Journal of Applied Molecular Biology (JAMB)
MolecularBiology Research & Studies Institute, Assiut University, Egypt.
ISSN 2974-4008
Vol. 1(1): Sep.(2023)

Journal of Applied Molecular Biology (ekb.eg)

Analysis of fungal diversity and structure in the Nile River Nile water polluted with crude oil and naphthalene using microcosm experiments.

Fatma Elzahraa Abdelhamed Mohamed [1]*, Ameer ELfarash [2,3,4] ,Mohamed A. Abdel-Sater [5] and Elhagag Ahmed Hassan [5,6]

[1] Department of Molecular Biology, Molecular Biology Researches & Studies Institute, Assiut University, Assiut, Egypt
[2] Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt.
[3] Department of Applied Biotechnolgy, Molecular Biology Researches & Studies Institute, Assiut University, Assiut, Egypt
[4]School of Veterinary Medicine, Badr University in Assiut, Egypt
[5] Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut, Egypt.
[6]School of Biotechnology, Badr University in Assiut, Egypt
[*]Corresponding Author: Fatma Elzahraa Abdelhamed Mohamed
e-mail: Fatma-elzahraa.Abdelhamed188@mbi.aun.edu.eg

ARTICLE INFO
Article History:

Received:2023-06-14
Accepted:2023-08-14
online :2023-09-12

Keywords:
Crude oil; Aquatic ecosystem; Fungal biodiversity; Naphthalene; Microcosm experiment; Fungal communities

ABSTRACT

Petroleum pollution is considered one of the major environmental issues. Microorganisms play a significant role for removal and degradation of petroleum hydrocarbons. Microbial community, dynamics and functions are well studied in cases of terrestrial ecosystems, while cases with aquatic environments have received much less attention. The obtained data revealed that 22 fungal species related to 9 fungal genera were recovered from hydrocarbon-polluted water. The most common fungal genera recovered from naphthalene and crude oil-polluted water were *Aspergillus*, *Penicillium* and *Trichoderma*. Wherease, the fungal species *Penicillium purpurogenum*, *Trichoderma* sp. and *Aspergillus niger* were recorded in the highest occurrence at the control and the polluted microcosm experiments. The highest fungal diversity indices, number of taxa and individuals, dominance, Simpson index, and Shannon index were recorded in crude oil-polluted water. While the lowest fungal diversity indices were estimated at naphthalene-polluted water. Furthermore, the physio-chemical properties of polluted water samples exhibited obvious correlation and influence on the fungal occurrence during different time intervals compared with the control

microcosm experiment. The cluster analysis of isolated fungi showed a noticeable grouping of the fungal communities in the control microcosm experiment compared with the naphthalene and crude oil-polluted microcosm experiment. The current data indicated that the ecological impact of emerged pollutants on fungal communities gives a varied fungal grouping and structures.

INTRODUCTION

Hydrocarbons are the main energy and fuel sources that are employed most frequently worldwide. It is well recognised that hydrocarbons are harmful substances with detrimental impacts on the environment [1]. Alkanes, cycloalkanes, and other aromatic hydrocarbons with varying dangerous, poisonous, and carcinogenic potential are included in crude oil that is a naturally occurring heterogeneous chemical [2,3]. The manufacturing process of crude oil, its transportation, chemical extraction, and circulation are regarded as the biggest sources of human-caused environmental hydrocarbon pollution [4]. Evidently undesirable spillages that happen during routine processes of crude oil production, refining, and distribution, in addition to as a result of serious accidents, have sustained study interest in this area [5] As well as, The polycyclic aromatic hydrocarbons (PAHs), which include naphthalene, are a broad and diversified category of serious environmental contaminants. Generally speaking, these are environmental toxins that are often found in the air, sediments, and surface and ground waters [6]. The toxicological effects of crude oil or petroleum products differ considerably based on their composition, content, environmental circumstances, and the biological condition of the organisms at the time of contamination [7]. The toxicity of hydrocarbons is better tolerated by microorganisms, especially fungi, and they have the technique for cleaning up spilt oil from the environment due to their physiology and environmental adaptation, [8]. Fungi are an important group of microorganisms found in a variety of aquatic ecosystems ranging from coastal waters [9-11] to the deep biosphere [12-14] and associated with a variety of marine substrates such as intertidal sediments [15,16], floated wood [17], algae [18], and othe animals and mussels [19,20]. Fungal occurrence appears to be highly associated with organic matter, implying that fungi play essential roles as recyclers of complex polymers in the water, such as polysaccharides [21], lignocellulose [22], and even hydrocarbon-based polymers like microplastics [23,24]. Interstingly, it was reported that there are more than 100 fungal species are known to play significant roles in the bioremediation of hydrocarbons [25]. As a result, fungi play an important role in promoting the biodegradation of refractory hydrocarbons by secreting extracellular enzymes that convert these chemicals into intermediates with lower environmental toxicity and boosting further decomposition by bacteria [26]. There have been very little research on fungal bioremediation of petroleum in aquatic environments [27-29]. The effective biodegradation of crude oil by aquatic fungi was proven by measuring changes in total crude oil mass over time [30].

So, this study aimed to 1) isolate of fungal species from hydrocarbon polluted river Nile water as well as control untreated River Nile water using a microcosm design. 2) determine the diversity and fungal communities associated with hydrocarbon polluted

water 3) assay the interaction of recovered fungi with the physicochemical characteristics of water samples from the microcosm experiment 4) analysis the clustering of fungi in polluted River Nile water by crude oil and naphthalene hydrocarbons.

MATERIALS AND METHODS

Sampling and measurement of physio-chemical parameters of collected water

About 10 L of water samples were collected in sterilized flasks from the River Nile at Paradise Park in Assiut Governorate, Egypt, and then the flasks were transferred immediately to the laboratory, and kept in the dark at *in situ* temperatures for further experiments. The water temperature was measured *in situ* using a laboratory thermometer (liquid mercury in a glass tube), pH and water conductivity were measured *ex-situ* using a combined pH and conductivity meter (Jenway Model 3540 conductivity/pH meter), total dissolved solids (Tds), total dissolved organic carbon (TDOC), total dissolved organic nitrogen (TDON) and total dissolved organic sulphur (TDOS) were analyzed by using Analysensysteme GmbH, Donaustr-7, D-63452 Hanau-Germany (Department of Chemistry, Faculty of Science, Assuit University).

The microcosm experimental design

The microcosm experimental design was assayed to simulate the natural habitate according to the following three treatments design. T1, the control experiment containing natural water from the River Nile; T2 (crude oil treatment), was performed with concentrations (2.5 mL of Crude oil) and T3 (naphthalene treatment) was performed with concentrations (0.25 g of Naphthalene) each pollutant was added to 1000 mL River Nile water in 1 L a gas-tight glass bottle in three replicates and 2 g of fresh River Nile mud were added to each bottle. The microcosm experiment was incubated at room temperature with gentle shaking. The impacts of crude oil and naphthalene on microbial composition and their function dynamics were assayed at different time intervals (0, 15, 30, 45, 60, 75 and 90 days).

Isolation and identification of fungal communities at different time intervals during cultivation of previous treatment.

Samples of water were obtained from each treatment of the microcosm experiment and used for fungal isolation by pour plate method on potato dextrose agar (PDA) medium (Potato 200g, glucose 20g/L, agar 20g/L, Rose Bengal 30mg/L), the medium was supplemented with chloramphenicol (250 mg/L) to suppress bacterial growth [31]. The culture medium PDA was poured under aseptic conditions into Petri dishes (9 cm in diameter) containing 1mL of water sample and then Petri dishes (3 replicates) were incubated at 28 °C for 5 days. The growing fungi was purified to procure pure cultures, and then fungal species were described based on their cultural, morphological such as the following (macro and microscopic characteristics), fungal isolates were identified using an Olympus CX41 optical microscope (Department of Botany and Microbiology, Faculty of Science, Assuit University). The fungal taxa were mainly identified based on their unique characteristic conidial morphology as they produce conidiophores and conidiogenous cells according to the relevant taxonomical keys [32-35].

