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Revising the role of belowground fungi in pentachlorophenol pollution management: insights from Tunisian cork oak forests

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Abstract. This summarizes the doctoral thesis "Belowground fungi are key sentinels in forest soils vulnerable to pentachlorophenol pollution: a mechanistic study in *Quercus suber* forests". The PhD degree was awarded by ITQB NOVA in March 2018. It comprises a revision of the major issues, objectives, methodology and results, as well as a few possible recommendations for future work on bioremediation of halogenated aromatic pollutants. The working hypothesis of the thesis proposed that soils in Tunisian cork oak forests are likely contaminated with pentachlorophenol (PCP), a persistent organic pollutant (POP). This hypothesis is based on frequent reports of cork contamination with pentachloroanisole (PCA), which is likely formed through microbial conversion of PCP. Furthermore, PCP was considered a suitable model for investigating the role of soil mycobiota in pollution management due to its ability to travel long distances in the atmosphere, partition favourably into the soil layer, and moderate abiotic resistance, besides its life-threatening toxicity. Finally, fungi play a key role in colonizing soil in cork oak forests. These concepts have been

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applied in the "Preventive and remediation strategies for continuous elimination of polychlorinated phenols from forest soil" project (S*f*P-NATO 981674, 2006-2011). Overall the major findings of the study include the demonstration of the prevalence of PCP in the soils of Tunisian cork oak forests. Additionally, the study elucidated both the significance and impact of fungal activity in the mitigation and dispersion of PCP.
Keywords: Cork oak forest soil; pentachlorophenol; fungal communities; cultivable fungal strains; resorcinol, hydroquinone and catechol degradation branches.

Análise do papel dos fungos do solo na gestão da poluição por pentaclorofenol: perspetivas das florestas de sobreiro na Tunísia

Sumário. Este trabalho é o resumo da tese de doutoramento "Os fungos do solo são sentinelas-chave nos solos florestais vulneráveis à poluição por pentaclorofenol: um estudo em florestas de Montado, *Quercus suber*". O grau de doutor foi concedido pelo ITQB NOVA em março de 2018. Inclui uma revisão dos principais problemas, objetivos, metodologia e resultados, bem como algumas recomendações possíveis para trabalhos futuros sobre biorremediação de poluentes aromáticos halogenados.
A hipótese de trabalho da tese propôs que os solos das florestas de sobreiros na Tunísia estão provavelmente contaminados com pentaclorofenol (PCP), um poluente orgânico persistente (POP). Essa hipótese baseia-se em relatos frequentes de contaminação de cortiça com pentacloroanisole (PCA), que provavelmente é formado através da conversão microbiana do PCP. Além disso, o PCP foi considerado um modelo adequado para investigar o papel da micobiota do solo na gestão da poluição devido à sua capacidade de viajar longas distâncias na atmosfera, se distribuir favoravelmente na camada do solo e moderar a resistência abiótica, além da sua toxicidade mortal. Por fim, os fungos desempenham um papel chave na colonização do solo em florestas de sobreiros. Estes conceitos foram aplicados no projeto "Estratégias preventivas e de remediação para a eliminação contínua de fenóis policlorados do solo florestal" (S*f*P-NATO 981674, 2006-2011). No geral, as principais descobertas do estudo incluem a demonstração da prevalência do PCP nos solos das florestas de sobreiros na Tunísia. Além disso, o estudo elucidou tanto a importância quanto o impacto da atividade fúngica na mitigação e dispersão do PCP.
Palavras-chave: Florestas de sobreiro; pentaclorofenol; comunidade fúngica; fungos cultivávei; vias de degradação do resorcinol, hidroquinona e catecol.

