobic bacteria species. In addition, a few have used white-rot fungi, although there are hardly any in soil. The objective of the present work is to assess TCE removal efficiency using two species of the genus *Pleurotus* that have not been tested before: *Pleurotus ostreatus* and *Pleurotus eryngii*, growing on a sandy loam soil. These fungi presented different intra- and extracellular enzymatic systems (chytochrome P450 (CYP450), laccase, Mn peroxidase (MnP)) capable of aerobically degrading TCE to less harmful compounds. The potential toxicity of TCE to *P. ostreatus* and *P. eryngii* was firstly tested in a TCE-spiked liquid broth (70 mg L^{-1} and 140 mg L^{-1}) for 14 days. Then, both fungi were assessed for their ability to degrade the pollutant in sandy loam soil spiked with 140 mg kg^{-1} of TCE. *P. ostreatus* and *P. eryngii* improved the natural dissipation of TCE from soil by 44%. Extracellular enzymes were poorly expressed, but mainly in the presence of the contaminant, in accordance with the hypothesis of the involvement of CYP450.

Keywords: trichloroethylene; mycoremediation; *Pleurotus*; bioremediation; biodegradation; pollution

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1. Introduction

Healthy soils are essential to support an environment that provides a safe habitat for the worldwide human population. However, in reality contaminated soils are more and more frequently found, representing one of the core problems we face today [1]. Trichloroethylene (TCE) is a volatile chlorinated aliphatic hydrocarbon with limited solubility in water (~1 g L^{-1}), but it is miscible with ethanol, ether, and chloroform. Its log Kow is 2.29 [2] and it is considered to be non-polar. It is widely used as a solvent in industrial cleaning solutions. Once the solvent has been used, it is often spilled onto soil or placed in metal containers that leak into the soil, leaching TCE down to the groundwater [3]. In addition to its ubiquity in the environment, TCE is one of the United States Environmental Protection Agency´s (US EPA) 126 priority pollutants for control [4]. It was re-evaluated in 2012 by the International Agency for Research on Cancer (IARC), and was classified as a Group 1 carcinogen to humans. The reclassification was based on epidemiological evidence with regard to its involvement in cancer of the kidney, with strong support from studies using experimental animals and human exposure to TCE [5].

Several researchers have recorded the negative impact of TCE on the metabolism of soil microbiota. They have demonstrated that the presence of TCE reduces the ability

of soil microorganisms to obtain nitrogen, thereby affecting the soil nitrogen cycle. It was concluded that concentrations of 20 mg kg^{-1} TCE inhibited the energy metabolism of the microbiota, as well as the metabolism of other substances such as xenobiotics in the soil [6]. Moreno et al. [7] reported that TCE negatively affected some soil-enzyme activities, for example with regard to dehydrogenase, β-glucosidase, phosphatase, urease, and o-diphenol oxidase. Thus, the safe removal of TCE from soils represents a challenge that should be approached from various perspectives. Abiotic approaches were first employed, followed by a wide range of types of biotic remediation using microorganisms as well as plants. Among the abiotic methods, Fenton's reagent (H_2O_2 + Fe^{2+}), O_3/UV, O_3/H_2O_2, and photocatalysis with the mediation of the hydroxyl radical resulted in the oxidation of TCE. These procedures were successfully used to remediate contaminated water and soil [8–10]. Furthermore, some researchers have induced the production of the hydroxyl radical using the extracellular enzymes of ligninolytic fungi such as *Pleurotus eryngii* [11] or *Trametes versicolor* to degrade TCE with the concomitant dechlorination of the compound [12]. Most TCE biodegradation studies have been aimed at the reductive dechlorination by anaerobic bacteria, including *Dehalospirillium multivorans* [13], *Dehalobacter restrictus* [14], and *Dehalococcoides ethenogenes* [15]. This process most often causes the accumulation of less chlorinated but more toxic compounds in the environment such as cis-1,2-dichloroethylene and some well-known carcinogenic intermediates such as vinyl chloride. The aerobic degradation of TCE by bacteria has been investigated resulting in the formation of a range of intermediates such as chloral, formate, glyoxylate, dichloroacetate, and CO [16]. Co-metabolic biodegradation of TCE, reported through degradation by bacteria as a collateral effect (such as in the case of methanotrophic bacteria) has also been reported [17].

