Waste and Biomass Valorization (2019) 10:3821–3831
https://doi.org/10.1007/s12649-019-00802-x

ORIGINAL PAPER

Mycoremediation of Oily Slime Containing a Polycyclic Aromatic Hydrocarbon Mixture

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Received: 15 October 2018 / Accepted: 4 September 2019 / Published online: 9 September 2019

Abstract

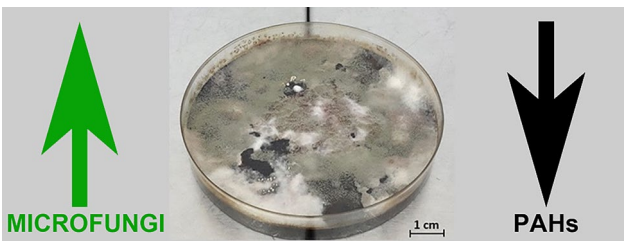
Purpose Polycyclic aromatic hydrocarbons (PAHs) are waste products, which today represent a serious problem in the world due to their high toxicity and difficult removal from the environment. For these reasons, they represent an important and challenging topic of study and research. PAHs may be degraded through biotic pathways including both aerobic and anaerobic degradation by bacteria, fungi, cyanobacteria and eukaryotic algae. In recent decades, fungi have proven very useful in the biodegradation of some of more toxic PAHs, such as anthracene, pyrene, benzo[a]pyrene and fluorene. However, there is a lack of information from an application point of view. This paper sheds light on real-world, polluted matrices that can be degraded by fungi.

Methods Fifteen fungal species were isolated from an oily slime derived from waste products of naval activities and screened to assess their ability to degrade PAH mixtures. The most suitable fungal strains were employed in the degradation treatment.

Results A set of selected microfungi (including *Fusarium solani* along with a fungal consortium of *Pseudallescheria boydii*, *Talaromyces amestolkiae* and *Sordaria fimicola*) was shown to degrade PAHs better than the other fungi considered. The greatest degradation activity was observed during the first week of treatment.

Conclusions The significant relevance of exploiting native fungi to recover marine and terrestrial areas contaminated by PAHs was shown. Moreover, the use of selected fungi isolated from the same contaminated substrate is highly effective in the mycoremediation of recalcitrant pollutants such as oily slime containing PAHs mixture.

Graphic Abstract



Keywords Micro fungal strains · Oily slime · Pahs · Mycoremediation

Statement of Novelty

Our research born from the need to find new techniques, possibly green and economic, as alternative to the traditional technique of remediation applied to the organic recalcitrant compounds deriving from vessel discharge. In our case, we treated real oily slime containing very high concentrations of polycyclic aromatic hydrocarbon. The biotechnique applied to treat this substance is based on the use of microfungi

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12649-019-00802-x) contains supplementary material, which is available to authorized users.

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directly isolated from the considered matrix. Extracted microfungi are adapted to grow up in very contaminated environment and therefore, they are the most indicated for this type of remediation. The obtained results are a valid starting point for the application of the method to treat large volumes at an industrial scale.

Introduction

Nowadays, waste products derived from human life and industrial activity represent a serious problem in the world due to environment removal and disposal difficulties. Although in the past little importance was put on the quality of the environmental, the risk tied to polycyclic aromatic hydrocarbon (PAH) contamination and its associated carcinogenic effects on human health is widely recognized today. Nevertheless, PAH pollution remains a very relevant problem because these compounds are often recalcitrant and highly toxic.

PAHs are a large group of environmental pollutants [1, 2] distributed in aquatic environments [3], sediments, soils [4] and air [5]. They are an incomplete combustion product of wood and fossil fuels, automobile exhausts, power generation plants, refuse burning and industrial emissions [1, 6]. They can also be derived from some natural sources, such as oil seeps, forest fires or volcanic eruptions [7]. PAHs end up in the atmosphere and then fall back on specific environmental compartments, such as soil or water, compromising their productive and ecological functions [8].

Nowadays, the removal of PAHs from environmental compartments is very difficult [9]. The removal effectiveness of these organic pollutants is related to specific physical properties, such as specific gravity (relative density), evaporation tendency, viscosity and the pour point [10], that usually influences both their behavior and persistence in the environment. Furthermore, these properties depend on the chemical composition of the PAHs mixture, proportion of volatile compounds and the presence or absence of more complex hydrocarbon chains [11].

In PAH removal plans, the pollutant solubility also plays an important role; for example, low molecular weight aromatic compounds and alkenes are more soluble in water than alkanes. Moreover, the water solubility of hydrocarbons decreases as the number of saturated substituents linked to the aromatic rings or to the double bonds increases [12]. Finally, site-specific factors (e.g. temperature, pH, salinity, granulometry) can play a significant role in removing these compounds [13, 14].