Analyse du rôle des champignons souterrains dans la gestion de la pollution par le pentachlorophénol: Perspectives des forêts de chênes-lièges en Tunisie

Résumé. Ceci résume la thèse de doctorat "Les champignons souterrains sont des sentinelles clés dans les sols forestiers vulnérables à la pollution par le pentachlorophénol: une étude mécaniste dans les forêts de *Quercus suber*". Le doctorat a été décerné par l'ITQB NOVA en mars 2018. Il comprend une révision des principaux problèmes, objectifs, méthodologie et résultats, ainsi que quelques recommandations possibles pour les travaux futurs sur la bioremédiation des polluants aromatiques halogénés. L'hypothèse de travail de thèse proposait que les sols des forêts de chênes-lièges en Tunisie sont probablement contaminés par le pentachlorophénol (PCP), un polluant organique persistant (POP). Cette hypothèse est basée sur des rapports fréquents de contamination du liège par le pentachloroanisole (PCA), qui est probablement formé par conversion microbienne du PCP. De plus, le PCP a été considéré comme un modèle adapté pour étudier le rôle du mycobiote des sols dans la gestion de la pollution en raison de sa capacité à voyager sur de longues distances dans l'atmosphère, à se partitionner favorablement dans les couches de sols et à modérer la résistance abiotique, outre sa toxicité mortelle. Enfin, les champignons jouent un rôle clé dans la colonisation du sol dans les forêts de chênes-lièges. Ces concepts ont été appliqués dans le projet "Stratégies préventives et de remédiatives pour l'élimination continue des phénols polychlorés des sols forestiers" (SfP-NATO 981674, 2006-2011). Dans l'ensemble, les principaux résultats de l'étude comprennent la démonstration de la prévalence du PCP dans les sols des forêts de chênes-lièges de Tunisie. De plus, l'étude a élucidé à la fois l'importance et l'impact de l'activité fongique dans la mitigation et la dispersion du PCP.

Mots-clés: Sol de forêt de chêne-liège; pentachlorophenol; communautés fongiques; souches fongiques cultivables; voies de degradation du résorcinol, hydroquinone et catechol.

Introduction

"Preventive and remediation strategies for continuous elimination of polychlorinated phenols from forest soil" project.

This project, funded by the NATO-S*f*P program, led by Prof. Cristina Silva Pereira, involved a multidisciplinary and multicultural consortium spanning five countries: Portugal, Scotland, Italy, Morocco, and Tunisia. Meetings were conducted in each of these countries to facilitate collaboration and progress. The project identified a critical issue, linking the cork taint defect in bottled wine to the likely presence of PCP in *Quercus suber* forests. Since the 1990s, scientific evidence has shown contamination of the bark of *Quercus suber* with PCP and its derivatives (SILVA PEREIRA *et al.*, 2000, MCLELLAN *et al.*, 2007). In response to significant concerns about pentachlorophenol contamination during the early stages of cork stoppers production, the doctoral thesis (VARELA 2018) focused on the taxonomic and functional diversity of mycobiota in forest soils potentially exposed to PCP. Preliminary data suggested the existence of saprophytic fungi that could play an important role in the mitigation of chlorinated aromatic compounds. Environment knows no boundaries; what goes up into the environment circulates around the world.

The doctoral thesis addressed critical aspects of PCP and of fungi colonizing forest soils that contribute to soil functioning, including to mitigation of soil pollution. To better understand the research undertaken, the key aspects will be analysed briefly in the next sections.

Pentachlorophenol

The work developed (VARELA 2018) provides a comprehensive and extensive review of the historical background of PCP application, from its early discovery and usage as wood preservative to its recognition as a banned persistent organic pollutant (POP). It emphasises aspects of its toxicity, environmental dispersion and microbial degradation. Moreover, it includes a general analysis of environmental pollution and microbial diversity, especially in soil, as well as the application of environmental proteomics to uncover the impacts of soil pollution in the colonising microbiota (VARELA *et al.*, 2017, MARTINS *et al.*, 2019). PCP was first synthesised in 1841 (ERDMANN 1841) and started to be applied in 1936. Since then, it has been extensively used for controlling pests and microbial pathogens in agriculture and industry, mostly due to its biocidal and physical properties (CARSWELL *et al.*, 1938). Throughout numerous reports, warnings against the use of PCP have been highlighted mostly due to its harmful effects to all life-forms. Advanced analytical techniques have enabled accurate detection of PCP in both human tissues/fluids and environmental samples (CHAPMAN *et al.*, 1965, BEVENUE *et al.*, 1966). PCP was only officially recognized as a Persistent Organic Pollutant (POP) by the Stockholm Convention, leading to extensive prohibition of usage in May 2015 (UNITED NATIONS 2015) (Figure 1). This recognition was extremely important, POPs can travel long distances from their application source, presenting a transboundary nature, meaning they can cross countries borders through pathways like water or air (CZAPLICKA 2004, HOFERKAMP *et al.*, 2010). It is intriguing to note that our studies were initiated in 2007, well before PCP was classified as a POP. At that time, PCP was considered a priority pollutant, highlighting the foresight and proactive approach of our research efforts.