Analysis of fungal diversity and communities

Fungal diversity, cluster analysis and determining the correlation between fungi communities and environmental factors (canonical-correlation analysis (CCA)) were analyzed for all sample using Paleontological Statistics PAST (4.03). As well as

Diversity indices including number of taxa which indicates the number of isolated fungal species from collected samples. Whereas fungal dominance determining the dominance of fungal taxa in treatments, Simpson index illustrates the evenness of the aquatic fungal communityand Shannon index is the diversity index that estimates the number of fungal individuals taking in account the number of fungal taxa. (entropy) [36]. The CCA was performed using the relative abundance of reads data for the fungal phyla and all the physic-chemical factors to identify environmental parameters that significantly correlate with fungi communities.

RESULTS AND DISCUSSION

Characterization of environmental variables

Data in Table (1) exhibited the physic-chemical characteristics of the analyzed water samples. Water temperature at zero time was recorded 36 ^{O}C in September whereas recorded 28 ^{O}C at 90 days in December. It was observed that, water temperature, pH, electrical conductivity, total dissolved solids (Tds), total dissolved organic carbon (TDOC), total dissolved organic nitrogen (TDON) and total dissolved organic sulphur (TDOS) were unevenly distributed and noticeably varied depending upon various pollutants. The highest values of these parameters were recorded in conductivity, which was measured for Naphthalene treatment at 90 days, The lowest conductivity value was recorded in Crude oil treated River Nile water compared to the control. Similarly, results showed that slightly lower or higher conductivity values were recorded in effluent wastewater samples in Turkey [37], in South Africa [38].

The highest value of total dissolved organic carbon (TDOC) parameter was at in the control sample at zero time, then it decreased at 90 days, whereas crude oil showed moderate values at zero time and then decreased at 90 days, and naphthalene showed the lowest value at 90 days. Total dissolved organic nitrogen (TDON) recorded a low value in the control as well as for crude oil, however the highest TDON value was recorded in naphthalene at zero time and 90 days. Total dissolved organic sulphur (TDOS) was recorded as the lowest in all treatments after 90 days.

Table 1*:* Some physicochemical features of water samples of microcosm experiment of River Nile water treated with crude oil and naphthalene.

Treatment	Control		Crude oil		Naphthalene	
Time intervals / Parameters	zero time	90 days	zero time	90 days	zero time	90 days
Temperature (°C)	36°C	28°C	36°C	28°C	36°C	28°C
PH	7.14	7.20	7.02	7.30	6.70	7
Conductivity (µS/cm)	279	279	280	327	343	800
Total dissolved organic carbon (TDOC) (mg/L)	10.17	5.87	5.93	2.94	2.71	1.27

Total dissolved organic nitrogen (TDON) (mg/L)	0.40	0.18	0.18	0.21	6.68	7.78
Total dissolved organic sulphur (TDOS) (mg/L)	2.99	0.49	4.41	0.54	3.34	0.37
Total dissolved solids (Tds) (mg/L)	0.40	0.40	0.40	0.40	0.40	0.60

Analysis of fungal Diversity and communities

The world's extensive consumption of crude oil products contributes to rising environmental pollution [39]. The obtained data presented fungal total count (TC), total count percent (TC%), and the number of case isolation (NCI) of fungi isolated from the River Nile water. The presented data revealed that 22 fungal species belong to 9 fungal genera emerged from River Nile. Seven fungal species were appeared in the three treatments namely: *Trichoderma* sp., *Penicillium purpurogenum, P. duclauxii*, *Neosartorya* sp., *Aspergillus terreus, A. niger, A. fumigatus*. Seven fungal species were appeared in control called: *Acremonium* sp., *Alternaria alternata, Aspergillus oryzae*, *Penicillium brevicompactum, P. citrinum, P.islandicum, Trichurus spiralis*. Two species were appeared in the treatment of crude oil called: *Aspergillus ustus*, *Penicillium glabrum*. Four fungal species were estimated in the treatment of naphthalene, *Aspergillus clavatus, A. flavus, A. sydowii, Epicoccum nigrum*, and one appeared in crude oil and naphthalene together, *Cladosporium cladosporioides*. Data in Table 5 showed that *Aspergillus niger* was the highest occurrence in crude oil treated water, *Penicillium purpurogenum* and *Trichoderma* sp. were the highest occurrence in naphthalene treated water compared with the control experiment. Data in Table 5 showed also that 14 fungal species belonging to 7 fungal genera, *Acremonium* sp., *Alternaria alternata*, *Aspergillus* spp. (*A. fumigatus, A. niger, A. oryza*, and *A. terreus*), *Neosartorya* sp., *Penicillium* spp. (*P. brevicompactum*, *P. citrinum*, *P. duclauxi*, and *P. islandicum*, *P. oxzalicum*, *P. purpurogenum*), *Trichoderma* sp., and *Trichurus spiralis* were isolated from control treated water. Whereas, 11 fungal species belonging to 5 fungal genera, *Aspergillus* (*A. fumigatus*, *A. niger*, *A. terreus*, and *A. ustus*), *Cladosporium cladosporioides*, *Neosartorya* sp., *Penicillium* spp. (*P. duclauxii*, *P. glabrum*, *P. oxalicum*, and *P. purpurogenum*), and *Trichoderma* sp. were isolated from crude oil treated water and 12 fungal species belonging to 6 fungal genera, *Aspergillus* spp. (*A. clavatus, A. flavus, A. fumigatus, A. niger, A. terreus*, and *A. sydowii*), *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Neosartorya* sp., *Penicillium* spp. (*P. duclauxii*, and *P. purpurogenum*), and *Trichoderma* sp. were isolated from naphthalene treated water[40,41].

In this respect, in a certain habitat, biodiversity refers to the variety of living forms. It is often used as a measure of the health of biological systems and is essential for anyone collecting or monitoring any fungi [42]. Both quantitative and qualitative results from the soil fungal diversity analysis show that the presence of hydrocarbons has a greater influence than the presence of heavy metals on the richness and taxonomic composition of the fungal community. Further studies have already shown that multi-pollution has a substantial impact on soil fungus populations [43,44]. The fungus's capacity to exist in surroundings polluted with petroleum products and petroleum fuels shows a mutual link

between these fungi and the goods indicated [45]. These results agree with the reports shown that various species and life stages of organisms are susceptible to pollution in different ways [46]. Due to their capacity to adapt to these challenging conditions by changing in quantity, microorganisms are valuable in forecasting the effects of a certain stress on the environment [47,48] and the elimination of some special types.Data in Table 2 showed that, *Aspergillus niger*, *Penicillium purpurogenum* and *P. duclauxii* were appeared from zero time to 90 days incubation of control microcosm experiment (without treatment). As well, *Trichoderma* sp., was recorded after 30 days to 90 days of incubation. Whearas *Alternaria alternata*, *Penicillium citrinum*, *P. brevicompactum*, *P. islandicum*, *P. oxalicum*, *Aspergillus fumigatus*, *A. oryzae* and *Trichurus spiralis* were recorded in different incubation times. Data in Table 3 exhibited that *Aspergillus niger* was appeared at zero time and continued to 90 days incubation of crude oil treated microcosm experiment and *Trichoderma* sp., was estimated from zero time to 45 days incubation. Whereas *Penicillium duclauxii*, *P. oxzalicum*, *P. glabrum*, *Aspergillus fumigatus*, *Cladosporium cladosporioides* and *Neosartorya* sp., was estimated at different intervals. On the other hand data in Table 4 reveled that *Aspergillus niger*, *A. terreus*, *Penicillium duclauxii* and *Trichoderma* sp., was estimated from zero time to 90 days incubation of naphthalene microcosm experiment and *Neosartorya* sp., was appeared from 15 days to 90 days. whereas *Aspergillus flavus*, was estimated after 60 days to 90 days incubation, *Aspergillus clavatus* was appeared two number of case isolation after 45 days and 60 days incubation. As well, *Penicillium purpurogenum* was recovered two times after 60 days and 75 days incubation of naphthalene microcosm experiment. Whereas *Aspergillus fumigatus*, *Aspergillus sydowii*, *Cladosporium cladosporioides* and *Epicoccum nigrum* were recovered only one time in different incubation time.