PAHs have been extensively studied by various researchers due to their environmental persistence, proven adverse effects on plants and soil micro-organisms and the well-known carcinogenic and teratogenic effects on humans [15–17]. The scientific interest in PAHs is mainly related to their known harmful action resulting from the metabolic transformations of these compounds into diol-epoxides that are able to bind to DNA and induce genetic mutations [18].

A serious issue of PAH pollution is the oily slime due to its often high PAH concentration and difficult disposal. Oily slimes are steadily produced by container ships, oil tankers, or merchant ships and disposed in dedicated systems where they are treated with chemical-physical techniques. While traditional remediation techniques for PAH treatment are often limiting due to their costs and poor efficiency while still produce a highly dangerous final waste, bioremediation constitutes a promising alternative. In fact, during the last two decades, bioremediation has received wide acclaim because it requires little energy and may efficiently detoxify recalcitrant organic compounds [19]. Conversion of PAHs can be induced by the action of specialized organisms, such as fungi and bacteria; as seen in several studies dealing with the degradation efficiency of macrofungi [20–23]. Only recently, attention was also addressed to the possible use of microfungi for bioremediation purposes [24, 25]. Some microfungal species have proven able to cope in adverse environmental conditions and can transform PAHs into non-toxic or less toxic compounds by obtaining carbon sources necessary to satisfy their nutritional needs [26–28]. Thereby, selected microfungal organisms may efficiently metabolize PAHs [29, 30]. Specifically, some microfungi have enzymes (e.g. laccase, lignin peroxidase and manganese peroxidase) playing a primary role in the PAH degradation [31].

Among the microfungal genera exhibiting a significant role in PAH degradation processes, there are: *Aureobasidium* [32], *Rhodotorula* [27, 32], *Sporobolomyces* [32], *Geotrichum* [27, 33] and *Rhizopus* [27, 34] isolated from soil and water samples. Garon et al. [35] found that *Absidia cylindrospora* Hagem removed about 90% of fluorene in 12 days from a hydrocarbon-contaminated soil, whereas in the absence of the fungus the process took 24 days. Ye et al. [36] showed that *Aspergillus fumigatus* Fresen was able to degrade anthracene: the anthracene molecular structure was modified into a series of compounds with lower toxicity levels as phthalic anhydride, anthrone and anthraquinone.

The novel aspect of the current investigation is checking whether fungi can degrade real-world polluted matrices. More specifically, we investigated which fungal strains, selected in the matrices themselves, can degrade hydrocarbons. The matrices consisted of oily slimes from a commercial vessel rich in PAHs and hydrocarbons. The use of autochthonous organisms (no foreign strains were employed) represents another intriguing aspect that may foster a possible exploitation of this kind of treatment on a large-scale.

Materials and Methods

Oily Slime Sampling and Laboratory Protocol

Samples of oily slime derived from commercial vessels and containing a high concentration of PAHs were collected from December 2015 to February 2017. The samples were taken directly upon arrival at the treatment plant, stored in sterile glass jars and stored in the dark in a fridge at 5 ± 1 °C to preserve their chemical and physical characteristics. A specific protocol, hereinafter discussed, was employed to isolate vital fungal strains that are biologically adapted to live in this highly contaminated substrate [37]. The complete sequence of laboratory activities is diagrammatically illustrated in Fig. 1.

Fungal Isolation and Identification

Microfungi were counted and isolated from oily slime with a modified dilution plate technique [28] using malt extract agar sea water (MEAs) culture medium (30 ml of medium for each plate). The dilution (performed as v/v) was obtained by mixing 0.5 g or 1 g of oily slime with 100 mL of sterile water in sterile vials. Each sample was plated in triplicate following 1:100 and 1:200 dilutions; later, serial dilutions to 1:50,000 and 1:100,000 were performed.