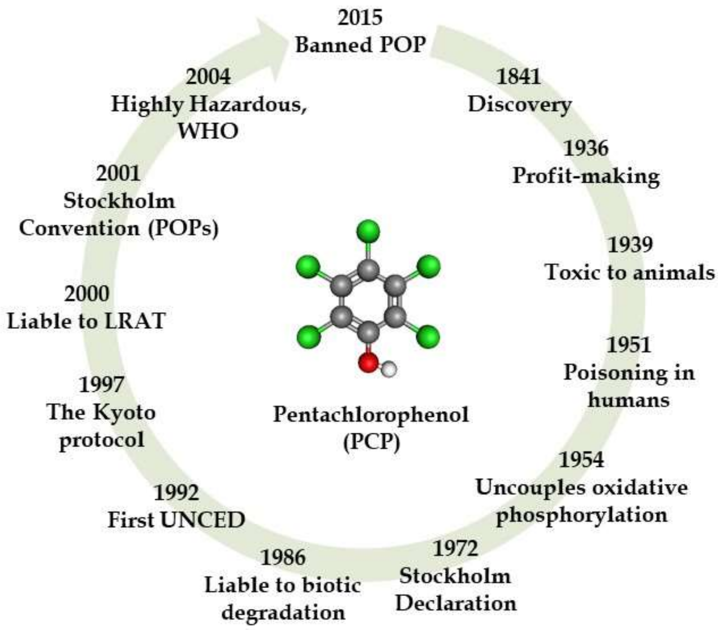


Figure 1 – Schematics of PCP history from its discovery in 1841 to its classification as POP in 2015

Soil Habitat

Soil habitats probably contain the greatest microbial diversity of all environments on Earth (DANIEL 2005). Soil is a complex and dynamic ecosystem where a large number of biological, chemical, and physical interactions occur (WAGG *et al.,* 2014). Indeed, soil often underestimated, harbours a vast diversity of organisms, ranging from microorganisms like bacteria and fungi to macrofauna such as earthworms and insects. Beyond its biodiversity, soil plays a critical role in regulating nutrient cycles, enhancing plant productivity, filtering water, and storing carbon – a proof to its multidimensional importance in ecosystem function and stability. Soil is recognized as the largest and most dynamic environmental compartment (COMMITTEE ON HEALTH EFFECTS OF WASTE INCINERATION 2000), serving as a diverse and invaluable resource. However, soil is also fragile, highly susceptible to atmospheric factors, pollution, and various management practices, among other factors. This vulnerability underscores the importance of adopting sustainable soil forestry management practices to preserve its integrity and ensure its continued functionality for future generations. Soil behaves as the main receptor (*e.g.* agroforestry inputs, air pollution deposition), transporter (e.g., migration to groundwater, root uptake), and decomposer (absorption, microbial transformation) of pollutants (VARELA 2018). Understanding the complexity of the soil habitat is essential for the sustainable management of natural resources and the development of responsible agricultural and forestry practices designed to optimize soil health, minimize degradation, and promote long-term productivity. A holistic approach is essential for safeguarding soil ecosystems and supporting global food security and environmental sustainability. Our studies are aligned with the emerging concept of One Health that underscores the interconnectedness of human, animal, and environmental well-being (PITT *et al.,* 2024). Soil health plays a fundamental role in this framework as it directly impacts the health and well-being of humans, animals, and ecosystems alike.

Cork Oak Forests

Cork oak forests are unique ecosystems found primarily in the Mediterranean region, known for their ecological and economic significance. They are vital for maintaining ecological balance, biodiversity, and climate regulation, while also generating added value-products (*e.g.* the cork bottle stoppers) through agro-forestry activities, contributing to global commercial and economic benefits.