Compared with most bacterial degradative enzymes, the intra- and extracellular enzymatic systems of white rot fungi (WRF) enable them to degrade several classes of organic pollutants such as polychlorinated biphenyls [18], polycyclic aromatic hydrocarbons (PAHs) [19,20], endocrine disruptors such as bisphenol A, 17 β-estradiol, or triclosan (among others) [21] and a wide variety of pharmaceutical compounds [22–25]. The ability of some ligninolytic fungi to degrade TCE in a liquid medium yielding less toxic intermediates than bacteria has been investigated in addition to their ability to mineralize it, as shown by research using *T. versicolor* [26], *Phanerochaete chrysosporium* [27], *Ganoderma lucidum*, and *Irpex lacteus* [28]. Thus, mycoaugmentation using WRF constitutes an interesting strategy for the treatment of contaminated soils. Their bioremediation potential is based on their growth habit of producing hyphal extensions and forming mycelial cords which are the main pathways for water and nutrient translocation [29]. These mycelial cords provide them with the advantage of being able to penetrate and grow across spatially heterogeneous soil. In addition, the secretion of oxidative ligninolytic enzymes, characterized by their low substrate specificity, enables them to degrade a wide range of organic pollutants. However, factors such as fungal competition with autochthonous soil microflora for nutrients and space are crucial to ensuring the successful mycodegradation of TCE. Some studies have shown that WRF can survive in soil and skillfully colonize it using the available lignocellulosic materials and other substrates present, because unlike bacteria, WRF can grow through areas with scarce nutrients by means of nutrient transport along their mycelial hyphae [30]. In addition, some researchers have demonstrated the ability of WRF to compete with complex soil microbiota [31], as well as to successfully remove several of the organic pollutants described above.

The starting hypothesis was that *P. ostreatus* and *P. eryngii* might be able to grow in the presence of TCE and effectively degrade it, as other WRF have been shown to do [25–27]. Therefore, the aim of this work was to assess the ability of two species of the genus *Pleurotus*, *P. ostreatus*, and *P. eryngii*, to remove TCE from soil, which to our knowledge has not been evaluated before. Firstly, both species were tested to see whether they could grow at high concentrations of TCE in a liquid broth, and then to evaluate the removal of TCE from a soil

spiked with TCE at double the threshold concentration for industrial soils (140 mg kg^{-1}) according to Spanish legislation [32].

2. Materials and Methods

2.1. Chemicals and Fungal Material

Trichloroethylene was purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium acetate, malic acid, $MnSO_4 \cdot H_2O$ 98%, and H_2O_2 33% were purchased from PanReac (Barcelona, Spain). Dimethyl sulfoxide, 2,6-dimethoxy phenol, malt extract, and malt extract agar (MEA) were purchased from Sigma Aldrich (St. Louis, MO, USA). All chemical reagents were of analytical grade and the solvents were of HPLC grade.

The strain of *P. ostreatus* was isolated from spent mushroom substrate collected from a commercial mushroom cultivation farm in Quintanar del Rey (Cuenca, Spain). This strain had been successfully tested for the degradation of PAHs and sulfonamide antibiotics in previous research [19,22]. *P. eryngii* was purchased from Gurelan Mycelium located in Huarte (Navarra, Spain). The fungi were maintained on malt extract agar (MEA) plates at 4 °C and sub-cultured every 28 days.

2.2. Soil

A 50-kg soil sample was collected from Burgos, Spain (42°20′27.8″ N 3°42.11′ O) The soil was homogenized, air-dried at room temperature, and finally passed through a 2-mm-sieve. The main results of the characterization of the soil are summarized in Table 1. According to the U.S. textural classification, the soil was classified as a sandy loam soil comprising clay (8%), loam (29%), and sand (63%). Microbiological activity was determined by measuring the total hydrolase [33], urease [34], and dehydrogenase [35] activity (Table 1). The soil was tested prior to the application of TCE to ensure that this pollutant was not present in the soil sample.

Table 1. Chemical and biological characterization of soil. Data present the mean ± standard deviation of three replicated analysis.