It was reported that, the technique, time, and amount of treatment are all crucial variables in defining the extent of hydrocarbons' detrimental actions for aquatic species and the environment as overall [49]. The results showed that some fungi have ability to live in contaminated environment with crude oil and naphthalene for three months but other fungi disappeared in zero time the agreement with previous studies [50].

Table 2: - Total count (TC), total count percent (TC%), No.of case isolation (NCI) of fungi isolated from river Nile water without supplement (Control).

Fungi	0 time			15 days			30 days			45 days			60 days			75 days			90 days			Total		
	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI
Acremonium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	7.14	1	3	50	2	4	9.09	3
Alternaria alternata	0	0	0	0	0	0	0	0	0	1	12.5	1	0	0	0	0	0	0	0	0	0	1	2.27	1
Aspergillus fumigatus	0	0	0	0	0	0	1	33.3	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2.27	3
A. niger	4	50	2	0	0	0	0	0	0	0	0	0	1	33.3	1	2	14.28	1	1	16.67	1	8	18.18	3
A. oryzae	0	0	0	0	0	0	0	0	0	0	0	0	1	33.3	1	0	0	0	0	0	0	1	2.27	1
A.terreus	0	0	0	1	50	1	1	33.3	1	1	12.5	1	0	0	0	0	0	0	0	0	0	3	6.81	3
Neosartorya sp.	1	12.5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.27	0
Penicillium brevicompactum	0	0	0	1	50	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.27	1
P. citrinum	0	0	0	0	0	0	1	33.3	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2.27	1
P. duclauxii	2	25	0	0	0	0	0	0	0	1	12.5	1	1	33.3	1	0	0	0	0	0	0	4	9.09	2
P. islandicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	14.28	1	0	0	0	2	4.54	1
P. oxzalicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	16.67	1	1	2.27	2
P. purpurogenum	1	12.5	1	0	0	0	0	0	0	0	0	0	0	0	0	4	28.57	1	0	0	0	5	11.36	2
Trichoderma sp.	0	0	0	0	0	0	0	0	0	5	62.5	2	0	0	0	4	28.57	1	1	16.67	1	10	22.72	4
Trichurus spiralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	7.14	1	0	0	0	1	2.27	1
Total	8	100	4	2	100	2	3	100	3	8	100	5	3	100	3	14	100	6	6	100	5	44	100	28

Fungi	0 time	15 days	30 days	45 days	60 days	75 days	90 days	Total

	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI
Aspergillus fumigatus	1	11.11	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.5	1
A. niger	1	11.11	1	1	100	1	0	0	0	0	0	0	0	0	0	0	0	0	22	88	1	24	60	3
A. terreus	1	11.11	1	0	0	0	2	100	1	0	0	0	0	0	0	0	0	0	0	0	0	3	7.5	2
A. ustus	3	33.33	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	1	4	10	2
Cladosporium cladosporioides	0	0	0	0	0	0	0	0	0	0	0	0	1	100	1	0	0	0	0	0	0	1	2.5	1
Neosartorya sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100	1	0	0	0	1	2.5	1
Penicillium duclauxii	1	11.11	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.5	1
P. glabrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	1	1	2.5	1
P. oxalicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	1	1	2.5	1
P. purpurogenum	1	11.11	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2.5	1
Trichoderma sp.	1	11.11	1	0	0	0	0	0	0	1	100	1	0	0	0	0	0	0	0	0	0	2	5	2
Total	9	100	7	1	100	1	2	100	1	1	100	1	1	100	1	1	100	1	25	100	4	40	100	16

Table 3: - Total count (TC), total count percent (TC%), No.of case isolation (NCI) of Fungi isolated from river Nile water supplement with Crude oil.

Table 4: - Total count (TC), total count percent (TC%), No.of case isolation (NCI) of Fungi isolated from river Nile water supplement with Naphthalene

Fungi	0 time			15 days			30 days			45 days			60 days			75 days			90 days			Total		
	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI
Aspergillus clavatus	0	0	0	0	0	0	0	0	0	1	50	1	3	17.64	1	0	0	0	0	0	0	4	5.79	2
A. flavus	0	0	0	0	0	0	0	0	0	0	0	0	3	17.64	2	0	0	0	2	8.69	1	5	7.24	3
A. fumigatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	10.52	1	0	0	0	2	2.89	1
A. niger	4	50	2	0	0	0	0	0	0	1	50	1	0	0	0	0	0	0	2	8.69	1	7	10.14	4
A. terreus	2	25	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4.34	1	3	4.34	3
A. sydowii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	8.69	1	2	2.89	1
Cladosporium cladosporioides	0	0	0	1	50	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.44	1
Epicoccum nigrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5.26	1	0	0	0	1	1.44	1
Neosartorya sp	0	0	0	1	50	1	0	0	0	0	0	0	1	5.88	1	0	0	0	2	8.69	2	1	1.44	1
Penicillium duclauxii	1	12.5	1	0	0	0	1	100	1	0	0	0	0	0	0	0	0	0	7	30.43	2	9	13.04	4
P. purpurogenum	0	0	0	0	0	0	0	0	0	0	0	0	9	52.94	1	8	42.11	2	0	0	0	17	24.63	3
Trichoderma sp	1	12.5	1	0	0	0	0	0	0	0	0	0	1	5.88	1	8	42.11	1	7	30.43	2	17	24.63	5
Total	8	100	6	2	100	2	1	100	1	2	100	2	17	100	6	19	100	5	23	69.56	10	69	100	29

Table 5*:* - Total count (TC), total count percent (TC%), No.of case isolation (NCI) of fungi isolated from river Nile water (control and treated with crude oil & naphthalene).

Fungi	Control			Crude oil			Naphthalene		
	TC	TC%	NCI	TC	TC%	NCI	TC	TC%	NCI
Acremonium sp.	4	9.09	3	0	0	0	0	0	0
Alternaria alternata	1	2.27	1	0	0	0	0	0	0
Aspergillus clavatus	0	0	0	0	0	0	4	5.79	2
A. flavus	0	0	0	0	0	0	5	7.24	3
A. fumigatus	1	2.27	3	1	2.5	1	2	2.89	1
A. oryzae	1	2.27	1	0	0	0	0	0	0
A. niger	8	18.18	3	24	60	3	7	10.14	4
A. sydowii	0	0	0	0	0	0	2	2.89	1
A. ustus	0	0	0	4	10	2	0	0	0
A. terreus	3	6.81	3	3	7.5	2	3	4.34	3
Cladosporium cladosporioides	0	0	0	1	2.5	1	1	1.44	1
Epicoccum nigrum	0	0	0	0	0	0	1	1.44	1
Neosartorya sp.	1	2.27	1	1	2.5	1	1	1.44	1
Penicillium brevicompactum	1	2.27	1	0	0	0	0	0	0
P. citrinum	1	2.27	1	0	0	0	0	0	0
P. duclauxii	4	9.09	2	1	2.5	1	9	13.04	4
P. glabrum	0	0	0	1	2.5	1	0	0	0
P. islandicome	2	4.54	1	0	0	0	0	0	0
P. oxzalicum	1	2.27	2	1	2.5	1	0	0	0
P. purpurogenum	5	11.36	2	1	2.5	1	17	24.63	3
Trichoderma sp.	10	22.72	4	2	5	2	17	24.63	5
Trichurus spiralis	1	2.27	1	0	0	0	0	0	0