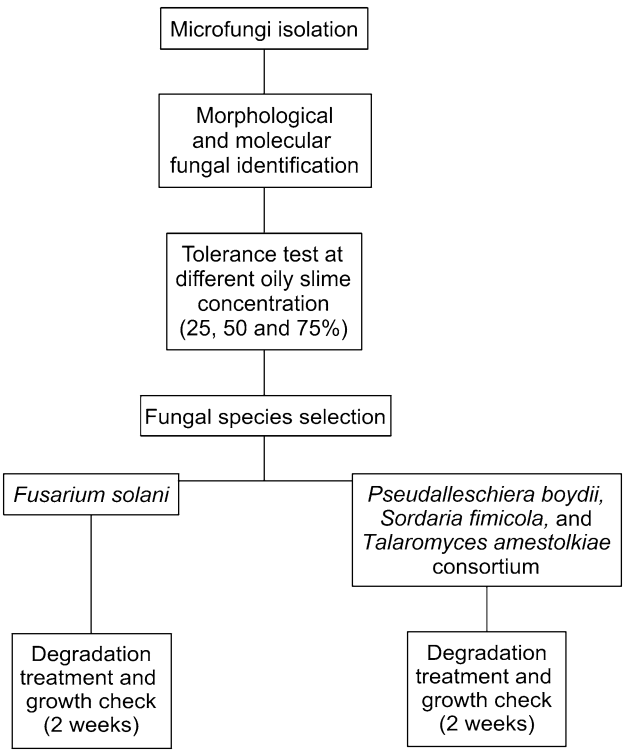

Fig. 1 Scheme of the complete sequence of laboratory activities

Fungal strains were identified by a combined morphological and molecular approach, which determined both the micro- and macro-morphological characteristics using specific taxonomical keys (as an example see references [38–42]) and then by optical microscopy (10×/0.30 to 40×/0.75). The identities of the isolated fungal strains were confirmed using nuclear DNA extraction, PCR amplification and DNA sequencing. The genomic DNA was extracted from 100 mg of fresh fungal culture using a modified CTAB method [43]. The morphological identifications were confirmed by amplification of the β-tubulin gene using Bt2a and Bt2b primers [44] along with the ITS region amplification using universal primers ITS1F/ITS4 [45]. The PCR products were purified and sequenced using the DNA sequencing services of MACROGEN Inc. (Seoul, Republic of Korea). Sequence assembly and editing were performed using the Sequencher® analysis software (version 5.2, Gene Codes Corporation, Ann Arbor, MI USA). Taxonomic assignment of the sequenced samples was carried out using the Nucleotide Basic Local Alignment Search Tool (BLASTN) algorithm to compare sequences obtained in the present study with the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/).

Later, the fungal strains were maintained in axenic cultures of MEA at 4 ± 1 °C and cryopreserved at -20 °C in the culture collection of the Mycological Laboratory of the Department of Earth, Environment and Life Sciences (DISTAV) of the University of Genoa. The sequences obtained were deposited in GenBank with accession numbers from MK499445 to MK499452 and MK503926 for the ITS and from MK519551 to MK519555 for the btub sequences.

Screening and Degradation Tests with Growth Check

A screening test was carried out to select the most suitable fungal strains from the isolates for the oily slime treatment. The oily slime was homogenized and added to the MEA medium at three different concentrations (25%, 50% and 75%) in sterile 9-mm Petri dishes. Later, Petri dishes were inoculated with 1 ml of a 10^8 conidial suspension of each isolated autochthonous fungal strain and incubated in the dark at 24 ± 1 °C. Fungal growth was then monitored daily for a total of 14 days (Fig. 2).

After the screening test, fungal strains exhibiting a higher oily slime tolerance were used in the degradation test. The modified culture medium, 25% of oily slime concentration, with the best fungal growth results in the screening test was used. For the degradation test, two distinct fungal sets were considered: the former consists of only one selected species, while the latter is a consortium of fungal species (fungal consortium, FC). During the degradation tests, the fungal growth was simultaneously checked (Fig. 3). Therefore, once inoculated (with 1 ml of a 10^8 conidial suspension for both

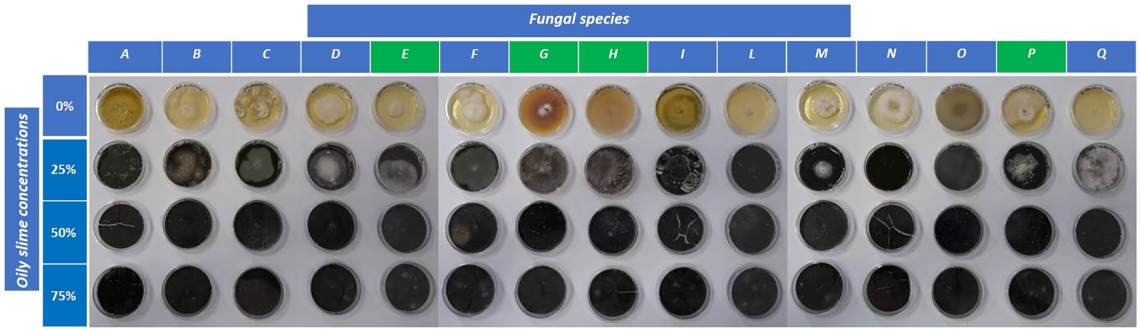

Fig. 2 Screening test of 15 isolated fungal strains to oily slime with different concentrations: from the top to the bottom, plates with 0%, 25%, 50%, and 75% concentrations of oily slime. *A. Trichoderma longibrachiatum*; *B. Aspergillus ochraceus*; *C. Penicillium crustosum*; *D. Galactomyces geotrichum*; *E. Pseudallescheria boydii*; *F. Penicillium antarcticum*; *G. Fusarium solani*; *H. Sordaria fimicola*; *I. Trichoderma harzianum*; *L. Eurotium niveoglaucum*; *M. Penicillium atramentosum*; *N. Rhizopus stolonifer*; *O. Mucor racemosus*; *P. Talaromyces amestolkiae*; *Q. Gibberella zeae*. In green, the only vital fungal species at the highest concentrations of oily slime