Chemical Analysis					Microbial Enzymatic Analysis (U g^{-1})		
pH H_2O	pH KCl	E.C. (µS cm^{-1})	O.M. (%)	N Kjeldhal (%)	Hydrolase	Dehydrogenase	Urease
8.14 ± 0.01	7.3 ± 0.2	449 ± 2	3.1 ± 0.2	0.20 ± 0.03	1.5 ± 0.6	11 ± 1	3142 ± 28

E.C.: Electrical conductivity; O.M.: Organic matter

2.3. Liquid Media Growth Assay

P. ostreatus and *P. eryngii* were inoculated by adding three 5-mm fungal plugs from a 7-day-old malt extract agar (MEA) culture into 500-mL Erlenmeyer flasks with 125 mL of 3% malt extract (ME) in the presence or absence of TCE (14 or 140 mg L^{-1}). The fungi were grown for 14 days at 28 °C under orbital agitation (120 rpm) in the dark. Fungal growth was monitored daily by visual observation. One-milliliter aliquots were sampled every four days to analyze ligninolytic activity (laccase and MnP).

Laccase activity was spectrophotometrically determined by the oxidation of 10 mM 2,6-dimethoxy phenol in 50 mM sodium acetate pH 5.0 at 477 nm (ϵ = 14,600 M^{-1} cm^{-1}) [36]. Mn peroxidase (MnP) activity was assayed by the oxidation of 1 mM $MnSO_4$ in 50 mM sodium malonate buffer (pH 4.5), in the presence of 0.1 mM H_2O_2. Manganic ions, by Mn^{3+} form a complex with malonate, which absorbs at 270 nm (ϵ = 11,590 M^{-1} cm^{-1}) [37]. One unit of enzyme activity (IU) is defined as the amount of enzyme that produces 1 µmol of product per minute under the assay conditions.

At the end of the assay, fungal pellets were studied by scanning electron microscopy (SEM, Hitachi S-3000N, Tokyo, Japan) to evaluate any potential damage to the structure of the hyphae by the presence of TCE in the liquid broth (14 or 140 mg L^{-1}). The fungal pellets were firstly washed in distilled water, then fixed in a modified Karnovsky's fixative consisting of a solution of glutaraldehyde 2.5% in 0.1 M phosphate buffer (PBS), pH 7–7.4

for 24 h, then rinsed three times with PBS for 5 min, and dehydrated by an increasing concentration of ethanol series for 5 min [38] before critical point drying; they were then sputter-coated with gold using a Sputter Coater Quorum, Q150T-S.

2.4. Mycodegradation of TCE in Soil

Three 5-mm fungal plugs from a 7-day-old malt extract agar (MEA) culture were inoculated into autoclaved sterilized spent mushroom substrate in autoclave (SSMS) and grown for two weeks before inoculation into soil. Eighty grams of 2-mm sieved soil were placed in 160-mL glass containers; then 20 g of SSMS-supporting fungi were mixed with the soil. Following this either distilled water as a biotic control or a TCE solution was instilled into the containers, which were sealed immediately, and cultured in a growth chamber at 28 °C in the dark, for four weeks. The final concentration of TCE in soil was 140 mg kg^{-1}. An abiotic control was carried out in the same conditions. The assay was performed in triplicate.

The TCE concentration was determined by static headspace GC coupled with EDC. A 200-mg soil sample from each experimental glass container was transferred to a 10-mL head space vial and 0.5 g NaCl, 5 mL MilliQ water, and 80 µL 2-bromine-1-chloropropane as an internal standard were added. The vials were sealed immediately with a Teflon-coated stopper. The test vial was placed in a headspace sampler Agilent 7694 (Agilent Technologies, Santa Clara, CA) and heated to 70 °C for 40 min. The chromatographic system consisted of an CG module from Perkin-Elmer coupled with an electronic capture detector (Waltham, MA, USA) with a CP-SIL 13 CB capillary column that was 25 m long with a diameter of 0.32 mm and a stationary phase thickness of 0.4 µm (Chrompack 7506). The detector was set at 290 °C, using He as the carrier gas at 14 psi.

Laccase and MnP were extracted from 3 g soil at 5 °C for 1 h using the buffered solution described by D'Annibale et al. [39]; then the aqueous suspension was centrifuged at 6000 g for 30 min, and the supernatant assayed for enzymatic activities as described above.

2.5. Statistical Analysis

Normality and homogeneity of the variances were checked using the Shapiro–Wilk and Levene tests, respectively, prior to one-way analysis of variance (ANOVA). To compare the differences between TCE degradation treatments, the Tukey post hoc test at $p < 0.05$ was used. All statistical tests were carried out using the IBM SPSS Statistics v25 software package.