The diversity of fungal communities recovered from the River Nile samples was calculated including fungal taxa (s) which indicates the number of isolated fungi species from collected samples and other treatments, dominance (d) determining the dominance of fungal taxa in each treatment, Simpson index illustrates the evenness of the aquatic fungal community and Shannon index is the diversity index that estimates the number of fungal individuals taking in the number of fungal taxa. So, the presented data in Figures (1 A, B, C, D, E) showed that the highest taxa of fungi recovered from water polluted with crude oil was recorded at zero time (7.0 fungal taxa) and followed by river Nile water treated with naphthalene after 90 days of microcosm incubation recording (6.0 fungal taxa), the highest dominance of fungi recovered from crude oil treated water was recorded after 15, 30, 45, 60 and 75 days incubation and then decreased after 90 days incubation. Whereas the lowest dominance was estimated after 90 days from naphthalene-treated water recording 0.1667. Whereas the highest Simpson and Shannon diversity indexes for fungi recovered from crude oil treated was estimated during zero

time and 90 days recording (0.8571 and 0.75), (1.946 and 1.386) and followed by naphthalene treated River Nile water at 90 days recorded 0.8333 and 60 days recorded 0.8. The highest Simpson and Shannon diversity indexes for fungi recovered from control treated was estimated at 75 days recording (0.83) and (1.792). Evenness in data refers to how close are the numbers to each species in the three treatments and intervals from zero time to 90 days. It is described mathematically as a diversity index, a measure of biodiversity that counts how numerically equal the community is. In agreement with our results Li et al.[51] showed that a greater Shannon's index and a closer Simpson's index to 1 generate larger fugal variation, which ultimately reflects superior potential for adapting to changes in the environment. Our results support and corroborate the findings of Pietryczuk et al.,[52] who revealed that aquatic fungi typically exhibit a decline in taxonomic diversity as a result of water contamination. Similarly, Selvarajan et al., [53] reported higher levels of contamination lead to reduced fungal species richness and diversity [54]. Long-term soil contamination with crude oil leads to the adaptation of fungi) and, consequently, the development of many adaptive mechanisms [55,56]. In comparison to control soil, polluted soils possess higher fungal diversity, according to several studies [57-59].

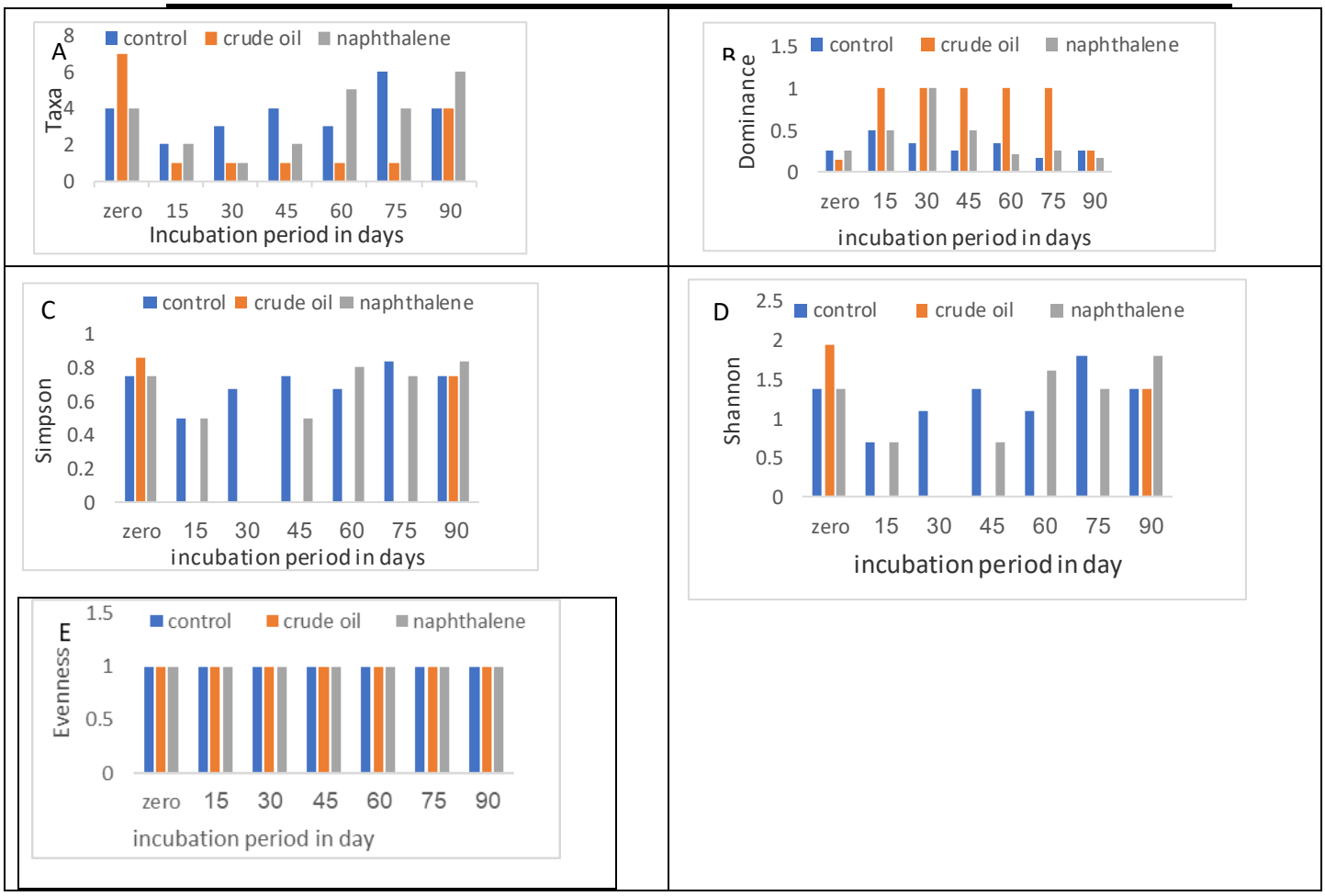
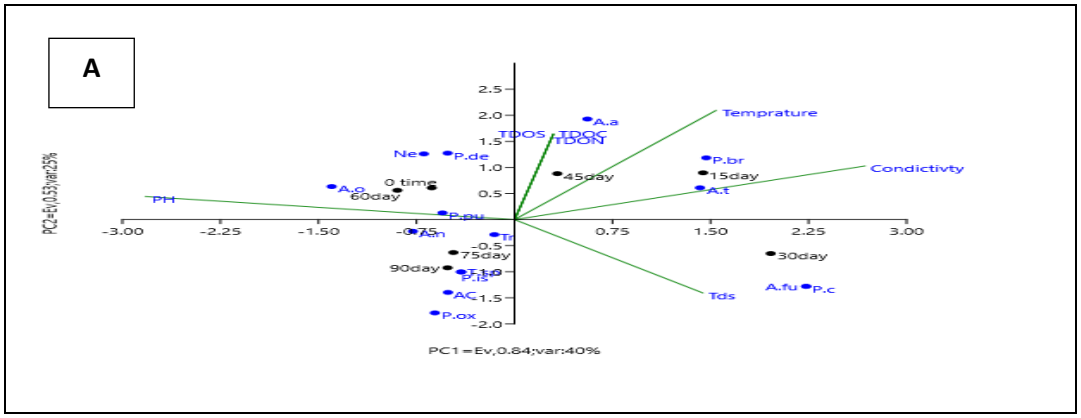


Fig.1: Fungal taxa (A), dominance (B), Simpson (C), Shannon (D) and Evenness (E) biodiversity indexes of mycobiota recovered from microcosm experiments.

