## NEW INSIGHTS INTO THE REMEDIATION OF POLLUTED SOILS USING ENDOPHYTIC FUNGI

Lorena- Roxana GURĂU<sup>1, 2</sup>, Ioan RADU<sup>2</sup>, Andreea COSOVEANU<sup>2</sup>, Leonard ILIE<sup>1</sup>

<sup>1</sup>University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania <sup>2</sup>Research Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd, District 1, 013813, Bucharest, Romania

Corresponding author email: lorenagurau@gmail.com

#### Abstract

Soil pollution is a growing global concern and one of the most challenging environmental issues today, involving the human sector as well. Innovative soil remediation strategies are needed to conserve the natural resources of the environment. Endophytes are present in all plants species, living symbiotically in a continuum dynamic mutualism. Among multiple changing roles, safeguarding the host in terms of increasing resistance to abiotic factors has been widely demonstrated. Following these physiological changes, host plants have beneficial potential in degrading pollutants from contaminated soils. Enzymes produced by host plants along with endophytes may also, degrade macromolecule compounds into small molecules or convert more toxic substances into less toxic substances to increase their adaptability. Phytoremediation technology with endophyte fungi is an ecological alternative strategy that has been documented as a promising technology for remedying contaminated soils. This review article aims to piece together the physiological, chemical and genetical mechanisms employed in phytoremediation techniques mediated by endophytic fungi and highlight the importance of the plant-microbiome ecology.

Key words: endophytic fungi, hydrocarbon degradation, soil, bioremediation.

### INTRODUCTION

Global industrialization has led to pollution of the environment, freshwater, and topsoil. Heavy metals are naturally occurring pollutants found in the Earth's crust and difficult to decompose. The compounds exist as ores in rocks and are recovered as minerals. High-level exposure to heavy metals can result in their release into the environment where remain toxic for extended periods (Masindi et al., 2020; Bala et al., 2022). The use of endophytic fungi for bioremediation polluted environments, particularly contaminated soils, is a promising approach. Studies have shown that endophytic fungi colonizing host roots can have a positive impact soil productivity and characteristics. Endophytic fungi during growth not only promote the nutrients in the soil decomposition, but also reduce the toxicity of the soil (Chen & Dai, 2013).

A literature survey was conducted to reveal the importance of endophytic fungi in the remediation of contaminated soils. The main databases used were CAB Direct and PubMed.

This review presents new perspectives on the bioremediation of soils polluted hydrocarbons by involving endophytic fungi and highlighting the relationships in host plants and their microbiome. A survey of the literature was performed to show the differences regarding the number of articles which focused on the significance of endophytic fungi in the remediation of contaminated soils. The abstract search was conducted using simple and combined keywords such as "endophytic fungi", "endophytic fungi in hydrocarbon degradation", "soil pollution", "fungi in polluted soil", "phytoremediation" and "phytoremediation with endophytic fungi". The number of indexed publications containing "endophytic fungi" was similar in both databases (CAB Direct – 10,923 and PubMed -11,227).

When we compared "phytoremediation" searchterm with "phytoremediation with endophytic fungi", the number of hits was highly different: CAB Direct and PubMed - 17,445 and 58,127 entries, respectively, for the simple keyword, and for the combined one, 79 hits and 365 hits, respectively. The low number of entries is due to the novelty of the topic regarding the involvement of endophytic fungi in the bioremediation of polluted soils. Conversely, the number of entries for "soil pollution" was very high for both databases, 161,792 hits in CAB Direct and 700,713 results in PubMed. The number of entries of "fungi in polluted soil", revealed a high interest with 105,466 entries in PubMed and only 8176 entries in CAB Direct. This survey reveals a keen interest in researching environmentally friendly methods and techniques for contaminated soils.