3. Results

3.1. Liquid Medium Growth Assay

Based on the visual observation of the growth of *P. ostreatus* and *P. eryngii* in the absence or presence of TCE at 14 and 140 mg L^{-1}, both fungi were able to grow normally in the presence of TCE at the two concentrations tested, forming round pellets similar in shape and size to those of the control. Therefore, the presence of TCE at concentrations of 14 and 140 mg L^{-1} in the liquid broth did not seem to be toxic to either *P. ostreatus* or *P. eryngii* at a macrostructural level (Figure 1).

Figure 2 shows the SEM images of *P. ostreatus* and *P. eryngii* in the absence and presence of TCE in the liquid medium. These images illustrate that the two fungi exhibited different effects at micro structural level in the presence of TCE. A morphological analysis of the scanning electron micrographs of *P. ostreatus* showed that their hyphae had been structurally altered to some extent when cultured in the highest concentration (140 mg L^{-1}) of TCE (Figure 2C). The ends of the hyphae appeared to be split and frayed and the hyphae did not maintain the typical tubular structure that can be seen in the control (Figure 2A). In the sample that had been cultured in TCE at 14 mg L^{-1} the hyphae retained the tubular structure, but the hyphal ends also appeared to be broken (Figure 2B). Therefore, although *P. ostreatus* tolerated the presence of TCE up to 140 mg L^{-1} and produced morphologically

similar mycelial pellets at macroscopic level, the structure of hyphae had been damaged by TCE, especially at the highest concentration.

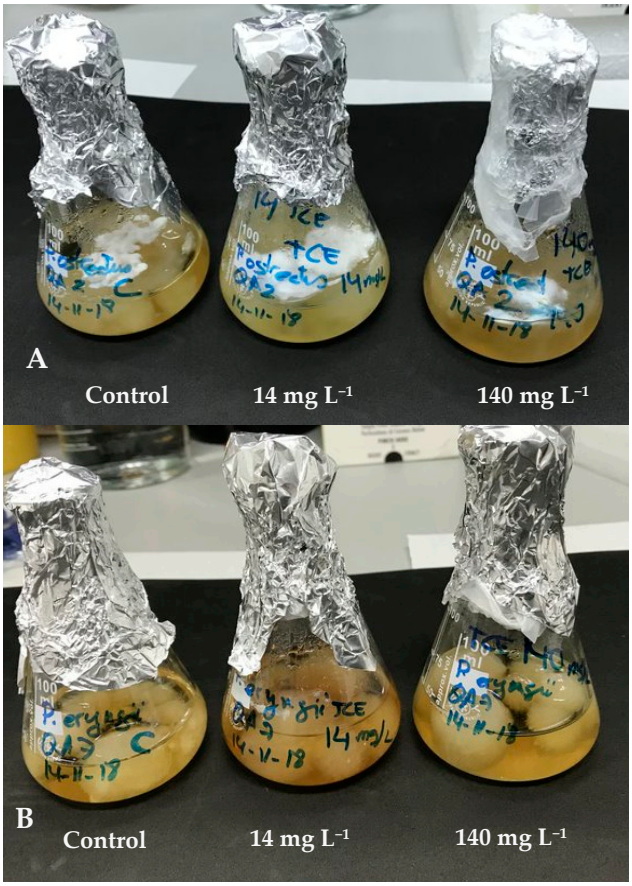

Figure 1. Pellets of *Pleurotus ostreatus* (**A**) and *Pleurotus eryngii* (**B**) growing in liquid broth without (control) and with trichloroethylene (TCE) at 14 and 140 mg L^{-1}.