Correlation between fungal communities and environmental factors

Canonical correspondence analysis (CCA) demonstrated that the measured physicochemical properties remarkably affected the occurrence of fungi in three treatments as shown in Figure (2 A, B, C). The physic-chemical parameters of River Nile sample in the control microcosm experiment (Fig 2 A) revealed that temperature, conductivity, TDOC, TDON, and TDOS influenced the occurrence of almost 20% the total number of fungi recorded in control after 15 days and 45 days incubation of microcosm, these fungi were *Alternaria alternate, penicillium brevicompactum, Aspergillus terreus.* pH had an influence on the occurrence of almost 30% the total number of fungi recorded in control at zero time and 60 days, these fungi were *Aspergillus* oryzae, *Penicillium purpurogenum, p. duclauxii, Neosartorya* sp. Total dissolved solids (Tds) had an influence on the occurrence of *Aspergillus fumigatus* and *Penicillium citrinum* estimated after 30 days incubation of microcosm experiment. The physicochemical parameters of River Nile in crude oil treated water (Fig 2 B) revealed that conductivity, TDON had an effect on the occurrence of almost 30% the total number of fungi in aquatic ecosystem recorded in crude oil at 90 days, these fungi were *Aspergillus ustus, Penicillium glabrum, P. oxalicum.* pH and tds influenced the

occurrence of almost 30% the total number of aquatic fungi recorded in crude oil at 15 days, 60 days and 75 days, these fungi were *Neosartorya* sp, *Aspergillus niger*, *Cladosporium cladosporioides*. Temperature, TDOC, and TDOS had an influence on the occurrence of almost 40% the total number of fungi recorded in crude oil at zero time and 45 days, these fungi were *Penicillium purpurogenum*, *Penicillium duclauxii*, *Aspergillus fumigatus*, *Trichoderma* sp. The physicochemical parameters of River Nile in Naphthalene treated (Figs 7 C) revealed that pH, TDON, Tds had an influence on the occurrence of almost 25% the total number of aquatic fungi recorded in naphthalene at 60 days and 75 days, these fungi were *Aspergillus fumigatus*, *Penicillium purpurogenum*, *Epicoccum nigrum*. The temperature had an influence on the occurrence of almost 25% the total number of fungi recorded in naphthalene at 15 days and 45 days, these fungi were *Neosartorya* sp., *Cladosporium cladosporioides*, *Aspergillus clavatus*. Total dissolved organic carbon (TDOC) and total dissolved organic sulpher (TDOS) had an influence on the occurrence of almost 15% the total number of fungi recorded in naphthalene at zero time and 30 days, these fungi were *Penicillium duclauxii*, *Aspergillus niger*. Interestingly the obtained data showed that the tested water parameters corresponding to the application of pollutants had an obvious impact on fungal community structure, in line with previous studies in other aquatic systems. Whereas the pH value of water samples did not exhibit any considerable influence on fungal diversity in different treatments, at least in the present study. This was in accordance with the results recorded in various water bodies [60]. Many authors described that pH, temperature, dissolved organic matter, total nitrogen, and electrical conductivity are factors driving the structure of microbial communities in different habitats [61-64]. And Other researchers indicated that of the environmental variables, total nitrogen, water temperature, and pH were the dominant factors affecting fungal community composition.[65].



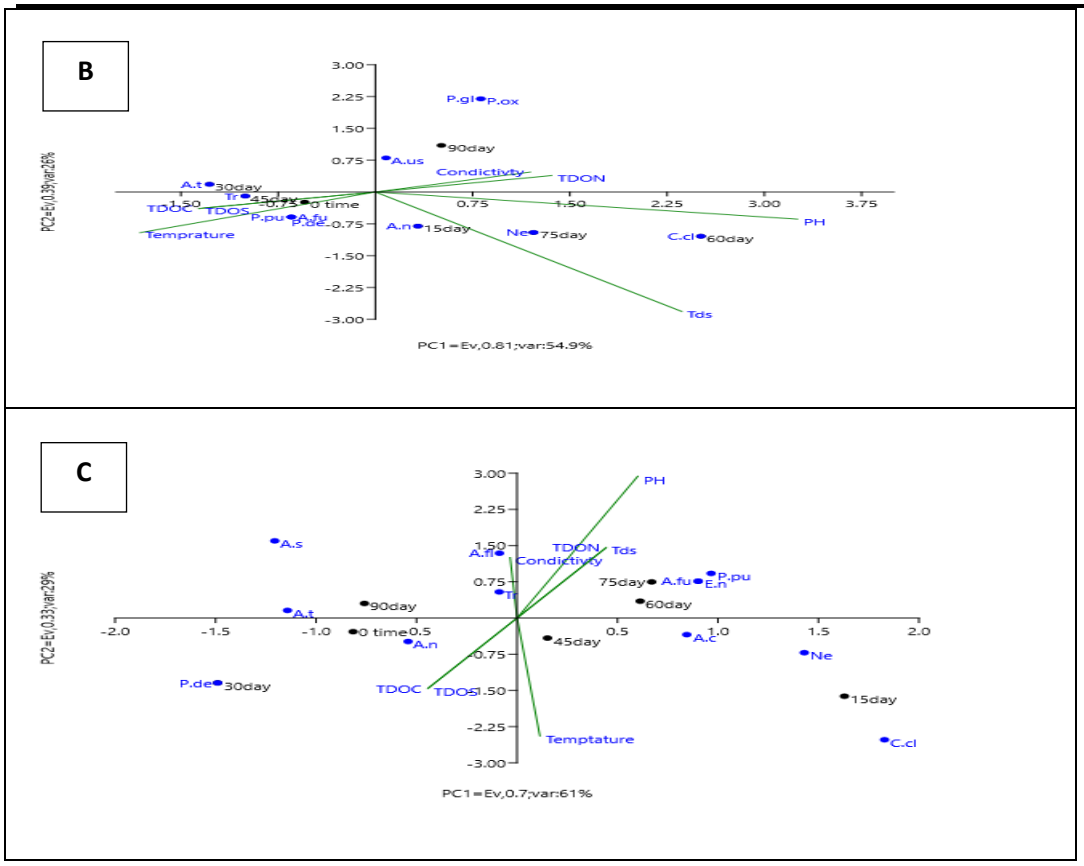
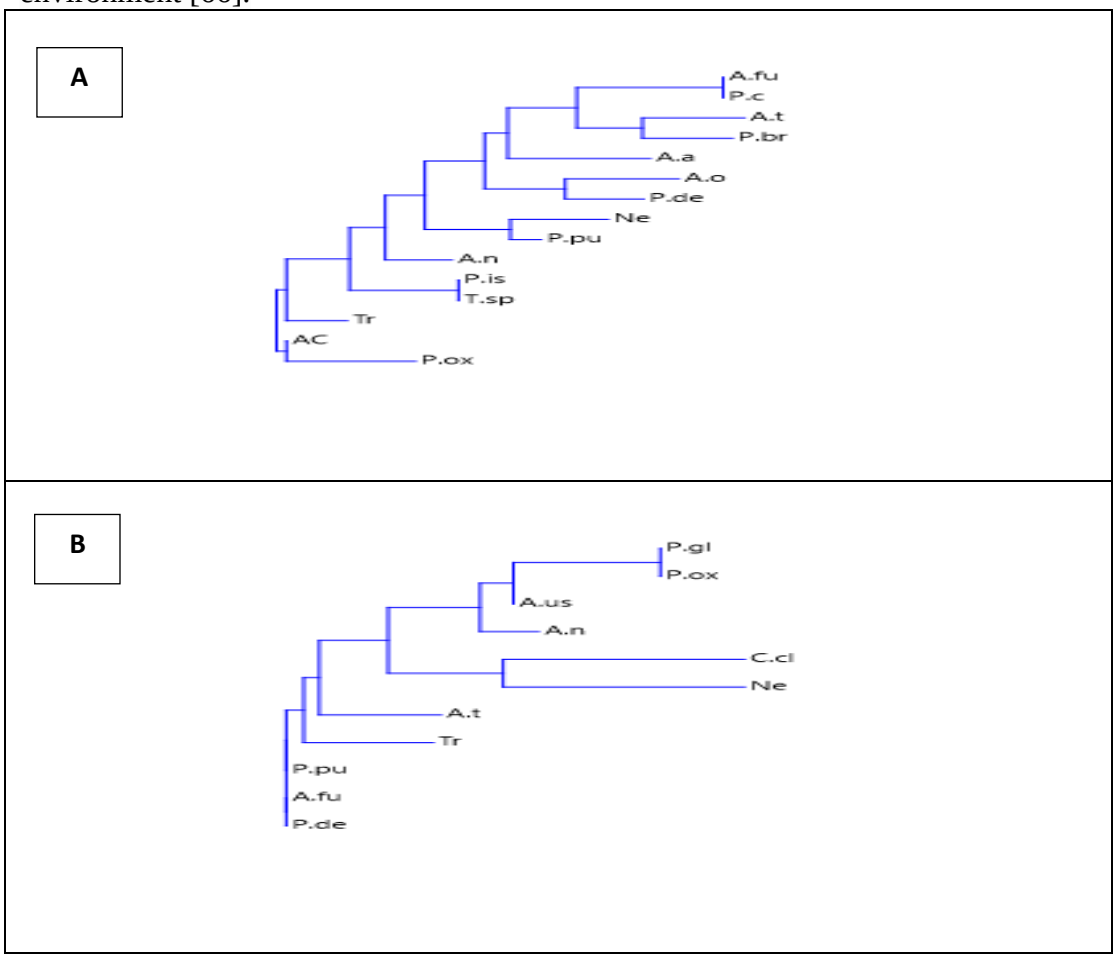