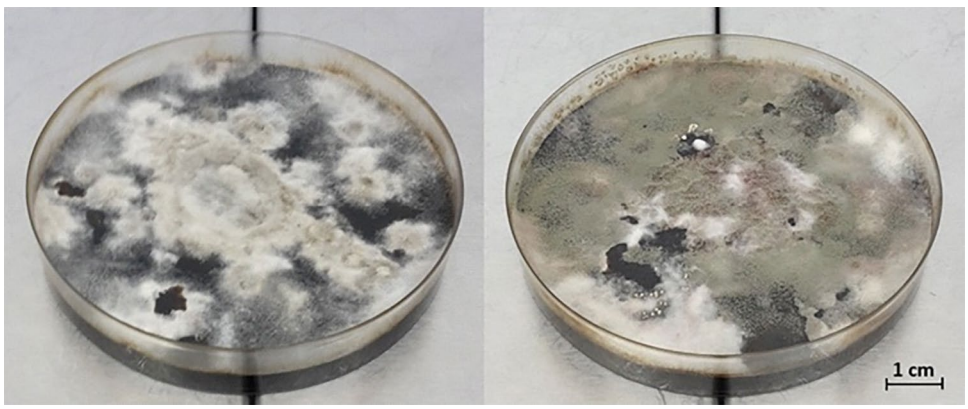

Fig. 3 The two fungal sets used: *Fusarium solani* (left) and fungal consortium (FC) (right) on modified culture media containing oily slime

fungal sets) the Petri dishes containing 25% of homogenized oily slime concentration were incubated at 24 ± 1 °C in the dark. The oily slime + culture medium contained in the Petri dishes was chemically analyzed at the starting time (t_0); the oily slime + biomass + culture medium was analyzed after 7 (t_1) and 14 (t_2) days. Five replicates for each fungal set and for each time (t_0, t_1 and t_2) were employed in the degradation test. To avoid changing the hydrocarbon concentrations and for safety reasons, the modified culture medium containing the oily slime was not sterilized before the treatment. No control plates were checked at time t_1 and t_2: this is motivated by the presence of an irrelevant spontaneous growth of microorganisms in the oily slime. This negligible fungal growth was observed in some preliminary screening tests performed on a portion of the oily slime under investigation. It is worth noting that our observations agree with the ones reported in the research by He et al. [46].

Chemical Analysis and Data Treatment

To determine the degradation efficiency of the mycological screening test and treatment, chemical analysis was carried out. The chemical analysis of the PAH hydrocarbon concentrations in the Petri dishes was assessed in a dedicated analysis laboratory using chromatography. The standard methods of EPA 3550C + EPA 8270E and EPA 3550C + EPA 8015D were carried out. Samples were weighed and extracted by sonication in solvent. The organic extract was then analyzed in a gas chromatograph with flame ionization detector (GC-FID) to carry out heavy hydrocarbon (C > 12) analysis. The chromatogram was integrated over the time range from the dodecane retention (C12) to the tetracontane retention (C40) and quantified through an aging line with a mineral oil standard. A gas chromatograph with a mass spectrometer (GC–MS) was employed to perform the PAH analysis. Later, the analytes were separated and the recognition of each PAH was verified considering the retention time and mass spectrum.

The fungal degradability efficiency was evaluated using the following formula:

$$\Delta C = \frac{C_0 - C_1}{C_0}$$

where ΔC is the fungal degradability efficiency, C_0 is the initial oily slime concentration before fungal treatment and C_1 is the final oily slime concentration after fungal treatment.

Results

The 15 fungal species, listed in Table 1, belonging to 12 genera were isolated from the oily slime. Among the isolates, *Penicillium* and *Trichoderma* were the most represented genera with three and two species, respectively. All of the species were identified with morphological and molecular analysis.

The morphological analysis showed that none of the isolated fungal strains showed different characteristics than those mentioned by the taxonomic keys used [38–42] due to the microhabitat characterized by high concentration of PAHs. Therefore, both the micro- and the macroscopic fungal characteristics are a typical result of the genera.

From the growth and degradative tests, strains showing tolerance to higher oily slime concentrations (50% and 75%), such as *F. solani*, *P. boydii*, *S. fimicola* and *T. amestolkiae*, were selected and used to assess their ability to use the oily slime as source of carbon for the growth. *F. solani* was individually tested while *P. boydii, T. amestolkiae and S. fimicola* were used together as a FC in the degradative test.