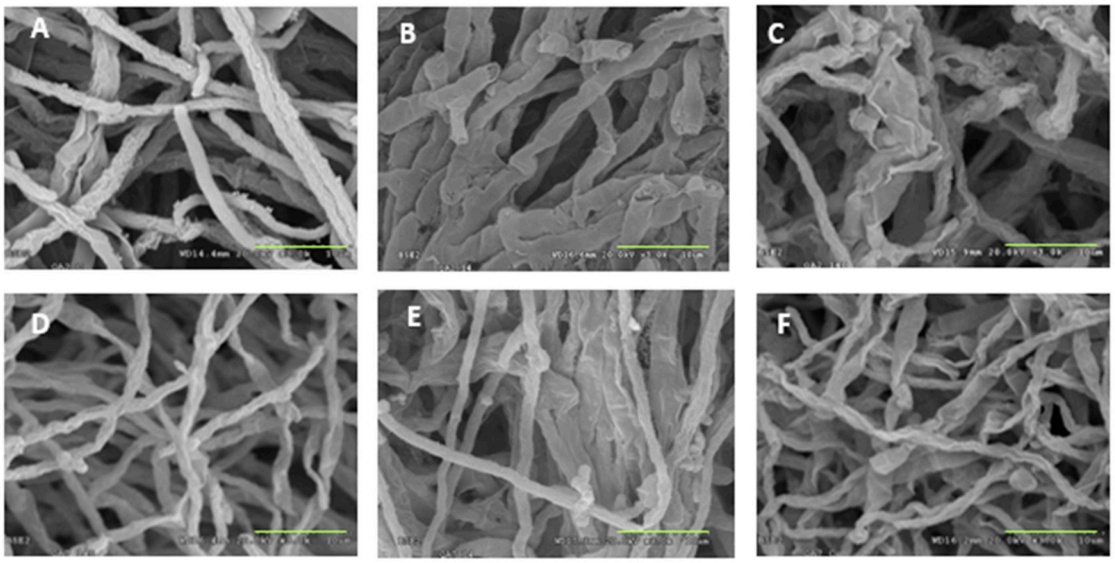

Figure 2. SEM images of *P. ostreatus* (**A**–**C**) and *P. eryngii* (**D**–**F**) grown in malt extract liquid broth for 14 days without (**A**,**D**) and with TCE at 14 mg L^{-1} (**B**,**E**) and 140 mg L^{-1} (**C**,**F**).

On the contrary, the hyphae of *P. eryngii* seemed to maintain their morphological structure and appeared unaffected by the presence of the pollutant at any concentration (Figure 2E,F) exhibiting the same the tubular structure both in the presence or absence of TCE.

The secretion of the extracellular enzymes laccase and MnP are another indication of fungal functioning [40]. Although *P. ostreatus* did not express laccase in the absence of TCE in liquid broth, low levels were detected in the presence of the contaminant (Figure 3A), despite the structural alteration of its hyphae. In addition, MnP was detected in both the absence (from day 9) and presence of TCE (14 mg L^{-1}) (Figure 3A). However, *P. eryngii* expressed laccase and MnP in the absence and presence of TCE (14 and 140 mg L^{-1}) (Figure 3B).