Mediterranean cork oak forests cover 1.5 million ha in Europe and 700 thousand ha in North Africa (BUGALHO *et al.,* 2011). These forests are dominated by *Quercus suber*, an evergreen tree that has adapted to thrive in the dry, hot climates of the Mediterranean basin, including Portugal (the largest cork oak forest area), Spain, southern France, Italy, North Africa, and Bulgaria. The last was introduced in 1954 and is the youngest and smallest forest area, 1500 ha. Cork oak forests also support a diverse array of flora and fauna, including many endemic species that rely on these ecosystems for habitat and food sources. Despite their importance, cork oak forests face various threats, including deforestation, climate change, and land-use changes. Understanding the ecological and economic value of cork oak forests is essential for effective conservation and sustainable management practices. By taking good care of cork oak forests and implementing conservation measures, these forests can continue to grow and offer important ecosystem benefits for many years.

Fungi

The work developed by the authors (MARTINS *et al.*, 2019) provides detailed insights into how filamentous fungi are major colonizers of forest soils and serve as key players in environmental *pollution* mitigation, particularly regarding pentachlorophenol. Fungi play essential roles as soil' colonizers, ensuring a wide range of ecosystem services, and can constitute up to 75% of the soil microbial biomass (HARMS *et al.,* 2011). As a highly diverse and heterogeneous kingdom of organisms, fungi are ubiquitous across all biomes. They exhibit remarkable adaptability, thriving in adverse conditions characterised by low nutrient availability, low water activity, and low pH, mostly due to their proficient enzymatic machinery capable of degrading complex chemical structures. The diverse array of fungi colonizing forest soil plays a critical role in maintaining its functionality. Currently, there are up to 120,000 fungi species described and approximately 2.2 to 3.8 million are estimated to exist (HAWKSWORTH *et al.,* 2017). Fungi possess the remarkable ability to form extensive networks of interconnected hyphae called mycelia. These mycelia networks create a large area of contact within the soil matrix, facilitating the mitigation of compounds present in the soil (VAN DER HEIJDEN *et al.,* 2008). Therefore, the intricate structure of mycelial networks enhances the efficiency of fungal-mediated degradation processes of pollutants.

Case-study adequacy for the research aims

This research aimed to achieve several objectives: (i) establish the presence of PCP in oak cork forest soils in the Tabarka region of Tunisia; (ii) investigate whether belowground fungi can act as a natural barrier against PCP pollution; and (iii) determine if PCP pollution influences the functioning of belowground fungal communities. To address these questions, a case study was conducted and the following objectives were outlined:

- Establish a soil sampling strategy and characterise the physicochemical properties of the sampled soils.
- Develop an optimal protocol for the recovery of PCP and define its contamination levels in the sampled soils.
- Characterise the belowground fungal community through taxonomic analysis of the cultivable members within the community.
- Evaluate the capacity of each cultivable fungal strain and the fungal community as a whole for PCP degradation.
- Propose the PCP degradation pathway for the fungal community.
- Identify functions within the fungal community altered by PCP exposure.

The Tunisian oak forest, *Quercus suber*, was identified as a preferential location for sampling due to its placement within a high-risk zone: some fungal metabolites, known to originate from poly-chlorinated phenols, are occasionally or frequently found in Tunisian cork. As the image illustrates (Figure 2) the three locations namely Aîn Hamraia (AH), Fej Errih (FER), and Ras Rajel (RR) (N. W. Tunisia) were selected because the cork collected there was occasionally contaminated with either PCP or its degradation products, including some anisole derivatives. Additionally, these forests exhibit signs of poor forestry management, including high vegetation density, a lack of adequate forest sanitation, and oak trees displaying not only very high mortality levels but also a very poor self-regeneration capacity (MCLELLAN *et al.*, 2007).

