SOIL FUNGI RESISTANT TO ORGANIC POLLUTANTS AS A POTENTIAL SOLUTION FOR AIR BIOFILTRATION

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Abstract

Human activity leads to the production and release of many aliphatic and aromatic compounds that have long been considered a major source of pollution that needs to be addressed. Especially those substances that possess one or several aromatic rings (MAHs and PAHs) and volatile organic compounds (VOCs) are of concern, and numerous studies have tried to understand mechanisms of degradation and develop biological solutions to this problem.

Many microorganisms, such as bacteria and fungi have been documented to be able to degrade and even use as a source of carbon and energy these types of organic pollutants. White-rot fungi are well documented for their ability to degrade aromatic compounds due to their enzyme system involved in lignin decomposition. However, several less studied anamorphic fungi and yeasts can grow on different organic compounds (including PAHs and VOCs) as a sole source of carbon. These species are of a great importance for developing strategies for organic pollutants biodegradation, and have often been isolated from sources traditionally contaminated, such as soil and industrial debris.

In this study, mycobiota from different types of soil contaminated with aromatic compounds has been studied to understand the way the pollutants affect the microbiome and fungal communities' composition. Several fungi have been demonstrated to grow well on contaminated soils and their potential for biodegradation of organic compounds is discussed. These insights contribute to moving forward the possible biological pathways involved in the biofiltration of the gaseous pollutants from urban automotive underground spaces and other related applications.

Keywords: *fungi, aromatic compounds, volatile organic compounds, biodegradation*

1. INTRODUCTION

Modern society makes use of many organic compounds, including oil derived, which leads to the release into the environment of different hazardous substances. From transportation to manufacturing of a large variety of goods, and from extraction of oil to its processing, all these activities result in production of organic pollutants with numerous side-effects [1]. A special attention is given to aliphatic and aromatic hydrocarbons, either with a single aromatic ring (monoaromatic hydrocarbons - MAHs) or several aromatic rings (polyaromatic hydrocarbons – PAHs), due to their strong toxicity towards human and animal health as well [2, 3]. These compounds have been proven to be cytotoxic, neurotoxic, nephrotoxic, carcinogenic, genotoxic and mutagenic [3], and therefore, there is a strong need for these pollutants to be eliminated. Some of them are highly volatile and often found or released as gases and are regarded as volatile organic compounds (VOCs), although the term is used for a wider range of volatile substances, and when polluting, such compounds are frequently found in mixtures [4]. Among the various VOCs associated with auto traffic pollution, it can be mentioned benzene, toluene, ethylbenzene, xylene etc. [5].

Generally speaking, MAHs and PAHs can be detected in various environmental matrix (soil, water, air) [6]. During the last decades, numerous attempts to address the issue have been performed, and there are several techniques proposed to clean up the soil, waters, and air of MAHs and PAHs, some of these methods involving physical processes (combustion) or physicochemical processes (adsorption, absorption) with limited applicability [7]. However, it is generally considered that such methods cannot be applied in all circumstances and, even more, are expensive and not always efficient [8]. For this reason, biological processes of cleaning hydrocarbon pollutants have been tested, either for mitigating the soil pollution, cleaning up the effluents resulted from industrial activities or for filtering the contaminated air [7, 9, 10]. Natural processes and the derived ones were involved in studies for bioremediation of organic pollutants of all the kinds.

This paper briefly discusses major achievements in the field of MAHs and PAHs biomitigation, pointing out the current limitations and opportunities for air biofiltration from the perspective of the microbiological component, which can be involved alone or along with their plants in such process (e.g. microbial biofiltration, botanical biofiltration). Particularly, a preliminary investigation on fungal diversity and abundance on toluene treated soil from rhizosphere of two plant species was realised by plating serial dilutions from soil samples, in order to point out several required aspects to be taken into account for further development of high-performance biofilters using plants and associated microorganisms.