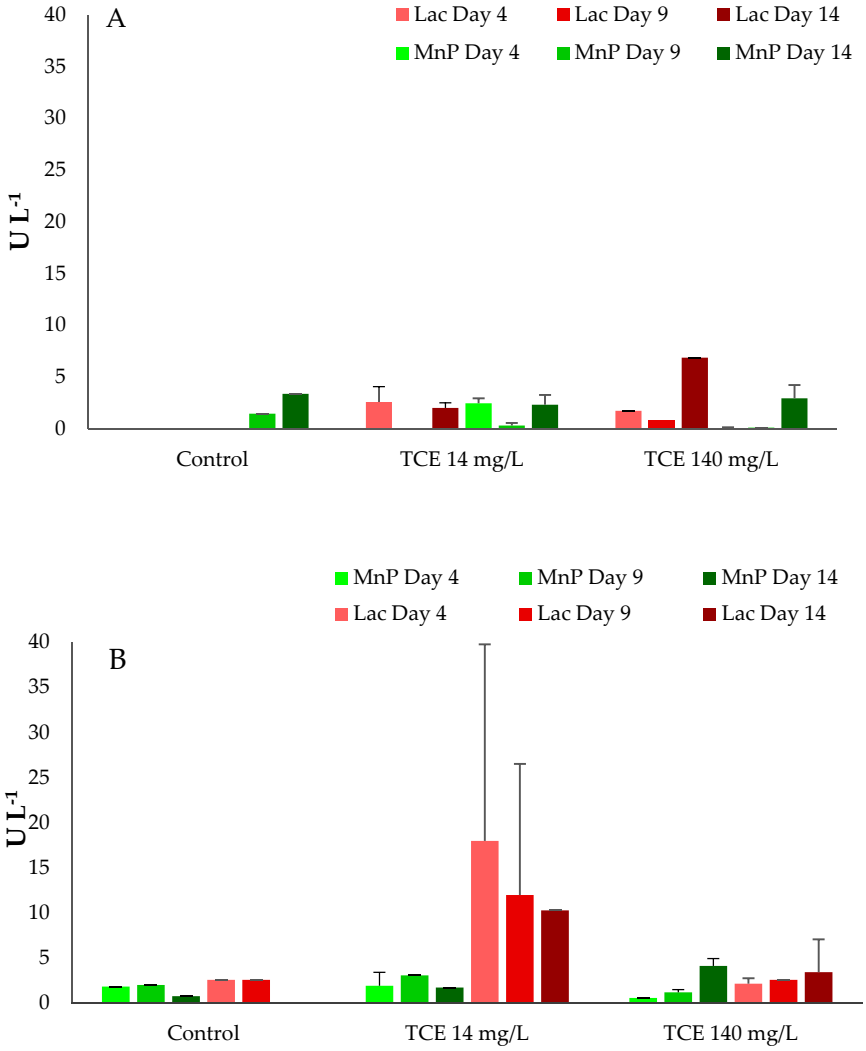

Figure 3. Laccase (Lac) and MnP activity of *P. ostreatus* (**A**) and *P. eryngii* (**B**) in the absence and presence (14 mg kg^{-1} and 140 mg kg^{-1}) of TCE for 14 days in malt extract liquid media. Error bars represent the standard deviation (n = 3).

3.2. Mycoremediation of TCE in Soil

P. ostreatus and *P. eryngii* were able to grow in the soil spiked with TCE at a concentration of 140 mg kg^{-1}, which is twice the threshold concentration for industrial soil and 20 times the threshold concentration for urban soil according to the Spanish legislation [32]. The percentage of TCE removal in the control soil and the two mycoremediation treatments are shown in Figure 4. The removal of TCE followed the same pattern in all the three treatments at the beginning of the assay. However, the percentage of removal was significantly higher ($p < 0.05$) for the two mycoremediation treatments by comparison with

the control after the second week. During the second week of treatment, *P. eryngii* achieved an increment in the removal of TCE of 8% and 32%, respectively, when compared with *P. ostreatus* and the control. Demonstrating that *P. eryngii* was faster at removing TCE than *P. ostreatus*. However, after 2 weeks incubation both species of fungi achieved a percentage removal greater than 70%. The results obtained at the third week of the assay showed the highest efficiency of *P. eryngii* for removing TCE with respect to *P. ostreatus*. At the final sampling time, both species of fungi had achieved 100% TCE removal, demonstrating the high effectivity of the mycoremediation procedure for this contaminant. At this point, the two mycoremediation treatments had increased the removal of TCE by 44% when compared to the control and were statistically significant ($p < 0.05$).

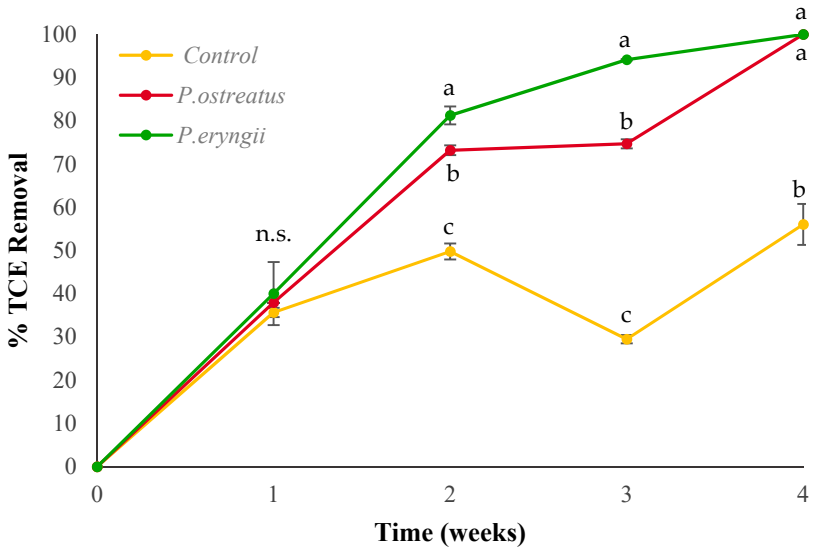

Figure 4. Percentage of TCE removal in polluted soil with TCE at 140 mg kg^{-1} for the control and mycoremediation treatments (*P. ostreatus* and *P. eryngii*). Error bars represent the standard deviation ($n = 3$). Different letters indicate significant differences among treatments at the same sampling time (Tukey post hoc test, $p < 0.05$). n.s.: not significant.