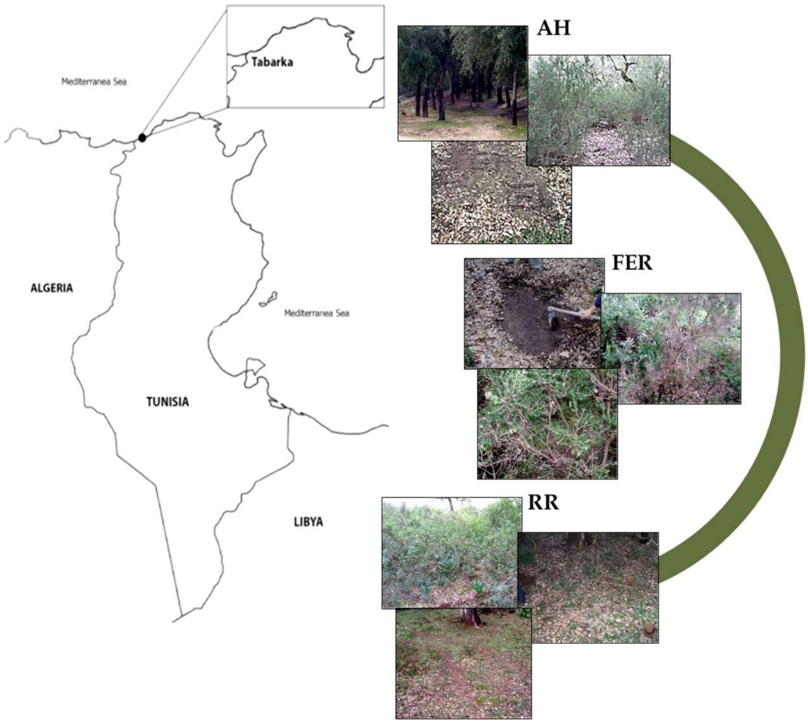


Figure 2 – The three locations sampled in Tabarka (N. W. Tunisia), AH, FER and RR

Determination of pentachlorophenol levels in Tunisian cork oak forest soils

The soil sampling of the cork oak forest occurred in collaboration with partners from Tunisia, among others, providing us access to them. Soil samples were collected in the three Tunisian demarked cork oak forests, namely AH, FER, and RR in February 2009, as described herein (MCLELLAN *et al.,* 2013). In brief, the sampling consisted of collecting five individual soil samples (0-20cm); which were then pooled and sieved to < 2 mm in the field and immediately conserved (dark, 4°C) until analysis. The analyses of the chemical and physical properties of the sampled soils were performed using standard methodologies (DEIVE *et al.,* 2011, VARELA *et al.,* 2015).

To evaluate the diversity of chlorinated compounds, as well as putative sub-products, present in the soil samples, a fast solvent extraction method was developed (MCLELLAN *et al.,* 2014). This method, which has been used as the basis for extracting PCP from the sampled soils, resulted in PCP recovery rates of > 70% from a certified reference material containing 2.04 ± 0.18 mg of PCP kg^{-1}

(ERM-CC008, LGC-Promochem, Spain) (Figure 3). These analyses were done in collaboration with researchers from the Barcelona University, namely Prof. Maria Teresa Galceran and Dr. Óscar Núñez. The analysis revealed that PCP levels were higher in AH soils compared to those of FER and RR. Consequently, AH soils were selected for further analysis (VARELA *et al.,* 2015).

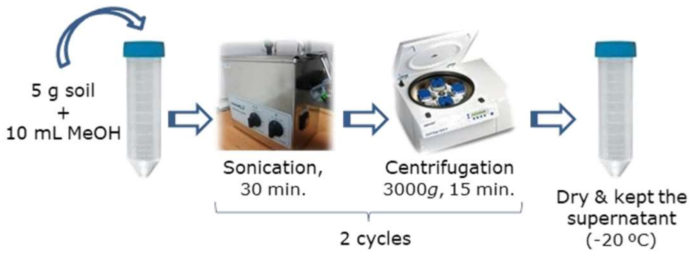
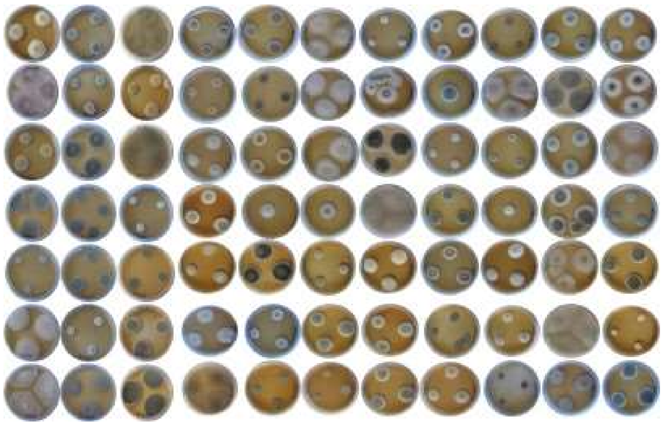


Figure 3 – Overview of method developed for PCP quantification

Can belowground fungi act as a barrier against PCP pollution?