2. MATERIALS AND METHODS

2.1. Materials

Literature published in the field has been used to emphasise the major findings of the researchers in the attempt to eliminate the hydrocarbon pollutants using living microorganisms - what has been done and what might be done to improve the biofiltration methods (either microbial or botanical related ones).

For plating microorganisms, standard media were used: potato dextrose agar (PDA) and malt extract agar 2% (MEA), Merck Gmbh, Germany, on 90 mm diameter Petri dishes. Toluene treated and untreated soils samples were taken from: a) the rhizosphere of commercial succulent plants; b) the rhizosphere from succulent plant grown on *in situ* prepared soil at the Botanical Garden of Iasi (Alexandru Ioan Cuza University of Iasi); c) soil rhizosphere from ivy (*Hedera helix*); d) leaves of ivy grown on treated and untreated soil. Controls consisting in soils samples that were treated or not with toluene but were not taken from rhizosphere were also used. The soil of the commercial succulent plant was fibrous and contained perlite, while the other above mentioned soils were amended with commercial compost (containing up to 0.4 g N_{total}/L, 0.25 g P_2O_5/L, 0.6 g K_2O/L). Toluene was used as a model VOC, taking into consideration that this monoaromatic hydrocarbon is often reported as being a dominant pollutant in the contaminated air due the auto traffic pollution in urban locations such as automotive underground spaces [5]. A toluene concentration of about 40 ppm in air was added in a sealed chamber containing the biological components (soil/plant), with a frequency of 3.5 days during the test period of five weeks.

Microscopical analysis was performed using an SZM2 stereomicroscope (Optika, Italy) and a contrast-phase trinocular microscope (Nikon Instruments, Japan).

2.2. Methods

Fungal diversity and number of living cells have been assessed through serial dilution method from soil samples. After plating (0.1 mL per Petri dish), the plates were placed in the dark, at 28 °C, for 3-7 days, in an aerated chamber (Microbiotest Inc). Leaves from Ivy grown on treated and untreated soils were firmly washed (using a vortex) in sterile distilled water and further serial dilution were performed. During incubation, all the plates were visually inspected periodically, and after 3 days the colonies were counted (using a colony counter) and the number of colonies forming units (CFU) per mL at 10^{-2} dilution were calculated. A visual inspection using the stereomicroscope was achieved and then colonies were microscopically analysed (water mounted slides) to assess the major type of grown microorganisms (yeasts, filamentous fungi).

3. RESULTS

As seen in Table 1, a significant decrease in number of CFU was observed for all toluene treated samples. The decrease was both qualitatively (diversity of filamentous fungi observed) and quantitatively (number of CFU). The strongest modification of mycobiota has been observed especially at the soil level, for all plants used, with a decrease in number of CFU to nearly half. Mycobiota recovered on MEA plates is illustrated in Fig.1.

Sample number	Type of sample	CFU mL^{-1}
S1	Ivy leave	130
S2	toluene treated Ivy leave	80
S3	untreated Ivy rhizosphere soil (control)	740
S4	toluene treated Ivy rhizosphere soil	**140***
S5	untreated succulent plant soil of commercial origin (control)	>4000
S6	toluene treated succulent plant soil of commercial origin	2750
S7	untreated succulent plant soil from BGI (control)	1820
S8	toluene treated succulent plant soil from BGI	1160

Table 1. Number of CFU mL^{-1} from 10^{-2} dilution obtained on MEA after 3 days at 28 °C (*total number of CFU exceeded 7000, with a strong increase in abundance of just few species of yeast; BGI – Botanical Garden of Iasi)