Table 2 shows the ligninolytic activity of the two mycoremediation treatments. Ligninolytic activity was not detected in the control treatment during the assay. By contrast with the liquid media assay, laccase was not detected in either mycoremediation treatments. The unique ligninolytic enzyme detected was MnP. No significant differences ($p > 0.05$) were found between *P. ostreatus* and *P. eryngii* during the four weeks of incubation.

Table 2. MnP (U kg^{-1}) activity in TCE-polluted soil with mycoremediation using *P. ostreatus* and *P. eryngii*. Data present the mean ± standard deviation of three replicates.

	Week 1	Week 2	Week 3	Week 4
P. ostreatus	12.8 ± 6.8	2.6 ± 1.5	4.7 ± 2.9	3.1 ± 1.9
P. eryngii	10.5 ± 4.8	n.d.	4.2 ± 1.5	2.4 ± 0.5

n.d.: not detected.

4. Discussion

4.1. Liquid Medium Growth Assay

The growth and activity of *P. ostreatus* and *P. eryngii* in the presence of TCE in liquid broth were evaluated as preliminary step in the assessment of the removal of TCE from soil. The results showed that both species of *Pleurotus* tolerated and grew in the presence of the highest concentration of TCE (140 mg L^{-1}) and developed functional pellets visually similar in shape and size to those in the control (Figure 1). Other researchers have reported that some species of ligninolytic fungi were not affected by the presence of TCE, such as the

case of *T. versicolor* (2–20 mg L^{-1}) [26], *P. chrysosporium* (10–75 mg L^{-1}) [27], *G. lucidum* and *I. lacteus* (5 and 10 mg L^{-1}) [28]. However, the concentration evaluated in this work was much higher, reaching 140 mg L^{-1}. Therefore, the results of this first test in liquid culture were promising because the two fungi were able to grow at a very high concentration of TCE. However, the observations carried out at a microscopical level using SEM showed that the hyphae of *P. ostreatus* appeared structurally affected by the presence of TCE at 140 mg L^{-1}, exhibiting hyphae with morphologically altered tips and a loss of their tubular structure. However, despite this they did not appear to have lost the ability to function properly, as was evaluated by the production of extracellular enzymes (Figure 3A). Yadav et al. [27] reported a slight inhibition of the growth of *P. chrysosporium* at TCE 100 mg L^{-1}. It would therefore appear that a high concentration of TCE produces toxic effects toward some white rot fungi. By contrast, the presence of this contaminant did not produce these effects on the structure of *P. eryngii* at any of the concentrations used (Figure 2). *P. eryngii* secreted higher levels of extracellular laccase and MnP than *P. ostreatus*. Laccase was not detected in the control by *P. ostreatus.* The highest levels of both laccase and MnP were produced in the presence of TCE 140 mg L^{-1} at day 14. The production of laccase by the two *Pleurotus* species seemed to be induced by the presence of TCE. This finding is in accordance with those of Marco-Urrea et al. [26], who stated that TCE could induce the production of laccase in *T. versicolor* because the laccase activity appeared to increase with increased initial TCE concentrations. Furthermore, the fungal growth did not seem to be greatly affected by the concentration of the pollutant, as the mycelial dry weight was only 15% lower in the highest TCE concentration treatment (20 mg L^{-1}). However, the fact that laccase was produced in the presence of TCE did not mean the two *Pleurotus* species would be able to degrade it. For example, *P. chrysosporium* produced ligninolytic enzymes in the presence of TCE, but these were not involved in the degradation of this contaminant. The highest rates of degradation were obtained when *P. chrysosporium* was cultured in a high N medium, which is well known for suppressing ligninolytic enzyme production [27]. Similarly, Marco-Urrea et al. [26] did not observe TCE removal by laccase. They reported that the main enzymatic system involved in the degradation of TCE was the cytochrome P450, and that degradation was favored by high levels of oxygen and glucose [28]. The resulting byproducts were 2,2,2-trichloroethanol and CO_2, which are less toxic than cis-1,2-dichloroethene and vinyl chloride, which are produced in bacterial systems under anaerobic conditions.