Fig.2: Canonical correspondence analysis (CCA) demonstrated interaction of fungal occurrence with the physicochemical parameters of water samples from the microcosm experiment of Control (A), Crude oil treated (B) and Naphthalene treated experiments (C). P.br= *Penicillium brevicompactum*, A.t= *Aspergillus terreus*, A.a= *Alternaria alternata*, Ne= *Neosartorya* sp, A.o= *Aspergillus oryzae*, P.de= *Penicillium duclauxii*, P.pu= *Penicillium purpurogenum*, A.fu=*Aspergillus fumigatus*, P.c= *Penicillium citrinum*, A.us= *Aspergillus ustus*, P.ox= *Penicillium oxalicum*, P.gl= *Penicillium glabrum*, A.n= *Aspergillus niger*, C.cl= *Cladosporium cladosporioides*, Tr= *Trichoderma*., E.c= *Epicoccum nigrum*, A.c= *Aspergillus clavatus*.

Cluster analysis of fungal communities

The cluster analysis of aquatic fungi showed an obvious grouping of the fungal communities. The obtained data revealed that four separated fungal communities were presented (Fig. 3A) at control microcosm experiment showing the first group of fungi comprising, *Aspergillus terreus*, *Penicillium brevicompactum*, *Aspergillus fumigatus*, *Penicillium citrinum*, *Aspergillus oryzae*, *Penicillium duclauxii*, *Aspergillus niger*, *Penicillium purpurogenum*, *Neosartorya* sp., *Alternaria alternata*, the second group such *Trichoderma* sp., the third group such *Penicillium oxalicum* and the fourth such *Acremonium*. Whereas the microcosm experiment treated with crude oil showed three separated fungal communities as shown in Fig. (3B), The first fungal group included *Aspergillus ustus*, *Penicillium oxalicum*, *Penicillium glabrum*, *Aspergillus niger*, the

second showed *Cladosporium cladosporioides*, *Neosartorya* sp. and the third group comprised from *Aspergillus terreus*. As well as the microcosm experiment treated with naphthalene exhibited three separated fungal communities as shown in Fig. (3C). The first group included *Epicoccum nigrum*, *Penicillium purpurogenum*, *Aspergillus fumigatus*, the second group showed *Cladosporium cladosporioides*, *Neosartorya* sp., *Aspergillus clavatus*, and the third showed *Penicillium duclauxii*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus sydowii*. These alterations in the microbial population are strongly influenced by the nature of the site's overall microbial community, the chemical structure of the crude oil, and the physicochemical variables controlling the specific environment [66].



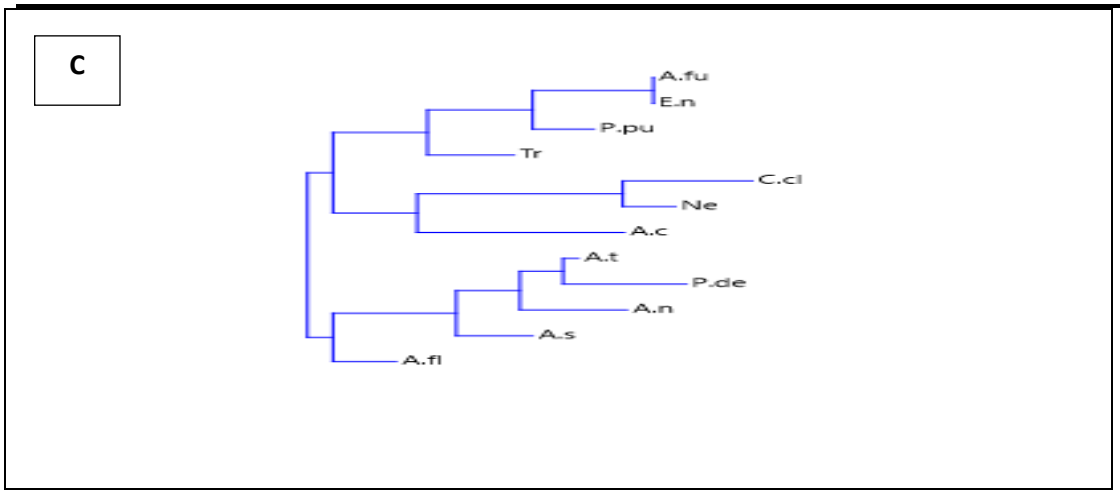


Fig 3: - Cluster analysis showing the fungal communities water River Nile of control (A), crude oil (B), naphthalene (C).

Conclusion

The present investigation revealed that isolating culturable fungi from hydrocarbon-treated water is a promising microbial resource for crude oil pollution removal and bioremediation. However, the specific limitations of bioremediation technique are the presence of metabolically competent microbial communities. In addition to the existence of sufficient levels of nutrients and pollutants, as well as the availability of suitable environmental growth conditions, are crucial site elements required for successful bioremediation. In contrast, the isolation of native fungal isolates from contaminated Nile River water may provide prospective candidates for bioremediation applications within the context of bioaugmentation or biostimulation processes.

REFERENCES

1. Chen, M., Xu, P., Zeng, G., Yang, C., Huang, D., Zhang, J., 2015. Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: applications, microbes and future research needs. Biotechnology advances 33, 745-755.

2. Head, I.M., Jones, D.M., Röling, W.F., 2006. Marine microorganisms make a meal of oil. Nature Reviews Microbiology 4, 173-182.

3. Tian, Y., Luo, Y.-r., Zheng, T.-l., Cai, L.-z., Cao, X.-x., Yan, C.-l., 2008. Contamination and potential biodegradation of polycyclic aromatic hydrocarbons in mangrove sediments of Xiamen, China. Marine pollution bulletin 56, 1184-1191.