The generic culture media MEA was employed to play the role of a starter, enabling fungal strains to develop up to a certain level at which the fungi begin the degradation process. Fungal species growth rate results, during the degradation test, showed that the FC grew faster than *F. solani* in the first week of the treatment (Fig. 4). This data also showed that both fungal sets reached at least 70% of the

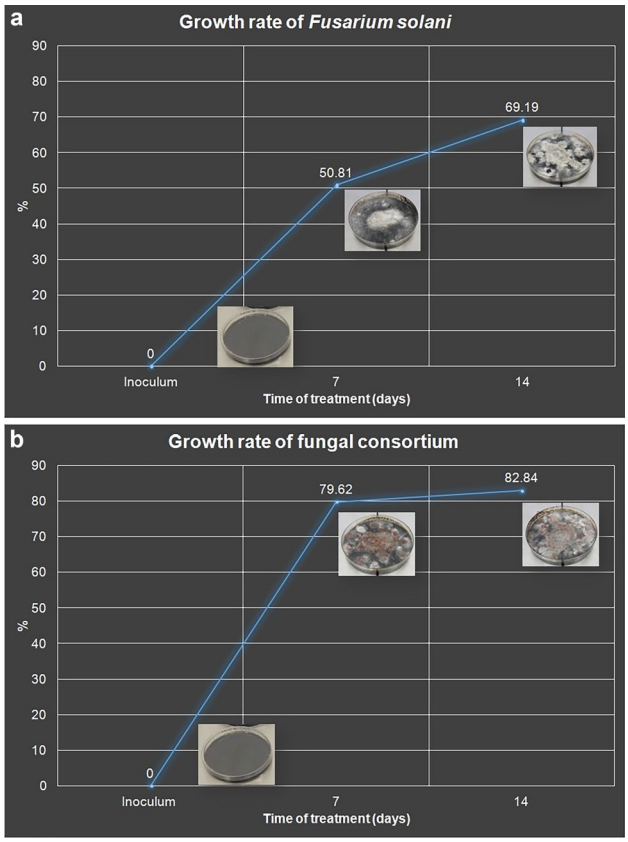

Fig. 4 Growth rate of fungal sets during the 14-day experiment. Above: *Fusarium solani*; below: fungal consortium of *P. boydii, T. amestolkiae* and *S. fimicola*

Table 1 Results of the fungal strains tested at concentrations of 25%, 50%, and 75% of oily slime

Fungal species	75%	50%	25%
Eurotium niveoglaucum (Thom & Raper) Malloch & Cain 1972	–	–	+
Penicillium antarcticum A.D. Hocking & C.F. McRae 1999	–	–	+
Pseudallescheria boydii (Shear) McGinnis, A.A. Padhye & Ajello 1982	+	+	+
Fusarium solani (Mart.) Sacc. 1881	–	+	+
Galactomyces geotrichum (E.E. Butler & L.J. Petersen) Redhead & Malloch 1977	–	–	+
Penicillium crustosum Thom 1930	–	–	+
Penicillium atramentosum Thom 1910	–	–	+
Gibberella zeae (Schwein.) Petch 1936	–	–	+
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not. 1863	+	+	+
Aspergillus ochraceus G. Wilh. 1877	–	–	+
Talaromyces amestolkiae N. Yilmaz, Houbraken, Frisvad & Samson 2012	+	+	+
Mucor racemosus Bull. 1791	–	–	+
Rhizopus stolonifer (Ehrenb.) Vuill. 1902	–	–	+
Trichoderma harzianum Rifai 1969	–	–	+
Trichoderma longibrachiatum Rifai 1969	–	–	+

"+" fungal growth; "–" no fungal growth

plate coverage during the two first weeks of test. The species employed in the FC displayed a good co-growth level, proved to be vital throughout the duration of the test and cooperated in the hydrocarbon degradation process.

Starting from these results, the degradation treatment was carried out using a real oily slime sample for two weeks. Results showed that after 14 days, both *F. solani* and FC revealed a great capability of degrading hydrocarbons. The total hydrocarbons decreased by 88% following mycological treatment with FC but only by 83% with *F. solani*. Acenaphthylene was totally biodegraded by applying both FC and *F. solani* (Fig. 5). Naphthalene decreased by 90% with both FC and *F. solani* while the benzo[a]pyrene concentration diminished 87% with FC but only decreased by 79% from applying *F. solani*. Benzo[e] pyrene was biodegraded by 92% with FC and 84% with *F. solani* while chrysene decreased by 81% with FC but only by 75% following *F. solani* treatment. The pyrene concentration diminished 76% with FC but only 69% after applying *F. solani*. Anthracene was degraded by 74% with FC but only 66% following *F. solani* treatment while phenanthrene decreased by 76% with FC and 69% applying *F. solani*. Fluoranthene, fluorene and benzo[a]anthracene were degraded of 76%, 77% and 68%, respectively, after applying FC whereas only 68%, 71% and 34%, respectfully, following *F. solani* treatment. All the graphs are reported in Online Resource 1.