4.2. Mycoremediation of TCE in Soil

To the best of our knowledge, all the prior research carried out on TCE degradation by fungi has been performed using liquid broth. Therefore, this work is unique because neither *P. ostreatus* nor *P. eryngii* or any other ligninolytic fungi have been assessed for their ability to degrade TCE in soil.

The characteristics of the soil may have had an influence on fungal development as well as on the fate of the contaminant. One of the most important parameters that influences the fate of TCE is the presence of organic matter (O.M.). If this content is high enough it facilitates the adsorption of higher concentrations of the pollutant. In addition, a high O.M. supports a greater population of autochthonous microbiota which will compete for space and nutrients with the allochthonous fungi. The O.M. content of the soil used in this investigation was 3.1 ± 0.2%, which supported a population of autochthonous microbiota that was estimated by the analysis of the activity of three enzymes: hydrolase, dehydrogenase, and urease. Microbial decomposing activity was determined through the activity of hydrolase, which provides a good estimate of total microbial activity [33], as well as dehydrogenase, another indicator of the general activity of the microbial community in soil [35]. Finally, urease activity was used to estimate the hydrolysis of the urea present in soil. The activity of the three enzymes indicated that the initial soil showed appreciable microbial activity; it was therefore evident that *P. ostreatus* and *P. eryngii* had strong competition from the autochthonous microbiota. Under these conditions, the two fungi were able to completely

remove the TCE after 4 weeks of incubation and clearly demonstrated a faster removal of TCE when compared with the control soil. The speed of removal by the two fungi and the control soil was similar after the first week of incubation. At this stage, the fungi were in the initial stages of growth and the colonization of the soil had not been completed. Hence, the removal of TCE was mainly biologically mediated by the activity of the autochthonous microbiota in addition to the abiotic transformation which might occur via two different pathways: one performed by elimination, yielding 1,1-dichloroethene, and the other via hydrolysis producing acetate [41]. TCE produces negative effects on the Actinobacteria population in soil, with activity such as nitrogen transformation. However, the negative impact of TCE on the fungal population is either very low or may even enhance fungal development in the long term [6]. This research demonstrates the greater degradation of TCE obtained by the two mycoremediation treatments with respect to natural attenuation (the control). Furthermore, the greater removal of TCE, and the higher slope shown in Figure 4 between the first and second week of incubation clearly indicate the important role of both *Pleurotus* species in TCE removal. During this period, the removal speeds of TCE were 2.82, 7.05, and 8.24 mg TCE kg^{-1} day^{-1} for the control, *P. ostreatus*, and *P. eryngii*, respectively. This time also represented the incubation period when TCE removal was at its greatest, with both mycoremediation treatments achieving levels of TCE removal greater than 70%. By contrast, during the final two weeks of incubation, the removal of TCE was reduced, with an increment of only 6.3% observed in the control treatment when compared with week 2. However, during the same period, the two test mycoremediation treatments were once again more efficient than the control, removing 26.8% and 18.7% of TCE for *P. ostreatus* and *P. eryngii*, respectively. Therefore, the use of mycoremediation for the removal of TCE in soil offers clear advantages with respect to autochthonous soil microbiota. The rate of TCE removal in soil by the two *Pleurotus* species was comparable to that of anaerobic degradation by bacteria in soil that ranged between 60% and 99%, as summarized by Shukla et al. [17]. Although both fungi were effective at removing TCE from soil, *P. eryngii* was demonstrably faster than *P. ostreatus* in this process.

5. Conclusions

This work provides the first evidence of the effectiveness of mycoremediation for the removal of TCE from soil. The two fungi assessed in this work, *P. ostreatus* and *P. eryngii*, were able to grow and produce ligninolytic enzymes in the presence of a very high concentration of TCE in both liquid broth and soil. Both fungi colonized soils with TCE concentrations that were twice the maximum amounts allowed for industrial use according to Spanish regulations. The two fungi clearly enhanced the TCE dissipation in the soil produced by autochthonous microbiota and caused the complete removal of TCE. However, more research should be done to clarify the complex factors and mechanisms that influence the mycodegradation of TCE in soil, which is probably one of the most difficult environmental matrices from which to clean pollutants.

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