4. Totolici, A.-I., Mitrea, S., Cioloca, A.T., Lupu, A., Móricz, P.M., Muntean, D., Negre, R., Topîrceanu, A., Țoc, M., Iordache, D., 2022. Breathing chemicals: a review of air pollution over the years. Studia Universitatis Babes-Bolyai, Biologia 67.

5. Hassanshahian, M., Amirinejad, N., Askarinejad Behzadi, M., 2020. Crude oil pollution and biodegradation at the Persian Gulf: A comprehensive and review study. Journal of Environmental Health Science and Engineering 18, 1415-1435.

6. Usman, S., Yakasai, H., Shukor, M., 2022. Growth Optimization of Naphthalene-Degrading Proteus vulgaris Isolated from Oil-spill Contaminated Soil at NNPC Depot in Northern Nigeria, 13, 502-514.

7. Mossa, A.R., Kasim, A.A., 2022. Isolation of Fungi from Oil-contaminated Soil from Maysan Province, Southern Iraq. Ecology, Environment and Conservation, S103-S110

8. Odili, U., Ibrahim, F., Shaibu-modagbe, E., Atta, H., 2020. Optimization of Crude Oil Biodegradation of Fungi Isolated from Refinery Effluent Site using Response Surface Methodology. Nigerian Journal of Technological Development 17, 257-268.

9. Khallil, A. M.; El-Hissy, F. T. and Ali, E. H (1995): Seasonal fluctuations of aquatic fungi recovered from Egyptian Soil (Delta region). Basic Microbioliology. 35 (2): 93-102.
10. Gutiérrez, M., Pantoja, S., Tejos, E., Quiñones, R., 2011. The role of fungi in processing marine organic matter in the upwelling ecosystem off Chile. Marine biology 158, 205-219.

11. Taylor, J.D., Cunliffe, M., 2016. Multi-year assessment of coastal planktonic fungi reveals environmental drivers of diversity and abundance. The ISME journal 10, 2118-2128.

12. Edgcomb, V.P., Beaudoin, D., Gast, R., Biddle, J.F., Teske, A., 2011. Marine subsurface eukaryotes: the fungal majority. Environmental Microbiology 13, 172-183.

13. Rédou, V., Navarri, M., Meslet-Cladière, L., Barbier, G., Burgaud, G., 2015. Species richness and adaptation of marine fungi from deep-subseafloor sediments. Applied and environmental microbiology 81, 3571-3583.
14. Pachiadaki, M.G., Rédou, V., Beaudoin, D.J., Burgaud, G., Edgcomb, V.P., 2016. Fungal and prokaryotic activities in the marine subsurface biosphere at Peru Margin and Canterbury Basin inferred from RNA-based analyses and microscopy. Frontiers in Microbiology 7, 846.
15. Li, W., Wang, M., Bian, X., Guo, J., Cai, L., 2016. A high-level fungal diversity in the intertidal sediment of Chinese seas presents the spatial variation of community composition. Frontiers in Microbiology 7, 2098.
16. 16- Park, M.S., Oh, S.-Y., Fong, J.J., Houbraken, J., Lim, Y.W., 2019. The diversity and ecological roles of Penicillium in intertidal zones. Scientific reports 9, 13540.

17. Rämä, T., Nordén, J., Davey, M.L., Mathiassen, G.H., Spatafora, J.W., Kauserud, H., 2014. Fungi ahoy! Diversity on marine wooden substrata in the high North. Fungal Ecology 8, 46-58.
18. Gnavi, G., Garzoli, L., Poli, A., Prigione, V., Burgaud, G., Varese, G.C., 2017. The culturable mycobiota of Flabellia petiolata: First survey of marine fungi associated to a Mediterranean green alga. PLoS One 12, e0175941.
19. Hatai, K., 2011. Diseases of fish and shellfish caused by marine fungi. Biology of marine fungi. Springer, pp. 15-52.
20. 2Van Dover, C.L., Ward, M.E., Scott, J.L., Underdown, J., Anderson, B., Gustafson, C., Whalen, M., Carnegie, R.B., 2007. A fungal epizootic in mussels at a deep-sea hydrothermal vent. Marine Ecology 28, 54-62.
21. Cunliffe, M., Hollingsworth, A., Bain, C., Sharma, V., Taylor, J.D., 2017. Algal polysaccharide utilisation by saprotrophic planktonic marine fungi. Fungal Ecology 30, 135-138.
22. Arifeen, M.Z.U., Liu, C.-H., 2018. Novel enzymes isolated from marine-derived fungi and its potential applications. United J. Biochem. Biotechnol 1, 1-11.
23. Paço, A., Duarte, K., da Costa, J.P., Santos, P.S., Pereira, R., Pereira, M., Freitas, A.C., Duarte, A.C., Rocha-Santos, T.A., 2017. Biodegradation of polyethylene microplastics by the marine fungus Zalerion maritimum. Science of the Total Environment 586, 10-15.
24. Lacerda, A.L.d.F., Proietti, M.C., Secchi, E.R., Taylor, J.D., 2020. Diverse groups of fungi are associated with plastics in the surface waters of the Western South Atlantic and the Antarctic Peninsula. Molecular Ecology 29, 1903-1918.
25. Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Gao, X., Li, F., Li, H., Yu, H., 2018. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. Frontiers in Microbiology 9, 2885.
26. Steliga, T., 2012. Role of Fungi in Biodegradation of Petroleum Hydrocarbons in Drill Waste. Polish Journal of Environmental Studies 21.
27. Hassanshahian, M., Tebyanian, H., Cappello, S., 2012. Isolation and characterization of two crude oil-degrading yeast strains, Yarrowia lipolytica PG-20 and PG-32, from the Persian Gulf. Marine pollution bulletin 64, 1386-1391.
28. McGenity, T.J., Folwell, B.D., McKew, B.A., Sanni, G.O., 2012. Marine crude-oil biodegradation: a central role for interspecies interactions. Aquatic biosystems 8, 1-19.
29. Simister, R.L., Poutasse, C., Thurston, A., Reeve, J., Baker, M., White, H., 2015. Degradation of oil by fungi isolated from Gulf of Mexico beaches. Marine pollution bulletin 100, 327-333.
30. Bovio, E., Gnavi, G., Prigione, V., Spina, F., Denaro, R., Yakimov, M., Calogero, R., Crisafi, F., Varese, G.C., 2017. The culturable mycobiota of a Mediterranean marine site after an oil spill: isolation, identification and potential application in bioremediation. Science of the Total Environment 576, 310-318.

31. Roberts, R., 1963. A study of the distribution of certain members of the Saprolegniales. Transactions of the British Mycological Society 46, 213-224.

32. Nilsson, S., 1964. Freshwater hyphomycetes. Symbolae Botanicae Upsalienses 18, 1-130.

33. Webster, J., 1981. Morphology, distribution, and ecology of conidial fungi in freshwater habitats. Biology of conidial fungi, 295-355.

34. Bärlocher, F., 2012. The ecology of aquatic hyphomycetes. Springer Science & Business Media.

35. Marvanová, L., 1997. Freshwater hyphomycetes: a survey with remarks on tropical taxa. Tropical mycology, 169-226.

36. Harper, D.A., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 4.

37. Tanyol, M., Demir, V., 2016. Correlations between some operation parameters and efficiency evaluation of domestic wastewater treatment plant in Tunceli (Turkey). Desalination and water treatment 57, 28115-28121.

38. Assress, H.A., Selvarajan, R., Nyoni, H., Ntushelo, K., Mamba, B.B., Msagati, T.A., 2019. Diversity, co-occurrence and implications of fungal communities in wastewater treatment plants. Scientific reports 9, 1-15.