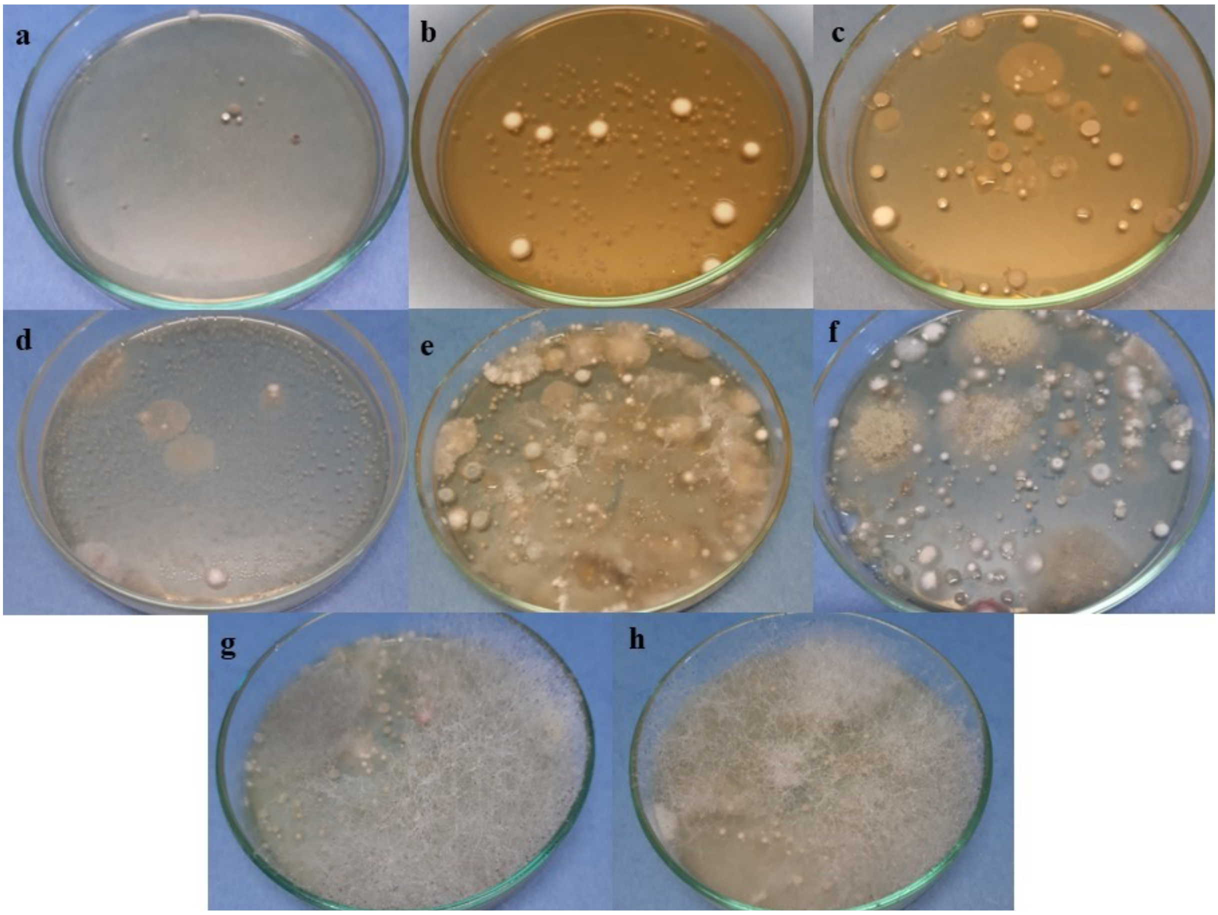

Fig. 1. Mycobiota recovered on MEA plates at 28 °C, after 7 days on incubation (a- Ivy leave; b- toluene treated Ivy leave; c- untreated Ivy rhizosphere soil (control); d- toluene treated Ivy rhizosphere soil; e-untreated succulent plant soil of commercial origin (control); f- toluene treated succulent plant soil of commercial origin; g- untreated succulent plant soil from BGI (control); h- toluene treated succulent plant soil from BGI)

For Ivy rhizosphere soil, the decrease was up to five-fold for filamentous fungi, however, a strong increase of CFU was recorded for one yeast that thrived in the contaminated soil (Table 1, Fig. 1). Diversity of filamentous fungi was different across the soil samples, with some more fungi belonging to Mucoromycota in the GBI soil samples, compared to all the other samples.