The next step was to isolate and taxonomically identify the cultivable fungal strains from AH soils (VARELA *et al.,* 2015). The taxonomic studies were done in collaboration with researchers from the CBS-KNAW Fungal Biodiversity Centre (The Netherlands), namely Dr. Jos AMP Houbraken and Prof. Robert Samson. The community comprised 77 cultivable fungal strains, the majority of which belonging to the phylum Ascomycota (91%), with a clear dominance of *Penicillium*. Other representative taxa were *Aspergillus, Cladosporium,* and *Fusarium* (VARELA *et al.,* 2015) (Figure 4). Out of these fungal strains three new species were identified. Notably, in AH soil, a new specie, *Penicillium vanoranjei*, was discovered (VISAGIE *et al.,* 2013), which received in 2014 the "Top 10 New Species Award", attributed by the College of Environmental Sciences and Forestry (ESF), State University of New York.



Genus	
Penicillium	52
Aspergillus	8
Absidia	6
Cladosporium	4
Fusarium	3
Phoma	2
Trichoderma	1
Zygorhynchus	1

Figure 4 – The cultivable fungal community comprised 77 fungal strains, most of which belong to the Ascomycota phylum, with a clear dominance of ***Penicillium*** **genus**

Then, each cultivable fungal strain was individually assessed for its PCP degradation capacity. PCP levels were systematically quantified by Liquid Chromatography (LC). The community-level physiological profile (CLPP) was also analysed using Biolog plates SF-N2. This assay allows screening the ability of fungi to utilise 95 different substrates, which are divided into major groups: Stress Response, Mitochondrial Functioning, Carbohydrate Metabolism, Amino acid Metabolism, and ATP Metabolism (LIU *et al.*, 2007). To describe the PCP degradation pathway utilized by each fungal strain, the diversity of PCP degradation intermediates and sub-products was analysed using high-resolution mass spectrometry (HRMS) (VARELA *et al.*, 2015). To assign the compounds' identities, a user-target database list was applied. Putative identifications were, subsequently validated, whenever possible, using standard compounds. The acquired data showed that out of the 77 tested fungal strains, 53 strains were able to degrade PCP, with 21 strains degrading it at the highest concentration tested (56□M).

Finally, to propose a pentachlorophenol degradation pathway for the fungal community, all individual PCP-metabolomes were integrated. After initial modification of PCP, either at meta (*m*), para (*p*) or ortho (*o*) position, respectively the resorcinol (R), hydroquinone (HQ) or catechol (C) branches, successive reductive dechlorination reactions occur. Some conjugate compounds of the lower chlorinated derivatives of PCP formed through *O*-methylation, sulfation or both, were also identified. The different branches intersect because of additional hydroxylation of R, HQ and C derivatives, either chlorinated or non-chlorinated, yielding the corresponding trihydroxybenzenes (THB). The identification of some non-chlorinated derivatives (*i.e.* THB and DHB) is consistent with the idea that some strains of the fungal soil community were capable of mineralizing PCP under the conditions tested. The identification of compounds derived from the resorcinol pathway is particularly relevant, as this association has not been previously documented in PCP degradation studies (VARELA *et al.*, 2015, VARELA *et al.*, 2017). The integration of the PCP-derived metabolites of each axenic culture matches the degradation pathway of the community, as well as, that of the sampled soils (Figure 5).