## BIOREMEDIATION WITH ENDOPHYTIC FUNGI

Bioremediation is a process that refers to the use of biological agents such as bacteria, fungi, algae and plants for the degradation and detoxification of hazardous substances and contaminants from soil, water, and other environments (Khatoon et al., 2021). It is considered a cost-effective and environmentally friendly technology under the biotechnology area (Khalid et al., 2021). Bioremediation of polluted environments, especially contaminated soils, using endophytic fungi is a promising approach. Over the years, studies have shown that endophytic fungi that colonize the host roots can positively affect the productivity of the soil and its characteristics. In bioremediation, endophytes are able to stimulate host plant growth in polluted soils by increasing the production of endogenous auxins and can positively influence nitrogen fixation process (Rajkumar et al., 2009; Nandya et al., 2020). Endophytic fungi can accumulate soil pollutants through the root parts of plants. For instance, Piriformospora indica a filamentous fungus with an endophytic character in a wide range of plant roots and with abilities similar to a mycorrhiza can colonize Zea mays roots, promoting plant growth and root development. Therefore, since plant roots penetrate deep soil layers, P. indica-maize mutualistic combination enhanced petroleum breakdown by relying on host-derived shelters and nutrients for their endophytic partner. This is especially helpful for treating deep-reaching contamination (Zamani et al., 2016; He et al., 2020). The symbiotic interaction between plants of meadow fescue (Festuca pratensis) and two endophytic fungi of the *Neotyphodium* species can support the synthesis of dehydrogenase, which assists in the degradation of PAHs (Soleimani et al., 2010; Saravanan et al., 2020). Another study points out the benefits of an endophytic fungi consortium on Lactuca sativa plants cultivated in cadmiumcontaminated soil. The endophytic fungi-treated group had a greater foliar and root biomass compared to the control group (Bibi et al., 2018; Soldi et al., 2020). In a laboratory experiment, endophytic fungi were also inoculated on Festuca arundinacea samples to asses their positive effects on this host at increasing lead concentrations. The results showed a better performance of the plants treated with the endophyte consortium for each of the growth parameters recorded, thus reducing the harmful effects of the heavy metal (Soldi et al., 2020). Aspergillus strain G16 associated with Brassica juncea and Trichoderma strain H8 associated with Acacia auriculiformis improved plant growth (44% and 167% respectively) in cadmium and nickel contaminated soils. Together, these two strains induced a higher plant yield (178%) compared to control plants in contaminated soils (Jiang et al., 2008). Plants which can colonize heavy metal polluted soils and highly accumulate heavy metals in their above-ground tissues are called hyperaccumulator plants, a promising tool phytoextraction of heavy metal-contaminated soils (Baker & Brooks, 1989; Baker, 2000; Sigel et al., 2013). A non pathogenic F. oxysporum, isolated from the Zn/Cd co-hyperaccumulator Sedum alfredii grown in a Pb or Zn mined area, was able to increase S. alfredii root systems and function, metal availability and accumulation, and plant biomass, and thus phytoextraction efficiency (Zhang et al., 2012; Zheng et al., 2016).

The endophytic fungi *Pestalotiopsis microspora* was uniquely able to grow on synthetic polymer polyester polyurethane as the sole carbon source under both aerobic and anaerobic conditions (Russell et al., 2011; Deng & Cao, 2016) suggesting its potential use for treatment of white plastic pollution. Toxic metals can be accumulated in large amounts within the mycelia of endophytic fungi. Yet, studies on the mechanisms behind metal transport and detoxification are scarce (Zahoor et al., 2017; Khalid et al., 2021).

## FUNGAL BIO-MECHANISMS INVOLVED IN HYDROCARBONS DEGRADATION

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds that are dangerous environmental pollutants and can cause health problems. Of the more than one hundred known PAHs, 16 have been identified as priority pollutants. The use of very diverse biological machineries, including endophytic collected from contaminated sites, has emerged as a safe and sustainable ecological approach to the degradation of PAHs (polycyclic aromatic hydrocarbons) and PHs (petroleum hydrocarbons). Since the first isolation of hydrocarbon-degrading bacteria in 1913 by Söhngen (Söhngen, 1913), over 79 genera of bacteria were found to be able to use hydrocarbons as a sole source of energy and other were detected as able to degrade or transform hydrocarbons (Head et al., 2006; Prince et al., 2010). Fungi can remediate pollutants through several mechanisms and various mechanisms such as bioabsorption, precipitation, biotransformation sequestration were proposed. Filamentous fungi have been reported as bioremediation agents due to their extensive mycelial networks and enzyme-secreting activities. These high redox such as potential enzymes, manganese peroxidase (MnP), laccases (Lac) and lignin peroxidases (LiP) for the oxidation of lignin are generally not substrate specific as they can oxidise a wide range of xenobiotics including pesticides. plastics and hydrocarbons (Asemoloye et al., 2020; Daccò et al., 2020). Mycoremediation significantly removes or degrades metals, persistent organic pollutants and other emerging contaminants (Kumar et al., 2021).