4. DISCUSSIONS

As earlier mentioned, hydrocarbons, in numerous forms, resulted from human activities are of major concern due to their impact on various environmental factors [1]. For decades, attempts to eliminate them through biosorption and biodegradation have been done, often referring to soil or water decontamination. Until recent years, most studies conducted in this sense were focused on *in vitro* screening of microorganisms that can degrade or absorb/adsorb pollutants cultivated axenically [11]. Although many achievements were leading to an increased optimism regarding further method development and implementation, applied methods on field did not always yield the expected results [12], as *in situ* there are present communities of microorganisms that interact to each other and many factors that can affect the process. Many authors reported the biodegradation of different hydrocarbons, especially aromatic ones using white-rot fungi [11, 13, 14], a group of fungi that usually grow on wood and posses the ability to efficiently degrade lignin (a natural polymer with an aromatic structure itself) via their ligninolytic enzymes (Ashan *et al.*, 2021; Premnath *et al.*, 2021). The most important such enzymes are Mn-peroxidase, lignin peroxidase and laccases [8, 15, 16]. Species from genera *Peniophora*, *Phanerochaete* and *Phlebia* were degrading at high rates a mixture of four PAHs – phenanthrene, anthracene, fluoranthene and pyrene [11]. *Anthracophyllum discolor* was found to degrade phenanthrene (62%), fluoranthene (54%), anthracene (73%), pyrene (60%) and benzo[a]pyrene (75%) but not in non-autoclaved soil [12], meaning that the presence of other microorganisms is affecting the process. The biodegradation process can be improved when a co-substrate (i.e., glucose) and surfactants are added [17].

Such reports are encouraging, however, in many cases, these fungi would not grow without an additional organic support in soils, waters or packing beds of the filters designed for air cleaning. Even more, the degradation is not fully achieved alone with these species – e.g. laccase from *Ganoderma* sp. degrades naphthalene to catechol and benzoic acid, phenanthrene to 2,2'-diphenic acid, fluorene to phthalic acid [14], compounds that require further degradation.

Other researchers have focused on bacteria or fungi that are able to grow on aromatic hydrocarbons as a sole carbon and energy source. Particularly, it appears that fungi might be more effective in degradation of volatile organic compounds such as toluene [18]. Especially telluric fungi from historical contaminated sites were considered good candidates for PAH degradation and could be considered for the bioremediation of soils contaminated with such compounds, as a sustainable alternative to other classical physical-chemical methods and in response to the actual needs of emergent solutions for saving the polluted environment [19]. Common fungi form genera *Penicillium*, *Cladosporium*, *Fusarium* proved the ability to degrade benzo[a]pyrene. Similarly, species of *Penicillium* and *Aspergillus* isolated from marine sediments on contaminated sites were reported to degrade multiple PAHs up to a level of 75% [20]. Non-ligninolytic fungi degrade PAHs through oxidation via cytochrome P450 monooxigenase [21]. Another involved mechanism refers to the sorption in lipid vacuoles [16] as PAHs are often lipophilic [1]. Isolating species from aged-contaminated sites gives the advantage of the bioaugmentation, making the species that survive in that environment to be more efficient in biodegradation [22]. Despite these promising results, a limited success in applying the technology *in situ* was obtained, as earlier mentioned, likely due to the significant difference between the natural conditions and the laboratory ones.