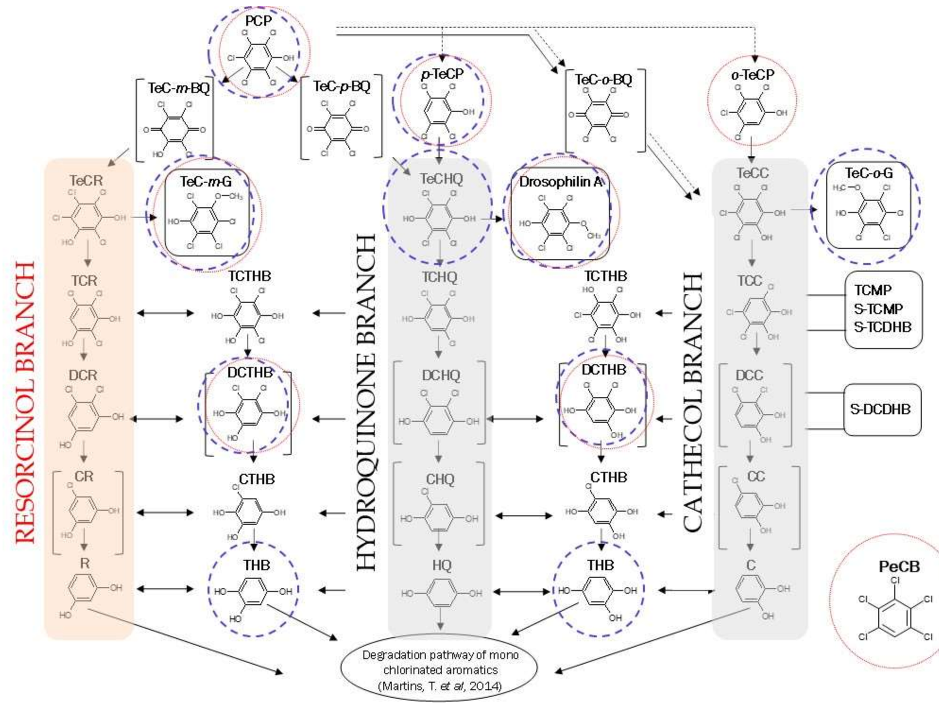


Figure 5 – The PCP degradation pathway herein proposed integrates all the PCP-derived metabolites identified by HRMS in the axenic fungal cultures. It comprises the resorcinol, the hydroquinone, and the catechol branches (VARELA *et al.*, 2015). Dashed lines represent compounds that can be formed abiotically, while solid lines represent those formed biotically. Compounds in brackets were predicted to exist. Conjugated compounds are shown within boxes.

To validate the proposed pathway, PCP-derived metabolites formed by the fungal community as a whole (*i.e.* community-based cultivations) or present in the sampled soils were analysed as well (blue and red circles, respectively). In the soil, a compound identified as a microbial precursor of PCP (pentachlorobenzene, PeCB) was identified.

Can PCP pollution influence the functioning of belowground fungal communities?

The compounds derived from the three branches could also be identified in community assays as well as in the soils (VARELA *et al.,* 2015, VARELA *et al.,* 2017). Therefore, these results challenge us to determine whether PCP pollution alters the functioning of the belowground mycobiota (VARELA *et al.*, 2015, MARTINS *et al.*, 2018).

The used strategy has focused on the activity of the entire community (not its individual members) during PCP mitigation. The analyses involved proteomics (mycelial) and community level physiological profiles (CLPP). The acquired data revealed that PCP induced major stress. Based on the proteomics analysis, PCP caused significant alterations in the mycelial proteome, especially in proteins associated with Stress Response, Mitochondrial Functioning, Carbohydrate Metabolism, Amino Acid Metabolism, and ATP Metabolism. The utilization profiles of the fungal metacommunity exposed to PCP, compared to the control, were dramatically affected by PCP even after its major depletion (CLPP assays). In particular while the utilisation of Carbohydrates was greatly hindered, that of Amino Acids and Miscellaneous compounds increased. Both analyses support the conclusion that PCP exposure impaired the capacity of the fungal metacommunity to utilize carbohydrate substrates, leading to a shift towards the usage of substrates containing nitrogen. Additionally, along PCP depletion the functioning at the community level showed slowly a capacity to recover, regardless that key functional alterations persisted even after major PCP depletion (VARELA *et al.*, 2017, MARTINS *et al.*, 2018). This study was further complemented with metagenomics and secretome analyses as well as measurements of the community susceptibility to an antifungal. Taken together, the collected data showed that the metabolic cost of PCP degradation is suggestive of augmented pathogenic potential (MARTINS *et al.*, 2018). This new hypothesis was further investigated by colleagues, leading to the demonstration that PCP (and other halogenated aromatic pollutants) can indeed led to a rise in the production of virulent airborne spores of aspergilla, mostly *Aspergillus fumigatus* (MARTINS *et al.*, 2023). This further shows the significance of studies focussing on the link between pollution and fungi, highlighting further its significance for the One Heath concept. Changes at the human-animal-environment interface, such as those driven by climate change and pollution, can impact the spread and emergence of fungal diseases as recognised by the Centers for Disease Control and Prevention (CDC) (2022).