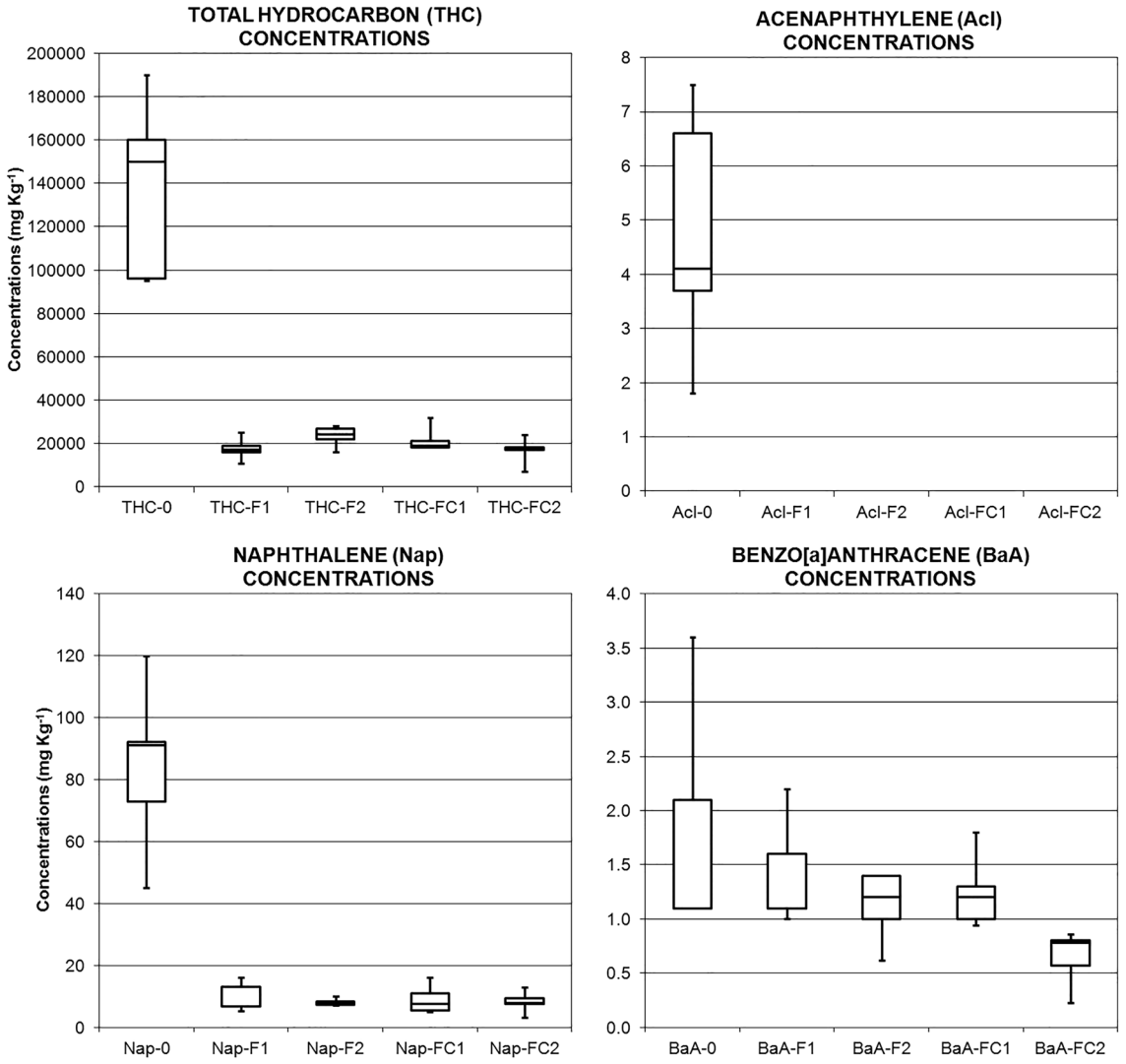

Fig. 5 Boxplots of total hydrocarbons (THC), acenaphthylene (Acl), naphthalene (Nap) and benzo[a]anthracene (BaA) in the samples at time t_0 (0, 5 replies) and in the treated samples with *Fusarium solani* (F) and fungal consortium (FC) at the two treatment times t_1 and t_2 (1: after 7 days, and 2: after 14 days of treatment)