Several recent studies undertaken on bioremediation / biofiltration of aromatic hydrocarbons are particularly approaching the synergism between fungi and bacteria or between plants and microbial population [23], as these complex interactions allow for pollutants to be degraded or incorporated at a higher efficiency. After bacteria, fungi belong to the most abundant rhizosphere microorganisms, along with protozoa and algae as well [24]. It appears that due to their strong association with plants, some telluric fungi posse great ability to eliminate aromatic hydrocarbons [25], and the plants their self are also contributing to the removal of these compounds [25], along with their epiphytic fungi as well [26]. Even more, simultaneously involvement of multiple microorganisms leads to a cooperative degradation process [27], while many of them became interdependent [21]. Some products of degradation are further mineralized by other species. The synergism between *Acinetobacter* sp. (bacteria) and *Scedosporium* (fungi) was proved by Atakpa and co-workers [28] with a degradation of up to 58.6% of crude oil when co-cultured. Filamentous fungi have, also, the ability to grow and explore porous materials that are inaccessible to many bacteria and yeasts, and during the elongation of the hyphae, they become vectors for unicellular microorganisms, adding effect on biodegradation [21]. As in the case of the white-rot fungi, for the telluric fungi the presence of co-substrates has a positive effect on the biodegradation process, especially when an organic natural matter (such as lignin) is available [29]. Different other factors, such as the pH or temperature are also important [30]. A limitation of this approach is represented by the difficulty of studying the microbial communities in real-time, as some species cannot be grown on standard media [31].

One of the biofiltration challenges involves the design of biofilters for the abatement of VOCs (including MAHs and PAHs), regardless of species or combination used. Gospodarek and coworkers [7] reviewed the literature published on some VOCs biofiltration techniques and show the successful elimination, for example, of styrene, acetone, trichloroethane, hexane, alpha-pinene and toluene with fungi grown on inert packing materials such as perlite. In recent years, biofiltration using fungi became attractive [9, 10]. Comparing to bacteria, fungi are more resistant to low humidity [32, 33] and increased temperature variation [2, 32, 33]. Moreover, co-cultures of yeast and telluric fungi can degrade a wide variety of hydrocarbons (n-hexane, alpha-pinene, trichloroethylene) at very low costs [33]. Zhang and co-workers [34] reported high efficiency in toluene removal with biotrickling filters comprised by *Fusarium oxysporum* continuously operated for 172 days. Some melanized fungi can grow on VOCs as sole carbon source [35], leading the way for self-sustainable biofilters.

It is know that plant soil naturally hosts a variety of microorganisms and this opportunity can can be further explored for developing environmental-friendly cleaning methods of contaminated air. Our own results showed the resistance of some fungal species to the toluene treatment and no visible negative impact on plants. There was a decrease in fungal diversity with the treatment, which was expected, as many microorganisms that inhabit soil are sensitive to such pollutants. However, fungi were recorded on every sample, regardless of the treatment, suggesting that some of the fungi can tolerate and might even feed on toluene. Differences between different soil samples related to the origin of soil have also been recorded, and the commercially available soil appeared to be richer in fungal species, which might be given by the organic matter added in the formulation of soil. A strong decrease in fungal diversity has been observed for the Ivy rhizosphere soil, however, many colonies from a yeast species developed on the sample, suggesting that the mentioned microorganism thrived after the toluene treatment, either using it as a source of nutrients, or by means of decreasing the competition from other species. Increasing the number of CFU in this case, should be seen in the perspective of the biology of the yeast compared to the filamentous fungi – in contrast to the yeast, elongation of the hyphae of the filamentous fungi, if not producing spores, doesn't result in a visible increase in CFU. Therefore, additional studies are required for a better understanding of the process.

5. CONCLUSIONS

Biofiltration of VOCs, including the vapours of the aromatic hydrocarbons, is an attractive method for the removal of these pollutants from air, and therefore an increasing interest for this subject occurred in the recent years. In order to develop such kind of technology, an interesting direction refers to the study of specific microorganisms and especially fungi. Synergism between plants and fungi is of great importance in the efficient and sustainable biotools development. Our preliminary results [5, 36, 37] indicate a good resilience of the used plants for creating filtering walls. For instance, some of the associated fungal species survived (despite the decrease in filamentous fungal diversity and the increase of some yeasts' abundance) after toluene treatment, indicating that there might be an interaction fungi-plant in the process, with no physically affectation of the studied plants. The obtained data suggests that the rhizosphere plays an important role in plant adaptation to toluene exposure and this aspect can be further explored to develop high-performance biofilters using plants and associated microorganisms. Further studies are required to assess the involved mechanisms.

ACKNOWLEDGMENTS

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PCE 39/2021, within PNCDI III.

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