Endophytic fungi Penicillium atrovenetum, Thermomyces lanuginosus, Penicillium canescens, Trichocladium opacum Aspergillus niger were assessed for the efficacy to utilize PHs as their source of carbon. All fungi were isolated from Eichhornia crassipes samples on minimal salt broth (MSB) containing petroleum hydrocarbons and were observed to have increased growth rates (Wahab et al., 2022). The plant-endophyte interaction system remediate polycyclic could aromatic

hydrocarbon contamination in vivo promoting the expression of metabolism-related The endophytic fungi *Phomopsis* liquidambaris, isolated from stem of Bischofia polycarpa demonstrated a good capacity in the remediation of phenanthrene in interaction with rice. The results showed that the removal rate of phenanthrene in the root was higher than that in the leaf and that in large part was due to the presence of the endophytic fungus (Hussain et al., 2018; Chen et al., 2019; Igbal et al., 2019; Fu et al., 2022). Crude oil and diesel were efficiently degraded in liquid culture by two filamentous fungi, Aspergillus ustus and Purpureocillium lilacinum, isolated from an artificially contaminated soil. P. lilacinum was more potent in degrading hydrocarbons and exhibited the highest crude oil (44.55%) and diesel (27.66%) removal rates and the highest biodegradation constant (Benguenab & Chibani, 2021). Endophytic species of Verticillium, Colletotrichum, Clonostachys Saccharicola, Phomopsis and Aspergillus were isolated from plants collected in a natural habitat contaminated with crude oil and were reported as able to degrade petroleum hydrocarbon, quantified by infrared spectroscopy and gas chromatography (Marín et al., 2018). Also, assessment of seven endophytic fungi living in mangroves showed the ability of Nigrospora sp., Aspergillus niger, Aspergillus sp., Curvularia sp., Pestalotiopsis aduinta, Fusarium sp., and Cladosporium sp. to degrade hydrocarbons, using FT-IR spectroscopy analysis (Sawant & Rodrigues, 2020). Effective bioremediation strategies require consideration physicochemical parameters and catabolic properties of degrading microbial communities. It is important to note that most microorganisms are not readily cultivable and cannot be easily characterized, which poses a significant challenge for microbiologists (Mishra et al., 2020). Yet, multiple fungal strains were reported to be able to remove or metabolize contaminants (Table 1).

Still, technological and economic constraints remain. Despite the existence of problems, the use of high-throughput genomic approaches in bioremediation is still in its early stages of development.

Table 1. Overview of the bioremediation potential of fungi - contaminants and bio-active fungal species

Compound	Fungal species	Reference
Crude oil	A. niger, Rhizopus sp., Candida sp., Penicillium sp., Mucor sp., Verticillium sp., Xylaria sp.	Damisa et al., 2013 Marín et al., 2018
Gasoline	Exophiala xenobiotica	Isola et al., 2013
Diphenyl ether (PAH)	White rot fungi Pleurotus ostreatus Trametes versicolor	Wu et al., 2013 Rosales et al., 2013
Anthracene (PAH)	Armillaria sp.	Hadibarata et al., 2013
Naphthalene (PAH)	White rot fungi Pleurotus eryngii	Hadibarata et al., 2013
Chlorinated hydrocarbons: Heptaclor	Pleurotus ostreatus	Purnomo et al., 2013
Chloropyriphos	Aspergillus terreus	Silambarasan & Abraham, 2013
Heavy Metals	Aspergillus sp., Curvularia sp., Acremonium sp., Aspergillus flavus, Alternaria sp. Penicillium sp., Fusarium sp. Arbuscular mycorrhizal fungi (AMF), Trichoderma sp., Mucor sp., Rhizopus sp., Pleurotus sp.	Akhtar et al., 2013 Kurniati et al., 2014 Neagoe et al., 2014 Borozan et al., 2021
Engine oil	Mucor irregularis; Aspergillus oryzae	Asemoloye et al., 2020
Petroleum hydrocarbons	Nigrospora sp., Aspergillus niger, Aspergillus sp., Curvularia sp., Pestalotiopsis adusta, Fusarium sp., Cladosporium sp., Piriformospora indica Aspergillus ochraceus, Cunninghamella elegans, Phanerochaete chrysosporium	Sawant & Rodrigues, 2020 D'Souza et al., 2015

### PLANT-MICROBIOME

Plant microbiome ecology is a multidisciplinary field that explores the complex relationships between plants and their associated microbial communities. It provides insights into the fundamental processes that shape plant-microbe interactions and the potential for using the plant microbiome for sustainable agriculture and environmental management.