Conclusions

The delicate state of cork ecosystems poses significant challenges, particularly the potential for damage due to pollution. Pressures to exploit resources for shortterm gains can lead to the improper use of chemical agents, exacerbating the situation (MCLELLAN *et al.*, 2014).

Understanding how PCP pollution affects the functioning of fungal communities is vital, especially in environments like the Tabarka cork oak forest soil. The aforementioned studies, for the first time, demonstrated the prevalence of PCP in Tunisian cork oak forest soils at levels similar to those where PCP is actively used, raising serious concerns and suggesting atmospheric deposition as a significant contributor (ZHENG *et al.,* 2012). Additionally, this research underscores the importance of belowground fungal communities in mitigating PCP pollution, acting as a buffer against it (HARMS *et al.*, 2011, VARELA *et al.*, 2015, HECHMI *et al.,* 2016). Fungi play a key role in the mineralization of chlorinated phenols, while bacteria typically produce highly toxic and resistant derivatives of both non-chlorinated and chlorinated phenols (MARTINS *et al.*, 2014).

The identification, for the first time, of the resorcinol branch of the fungal PCP degradation pathway, apart from those already described before: the hydroquinone and catechol branches, highlights the uniqueness of PCP degradation by fungi. Metabolites from the resorcinol branch could be found in community-based cultures and in soils; an indication that fungal activity is contributing to PCP degradation in either system. Additionally, new fungal metabolites of PCP, such as Drosophilin A, were discovered, expanding our understanding of fungal degradation pathways in the Ascomycota phylum. The three different branches intersect, forming compounds containing three or less chloride atoms, including non-chlorinated compounds, consistent with PCP subsequent mineralization. Further analyses of the functioning of belowground fungal communities were done to better understand microbial specialisation, events and/or shifts in functional biodiversity that may happen during the mitigation of the biocide (MARTINS *et al.,* 2018).

Our findings reinforce that community-based studies provide means to reveal key functional trends within communities, identifying new chemical markers for the assessment of environmental pollution and highly efficient pollutant-degrading strains or taxa. However, the process of PCP degradation and mineralization comes at a high cost, as evidenced by alterations in the mycelial proteome and disruptions in carbon and nitrogen utilization profiles of the fungal metacommunity. These alterations are significantly impacted by PCP presence; even at very low levels, raising questions about how these alterations affect the multifunctionality of the entire ecosystem (MARTINS *et al.*, 2018).

Fungi high remedial potential, in particular Ascomycota, for the remediation of polluted soils should not be ignored, especially if their ubiquity, resilience, and extensive hyphal networks in the soil are considered. In addition, most fungal strains in the community demonstrated significant ability to degrade PCP, highlighting the widespread capacity of fungi to break down this biocide.

Possible recommendations

One of the key recommendations for preserving biodiversity and the quality of different ecosystems is to minimize air, water, and soil pollution by implementing stronger legislation. This helps safeguard the health of ecosystems and the species within them by reducing the introduction of harmful chemicals into the environment. Also, understanding how microorganisms break down pollutants can inform the design of remediation technologies. Focusing on the production of environmentally friendly products is crucial. Additionally, we should refrain from exporting such products to other countries, as some may undergo long-range atmospheric transport. Further research is needed to explore the long-term effects of pollution on belowground fungi and their ecosystem functions. Moreover, the development of novel bioremediation strategies for sites contaminated with PCP or other halogenated aromatics is yet a necessity.

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