Discussion

Fungal Identification

Among the isolated fungal strains, *Eurotium niveoglaucum* was identified as a fundamental agent of the fermentation process in the indigenous Chinese dark tea by Mao et al. [47]. *Galactomyces geotrichum* is the most important mold for the dairy industry and is present in milk, cheeses and alcoholic beverage in which it is inserted to obtain characteristic aroma and taste for consumers [48]. *Gibberella zeae* is an important pathogen on major cereal crops such as wheat, barley and maize [49]. *Rhizopus stolonifer* is commonly known as black, bread mold and is an agent of plant disease [50]. *Trichoderma harzianum* is used as a fungicide in foliar application, seed treatment and soil treatment for suppression of diseases generated by other fungal pathogens [51]. *Aspergillus ochraceus* was isolated from Spanish and Brazilian grape samples [52] along with coffee pulp [53]. *Penicillium antarcticum*, *Penicillium crustosum*, *Mucor racemosus* and *Trichoderma longibrachiatum* have a worldwide distribution and colonize many habitats such as vegetation products, soil and food.

The particularity and relative rarity of some fungi found in our oily slime sample can provide further information. For example, *E. niveoglaucum* found previously only in Chinese dark tea can provide insight into the origin (China) of the ship from which the oily slime was obtained, the route that the ship makes during its stopovers or the goods that it carried.

PAH Degradation

The results of the screening test highlighted the different growth response that each fungal species found in the oily slime reached inside the studied matrix. At higher concentrations of oily slime (75%) 11 out 15 strains didn't grow due to limiting pollution conditions. These fungal species could have ended up in the original matrix by chance and may have lived in latent conditions, waiting for more suitable growth conditions. The culture media, having more assimilable sources of nutrients (at 25 and 50% of oily slime), may have been just such suitable conditions.

This first phase of the study allowed identification and choice of the most suitable fungal species for PAH degradation purposes. This result was already seen in the degradation of PAHs and other chemical contaminants. For example, Godoy et al. [54] explored the potential of fungi isolated from PAH polluted soil and Mouhamadou et al. [55] studied the potential of autochthonous fungal strains isolated from contaminated soils for degradation of polychlorinated biphenyls (PCBs).

Most studies in the literature have faced a similar problem, addressing however the ability of fungi to degrade a single hydrocarbon compound and starting from samples synthesized in laboratory. Verdin et al. [26] demonstrated fungal ability to break up benzo[a]pyrene; similarly, Ravelet et al. [56] and Romero et al. [57] focused on fungal degradation of pyrene and Wu et al. [58] proved their fungal remediation activity on anthracene. Starting from similar research carried out by He et al. [46] on a real oily slime containing aliphatics, aromatics, N–S–O compounds, asphaltenes and Total Petroleum Hydrocarbon, this work dealt with the fungal capability to degrade a real complex mixture of PAHs (oily slime) produced by human activity. Moreover, compared to existing studies, the novelty of this research lies in suggesting a pool of native filamentous microfungal species for simultaneously degrading a mixture of PAHs over a short treatment time (2 weeks). Such results could open the way for future industrial applications.

Among the selected species, *F. solani* was employed both for its good resistance obtained by the screening test and for its rapid growth rate on the modified culture medium. Additionally, this fungal species was employed for it's well known PAH degrading capacities reported in previous studies [57, 58]. *F. solani* has a cosmopolitan distribution and is active in decomposing cellulolytic plant substrates [42]. It has often been isolated from environments contaminated by PAHs [57–59]. Wu et al. [58] showed that *F. solani* isolated from PAH-contaminated mangrove environments in Ma Wan (Hong Kong) removed anthracene and benzo[a]anthracene by 40 and 60% respectively, after 40 days of fungal treatment. Romero et al. [57] isolated *F. solani* from contaminated sediments of the industrial area near an oil refinery (La Plata, Argentina). These sediments contained 1773 ppm total hydrocarbon and 159 ppm pyrene [60]. *F. solani* was highly active and metabolized pyrene as the sole source of carbon. Hong et al. [61] isolated five strains of *F. solani* from petrol station soil showing over 60% degradation of the supplied pyrene within two weeks.

To evaluate the synergy relationship of more fungal species, fungal consortium formed by strains having tolerance to the highest concentration of oily slime (75%) (*Pseudallescheria boydii, Talaromyces amestalkiae, Sordaria fimicola)* was tested.

Pseudallescheria boydii has a worldwide distribution, particularly in soil [42]. It was isolated from polluted water, composted municipal waste and diesel fuel [42]. It is a well-known saprotrophytic filamentous fungus recognized as a potent agent of infection (human pathogenicity code 2) in both immunocompromised and immunocompetent patients [62, 63]. This fungus causes human infections, called hyalohyphomycosis, which are characterized by the growth of

non-pigmented septate hyphae in the infected tissue [64]. *P. boydii* may also cause invasive disease which can involve the central nervous system [65]. Its resistance to the highest oily slime concentrations (50–75%) shown in the screening test and its good biodegradable potential [29, 60] justify use in our tests. The good hydrocarbon degradation capacities of *P. boydii* and its pathogenicity may not limit a possible industrial application. Our goal is to open new venues in the mycological field aimed at investigating metabolic pathways and extracting enzymes that can play an important role in the degradation process of PAHs [27].