The microbiome of plants comprises bacterial, fungal, and archaeal communities that are associated with their host plants in the rhizosphere, phyllosphere and endosphere

(Tardif et al., 2016; Mitter et al., 2019). The microbiome has gained significant attention in both research and applied science. Some key areas where the plant-microbiome has proven to be an important and useful technique are agriculture and crop production, industrial processes and biotechnology, environmental monitoring and restoration, as well as lately bioremediation and waste management. The microbiome plays a vital role in bioremediation, which involves the use of microorganisms to degrade or transform pollutants into less harmful forms (Bala et al., 2022). The microbiome is used in bioremediation in hydrocarbon

degradation, heavy metal remediation, chlorinated compound degradation, xenobiotic transformation, land and water remediation. Microbes can colonize the rhizosphere, phyllosphere, and live inside plant tissues as endophytes. Fungal microorganisms, which have an endophytic lifestyle, play an important role in all plant processes.

Plant-associated microbes have an essential mission in the growth and development of their hosts by producing various plant growth hormones, including auxins and gibberellins. These two hormones are known to promote stem elongation, seed germination, and flowering in plants. By producing gibberellins, root-associated microbes contribute to the overall growth and development of plants. Auxin production is a common trait found in all plant-associated microbes (Yadav et al., 2017). Environmental factors, such as soil type, climatic conditions, land management practices, and plant genotype, shape the composition and functioning of the plant microbiome.

Plant microbiome ecology investigates how these factors influence microbial community structure, diversity, and activity, and how they, in turn, impact plant performance and ecosystem functioning (Fonseca et al., 2017; Dastogeer et al., 2020). Certain species of arbuscular mycorrhizal fungi (AMF) that promote plant growth have been found to hinder it in specific circumstances, such as low light, low temperature, or phosphorus (P) availability (Smith & Smith, 1996; Johnson et al., 1997; Sergaki et al., 2018). The activity of AMF can be inhibited by the soil microbiota, as demonstrated by Svenningsen et al. (2018). This emphasises the importance of conducting field experiments to gain a comprehensive understanding of microbe behaviour. The study of microbiomes in polluted soils is a relatively recent field, and pinpointing the exact first study on this topic can be challenging. However, one of the pioneering studies that laid the foundation for understanding the microbiome of polluted soils was published in 2003 by Anne Spain and Elizabeth Alm (Spain & Alm, 2003). Genomic analysis is a culture-independent technique that enables the quick study of various samples, such as water and soil, through the development of sequencing technology. Recent advances in next-generation sequencing (NGS)

allowed for comprehensive genomics, metagenomics, and bioinformatics analysis of microbial communities, providing unparalleled insight into key bioremediation mechanisms. Nucleotide databases and in silico tools are used to advance research on the role of microbes in pollutant degradation and the identification of new genes responsible microbial remediation (Sharma & Kumar, 2021). These recent advances in metagenomics and amplicon sequencing have provided new insights into plant symbioses, including the structure and assembly of symbiotic microbial communities (Vandenkoornhuvse et al., 2015).

# MICROBIOME DIVISIONS INTO MICRO-ECOSYSTEMS - RHIZOSPHERE, ENDOSPHERE AND PHYLLOSPHERE

The rhizosphere zone is the 1-10 mm part of soil immediately surrounding the roots, which is influenced by the plant through its root exudates. mucilage and dead plant cells (Cai et al., 2017). The rhizosphere organisms that are most frequently studied for their benefits include mycorrhizae, rhizobium bacteria, plant growthpromoting rhizobacteria (PGPR), and biocontrol microbes (Hinsinger et al., 2009; Dastogeer et al., 2020). Symbiotic fungi colonize the rhizosphere region in soil or the internal tissues of plants. They usually obtain carbon from the host plant and return essential soil elements to the plant, while also improving water and nutrient uptake (Privashantha et al., 2023). Studies confirmed the existence of a central microbiome by identifying members Xanthomonadales. Rhizobiales. Sphingomonadales, Burkholderiales in the rhizosphere of six plant species: Artemisia argyi, Ageratum conyzoides, Erigeron annuus, Bidens biternate, Euphorbia hirta and Viola japonica (Lei et al., 2019; Santos et al., 2021). Experimental data have shown the importance ofthe rhizosphere microbiome rhizoremediation and plant contaminated sites. Thus, it is obvious that microbial assisted rhizoremediation has the potential to remove toxic compounds (Kotoky et al., 2018). Bacteria, and to some extent archaea, important members of endosphere communities and as microbes that interact with their host plants, provide them with benefits.