39. Nwaichi, E.O., Frac, M., Nwoha, P.A., Eragbor, P., 2015. Enhanced phytoremediation of crude oil-polluted soil by four plant species: effect of inorganic and organic bioaugumentation. International journal of phytoremediation 17, 1253-1261.

40. Garzoli, L., Gnavi, G., Poli, A., Prigione, V., Varese, G., 2015. Marine fungi in the Mediterranean Sea, hidden biodiversity and taxonomical challenges. Second Inrterntaional Workshop Ascomycete Systemtatics, Abstract p24, CBS, Amsterdam.

41. Alwakeel, S.S., 2017. Molecular identification of fungi isolated from coastal regions of Red Sea, Jeddah, Saudi Arabia. Journal of the Association of Arab Universities for Basic and Applied Sciences 24, 115-119.

42. Primicia, I., Camarero, J.J., de Aragón, J.M., de-Miguel, S., Bonet, J.A., 2016. Linkages between climate, seasonal wood formation and mycorrhizal mushroom yields. Agricultural and Forest Meteorology 228, 339-348.

43. Bourceret, A., Cébron, A., Tisserant, E., Poupin, P., Bauda, P., Beguiristain, T., Leyval, C., 2016. The bacterial and fungal diversity of an aged PAH-and heavy metal-contaminated soil is affected by plant cover and edaphic parameters. Microbial ecology 71, 711-724.

44. Okrasińska, A., Decewicz, P., Majchrowska, M., Dziewit, L., Muszewska, A., Dolatabadi, S., Kruszewski, Ł., Błocka, Z., Pawłowska, J., 2022. Marginal lands

and fungi–linking the type of soil contamination with fungal community composition. Environmental Microbiology 24, 3809-3825.

45. Alrumman, S.A., Standing, D.B., Paton, G.I., 2015. Effects of hydrocarbon contamination on soil microbial community and enzyme activity. Journal of King Saud University-Science 27, 31-41.

46. Bovio, E., Gnavi, G., Prigione, V., Spina, F., Denaro, R., Yakimov, M., Calogero, R., Crisafi, F., Varese, G.C., 2017. The culturable mycobiota of a Mediterranean marine site after an oil spill: isolation, identification and potential application in bioremediation. Science of the Total Environment 576, 310-318.

47. Liu, J., Wang, J., Gao, G., Bartlam, M.G., Wang, Y., 2015. Distribution and diversity of fungi in freshwater sediments on a river catchment scale. Frontiers in Microbiology 6, 329.

48. Brito, E.M., De la Cruz Barrón, M., Caretta, C.A., Goñi-Urriza, M., Andrade, L.H., Cuevas-Rodríguez, G., Malm, O., Torres, J.P., Simon, M., Guyoneaud, R., 2015. Impact of hydrocarbons, PCBs and heavy metals on bacterial communities in Lerma River, Salamanca, Mexico: investigation of hydrocarbon degradation potential. Science of the Total Environment 521, 1-10.

49. Tong, R., Yang, X., Su, H., Pan, Y., Zhang, Q., Wang, J., Long, M., 2018. Levels, sources and probabilistic health risks of polycyclic aromatic hydrocarbons in the agricultural soils from sites neighboring suburban industries in Shanghai. Science of the Total Environment 616, 1365-1373.

50. Maamar, A., Lucchesi, M.-E., Debaets, S., Nguyen van Long, N., Quemener, M., Coton, E., Bouderbala, M., Burgaud, G., Matallah-Boutiba, A., 2020. Highlighting the crude oil bioremediation potential of marine fungi isolated from the Port of Oran (Algeria). Diversity 12, 196.

51. Li, P., Wu, Z., Liu, T., Wang, Y., 2016. Biodiversity, phylogeny, and antifungal functions of endophytic fungi associated with Zanthoxylum bungeanum. International Journal of Molecular Sciences 17, 1541.

52. Pietryczuk, A., Cudowski, A., Hauschild, T., Świsłocka, M., Więcko, A., Karpowicz, M., 2018. Abundance and species diversity of fungi in rivers with various contaminations. Current microbiology 75, 630-638.

53. Selvarajan, R., Sibanda, T., Sekar, S., Nel, W.A., 2019. Industrial effluents harbor a unique diversity of fungal community structures as revealed by high-throughput sequencing analysis. Polish Journal of Environmental Studies 28, 2353-2362.

54. Gałązka, A., Grządziel, J., Gałązka, R., Ukalska-Jaruga, A., Strzelecka, J., Smreczak, B., 2018. Genetic and functional diversity of bacterial microbiome in soils with long term impacts of petroleum hydrocarbons. Frontiers in Microbiology 9, 1923.

55. Grządziel, J., Gałązka, A., 2019. Fungal biodiversity of the most common types of polish soil in a long-term microplot experiment. Frontiers in Microbiology 10, 6.

56. Wolińska, A., Gałązka, A., Kuźniar, A., Goraj, W., Jastrzębska, N., Grządziel, J., Stępniewska, Z., 2018. Catabolic fingerprinting and diversity of bacteria in mollic gleysol contaminated with petroleum substances. Applied Sciences 8, 1970.

57. Deshmukh, R., Khardenavis, A.A., Purohit, H.J., 2016. Diverse metabolic capacities of fungi for bioremediation. Indian journal of microbiology 56, 247-264.

58. Borowik, A., Wyszkowska, J., Gałązka, A., Kucharski, J., 2019. Role of Festuca rubra and Festuca arundinacea in determinig the functional and genetic diversity of microorganisms and of the enzymatic activity in the soil polluted with diesel oil. Environmental Science and Pollution Research 26, 27738-27751.

59. Khallil, A.-R.M., Ali, E.H., Hassan, E.A., Ibrahim, S.S., 2020. Biodiversity, spatial distribution and seasonality of heterotrophic straminipiles and true zoosporic fungi in two water bodies exposed to different effluents at Assiut (Upper Egypt). Czech Mycology 72, 43-70.

60. Chan, C.S., Chan, K.-G., Ee, R., Hong, K.-W., Urbieta, M.S., Donati, E.R., Shamsir, M.S., Goh, K.M., 2017. Effects of physiochemical factors on prokaryotic biodiversity in Malaysian circumneutral hot springs. Frontiers in Microbiology 8, 1252.

61. Khomich, M., Davey, M.L., Kauserud, H., Rasconi, S., Andersen, T., 2017. Fungal communities in Scandinavian lakes along a longitudinal gradient. Fungal Ecology 27, 36-46.

62. Li, S., Wu, F., 2018. Diversity and co-occurrence patterns of soil bacterial and fungal communities in seven intercropping systems. Frontiers in Microbiology 9, 1521.

63. Khallil, A.-R.M., Ali, E.H., Hassan, E.A., Ibrahim, S.S., 2020. Biodiversity, spatial distribution and seasonality of heterotrophic straminipiles and true zoosporic fungi in two water bodies exposed to different effluents at Assiut (Upper Egypt). Czech Mycology 72, 43-70.

64. 64- Bai, Y., Wang, Q., Liao, K., Jian, Z., Zhao, C., Qu, J., 2018. Fungal community as a bioindicator to reflect anthropogenic activities in a river ecosystem. Frontiers in Microbiology 9, 3152.

65. 65--Fernandes, E.G., Pereira, O.L., da Silva, C.C., Bento, C.B.P., de Queiroz, M.V., 2015. Diversity of endophytic fungi in Glycine max. Microbiological research 181, 84-92.

66. 66-Ortiz-Vera, M.P., Olchanheski, L.R., da Silva, E.G., de Lima, F.R., Martinez, L.R.d.P.R., Sato, M.I.Z., Jaffé, R., Alves, R., Ichiwaki, S., Padilla, G.,

2018. Influence of water quality on diversity and composition of fungal communities in a tropical river. Scientific reports 8, 14799.