The application of *Talaromyces amestolkiae* in the degradative test is justified by the adaptability to PAH environmental contamination reported by Greco et al. [28], which found this species on PAH-contaminated marine sediments during a survey in the Port of Genoa (North-Western Italy).

Sordaria fimicola has a worldwide distribution, though predominantly in temperate regions [42]. It was mainly isolated from the fresh droppings of horse, hare, rabbit, cow and many other mostly herbivorous animals [42]. Moreover, it is known to degrade various cellulose-containing substrates [42]. However, the use of *S. fimicola* in the degradation test is justified by its capacity for degrading lignin. Raghukumar et al. [66] found, during a study on the treatment of colored effluents with lignin-degrading enzymes, that *S. fimicola* secreted MnP (manganese-dependent peroxidase) and laccase in seawater media. Thus, this fungus belonging to the category of lignin-degrading fungi can be an important tool for bioremediation of matrices contaminated by PAHs. As is well documented, many ligninolytic micro-organisms can degrade PAHs because of the highly unspecific nature of their ligninolytic systems and the resemblance of the lignin structure with PAHs [61, 67].

Our data show that both *F. solani* and FC revealed a high efficiency in degrading most PAHs, especially during the first week of treatment. Moreover, FC compared to *F. solani* shows a better efficiency in the degradation process of PAHs mostly in the second week of fungal mycological treatment.

PAHs, defined as toxic to humans and aquatic organisms, in the environment and bio-accumulatives, such as benzo(a)pyrene, anthracene and fluorene, have been visibly reduced following mycological treatment using both *F. solani* and FC. During a week of mycological treatment, the concentration of total hydrocarbons decreased by at least 83% and acenaphthylene was completely biodegraded. The pyrene concentration was reduced below the limit value of 5 mg kg^{-1} fixed for soils of private, public and residential green areas by the Italian Legislative Decree 152/2006.

The results could be used to implement future large-scale applications considering that the degradability efficiency and the differences found in PAH degradation rate depend on several factors influencing the growth rate, sporulation and metabolite fungal production. Environmental factors such as oxygen concentration, nutrient accessibility, pH, temperature and humidity can affect fungal growth and degradation activity [68, 69]. Chemical characteristics of the matrix such as chemical composition, bioavailability rate and phase (liquid or solid) can also affect the fungal degradation efficiency. The initially low bioavailability rate of PAHs tends to increase with aging and this is a key-constraint to the fungal cleanup of PAH contaminated matrices [70]. Moreover, fungal physiological factors, such as specific enzymatic activity, mass and size of fungi play a fundamental role in the PAH degradation [71]. For example, *F. solani* has cellulosolitic capacity and produces specific lignin-degrading enzymes needful for the degradation process, as confirmed by the literature [72–75]. These enzymes can degrade not only lignin but also several recalcitrant environmental pollutants, such as hydrocarbons and PAHs [72–75].

Our data relating the first week of treatment also show that the FC achieving a higher growth rate and hydrocarbon degradation efficiency than what was obtained with *F. solani*, highlighting the positive effect of the synergistic relationship among the species of the FC. This joint fungal action allowed obtaining similar results to those observed by Anastasi et al. [76], Balaji et al. [77] and Chen et al. [78].

Conclusions

The PAH degradative capacity of filamentous fungi isolated from polluted oily slime was shown. This work represents a contribution to sustainable remediation techniques of polluted matrices and in the selection of specific PAH-degrading fungal strains. Our results highlight the importance of fungal strain selection for mycoremediation purposes and the importance of isolating species already present in the matrix to be treated. Isolation avoids the introduction of alien species and employs organisms already adapted to those environments.

It is necessary to conduct further studies to identify the enzymes involved in PAH degradation and optimize the corresponding biodegradation process. The study of the PAH bioavailable fraction during the fungal degradation activity will be of fundamental importance to devise any appropriate protocols of fungal efficiency augmentation. Furthermore, a deeper investigation on the synergistic relationship between fungi and isolated bacteria may be relevant to better understand how the environmental variables can influence the various degradation process parameters. Finally, a draft protocol could be devised for developing a procedure to exploit the synergistic relationship of fungi and bacteria for PAH biodegradation.

Acknowledgements The authors would like to thank Eurochem Italia for the collaboration in the chemical analysis. The research was

performed in the framework of the PhD STAT (Scienze e Tecnologie per l'Ambiente e il Territorio) curricula in Biology Applied to Agriculture and Environment (University of Genoa, DISTAV) and partially supported with internal funds.

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