Endophytic microorganisms colonize the endosphere and have been shown to metabolize pollutants and influence plant development ability (Taghavi et al., 2005; Tardif et al., 2016). The endophytic community in the roots can differ significantly from the adjacent soil community. Generally, the diversity of the endophytic community is lower than that of the microbial community outside the plant (Schlaeppi et al., 2014; Dastogeer et al., 2020). Scientists employ various methods, including DNA sequencing, metagenomics, microscopy, and culturing techniques, to study the endosphere microbiome.

The study of the endosphere microbiome has practical applications in agriculture, horticulture, and environmental management. By improving the beneficial interactions plants and endophytes, between productivity can be improved, with the need for chemical inputs reduced, stress tolerance enhanced, and sustainable agriculture practices promoted. The phyllosphere refers to the aerial surface of a plant, including the stem, leaf, flower, and fruit. It is considered to be relatively nutrient-poor when compared to the rhizosphere and endosphere (Dastogeer et al., 2020). The microbiome has phyllosphere important implications for plant health and ecology. It is a dynamic and unique environment that provides microhabitats for microbial colonization. It offers nutrients, moisture, and structures that support the growth and survival of microorganisms. The composition and dynamics of the phyllosphere microbiome can vary depending on factors such as plant species. age. environmental conditions interactions with neighboring microorganisms. The phyllosphere actively acquires microbiome through vertical transmission (from mother plants or seeds), horizontal transmission (from the environment), or mixed modes from neighbouring microbial reservoirs (Bright & Bulgheresi, 2010). Phyllosphere endophytes are primarily transmitted systemically through xylem but can also enter through leaf epidermal openings such as stomata, lenticels, and hydathodes (Compant et al., 2010).

The most common groups of bacteria (*Proteobacteria*, *Bacteroides*, *Firmicutes*, *and Actinobacteria*) are usually residing on the phyllosphere of different plant species including

model plants *Arabidospis thaliana* and *Citrus* species (Reisberg et al., 2013; Kembel et al., 2014; Durand et al., 2018; Carvalho et al., 2020). In addition to bacteria, the phyllosphere also supports a significant diversity of yeasts and filamentous fungi.

## MICROBIOME TOWARDS APPLIED SCIENCE - AT WHAT STAGE ARE WE?

Over the last decade, research on microbiomes has altered our understanding of the complexity and structure of microbial communities. The observations were complemented by progress that largely led to a significant reduction in highyielding screening costs (Cullen et al., 2020). We can mention some examples of real-world bioremediation projects that have successfully utilized the microbiome with the project Deepwater Horizon Oil Spill: Following the Deepwater Horizon oil spill in the Gulf of Mexico in 2010. where bioremediation played a significant role in oil degradation (Kimes et al., 2014). Microbial bioremediation techniques were employed to help mitigate the contamination radionuclides of soil and water in the affected ofChernobyl Nuclear Penicillium and Cladosporium, were found to accumulate effectively and immobilize radioactive isotopes, reducing their mobility in the environment (Zhdanova et al., 1991). Australia has a vast and diverse landscape, making it susceptible to various forms of soil contamination. The country has embraced bioremediation practices that leverage the microbiome to restore contaminated soils. Australian researchers and environmental practitioners have utilized microbial communities to remediate sites affected by mining activities, agricultural pollution, and other sources of soil contamination. Using structural equation modeling, a prediction model was developed to predict the impact of future climate change scenarios on soil microbial biodiversity in sub-Saharan Africa. The model predicts that increased temperatures and decreased precipitation will have a negative effect on soil microbial biodiversity in countries like Kenya, while the fungal biodiversity of Benin will benefit from the increase in annual precipitation. This study can provide important information to support conservation efforts in countries heavily reliant on rain-fed agriculture and most vulnerable to the impacts of climate change. (Cowan et al., 2022).

## CONCLUSIONS AND FUTURE PERSPECTIVES

Nowadays, the main biological means to clean soil are bioremediation and phytoremediation. These are two commonly processes used approaches for cleaning up contaminated soils. Either with microorganisms, such as bacteria and fungi, to degrade or transform contaminants in the soil into less harmful substances or using plants to remove, degrade, or stabilize contaminants in the soil.

Currently, specialized techniques for monitoring the genome, transcriptome, and proteome of microbial communities broad the path towards successful and efficient bioremediation techniques. The endophytes induce a quicker establishment of plants in these conditions, which accelerates the restoration of deteriorated areas.

Thus, reducing the presence of bioactive metals and improving the ecology, variety of soil microflora, and soil fertility, reestablishing healthy crop production. In conclusion, more research is required to study the mechanisms of degradation and in situ bioremediation of the fungal microorganisms.

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