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Evaluation the Fungal Biodiversity and Structure in Nile River Water Treated with the Emerging Pollutant Ibuprofen

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ABSTRACT

Freshwater is an essential resource in our life as it serves many human requirements including drinking, industrial and agricultural purposes. The aquatic ecosystem was negatively impacted by urbanization and anthropogenic water pollution. Ibuprofen is considered one of the most common pharmaceuticals used worldwide and is discharged into water by different anthropogenic activities, leading to water contamination and consequently affecting biodiversity and microbial communities of aquatic ecosystems. As the principal producers of extracellular enzymes that enable the breakdown of highly polymeric compounds in aquatic environments, fungi play a crucial function in the aquatic ecosystem. The reservoir system's fungal diversity and its mechanism for the breakdown of pharmaceuticals is still greatly unexplored. Using a microcosm experiment, the impact of ibuprofen (as an emerging pollutant) on fungal diversity was investigated. The obtained results revealed that 26 fungal species related to 16 fungal

genera were isolated from water collected from the microcosm experiment. The highest occurrence of fungal species from the control microcosm was recorded for *Trichoderma* sp. whereas the highest occurrence was recorded for *Aspergillus niger* recovered from ibuprofen treated ones. Furthermore, it was found that ibuprofen-contaminated water had adverse effects on fungal taxa, individuals, and Simpson and Shannon indexes compared to the control microcosm experiment (without ibuprofen contamination). whereas, fungal dominance was increased in ibuprofen-polluted water compared to the control. The current study provides a valuable vision for understanding the impact of the emerging pollutant ibuprofen on fungal diversity and clustering in the aquatic ecosystem regarding fungal adaptation and their putative role as bioindicators for ibuprofen contamination in aquatic ecosystems.

INTRODUCTION

Freshwaters are a group of the aquatic ecosystem on earth, they incorporate lakes, rivers, streams, ponds, wetlands, bogs, and springs [1]. They have a vital role in many human requirements such as drinking water, and agricultural and industrial requirements [2]. Fungi are considered one of the most diverse groups of microorganisms in aquatic ecosystems, which is important to nature as a decomposer, a mutualist, or a pathogen [3]. They make use of all of the polymeric substrates and nutrients present in aquatic bodies [4]. The majority of aquatic fungi were recorded in freshwaters [5] and their biodiversity is still unexplored till now and needs extensive research which challenges the microbial ecology today [6]. Some fungi break down wood and leaves [7] and others are important sources of food and nutrients for filter-feeding zooplankton and copepods [8,9]. Furthermore, fungi play an essential role in the bioremediation of pollutants in aquatic systems [10]. Pharmaceuticals, which include antibiotics, antidepressants, cancer therapies, antiepileptics, hormonal drugs, and medications for inflammation, are among the most diverse types of aquatic pollutants. These emerged pollutants are discharged in freshwater ecosystems due to urbanization and anthropogenic heavy activities and consequently lead to a decrease in water quality and changes in fungal communities and diversity. Interestingly, members of the Basidiomycota and Ascomycota, and other aquatic fungi can break down these compounds [11, 12] and various contaminants in

inland waters via four processes. In the first process, they create two different categories of extracellular enzymes: peroxidases and phenoloxidases [10, 13-15]. The second involves the synthesis of low-molecular-weight redox mediators outside of cells, such as reactive oxygen species (including singlet oxygen, peroxide, and the hydroxyl radical) [16, 17]. Whereas the third process consists of intracellular breakdown caused by enzymes such as the cytochrome P450 complex [18]. The fourth concerns the biosorption of substances on fungal cell walls [19].

Richardson, and Bowron [20] were the first who introduced pharmaceuticals such as environmental contaminants, pharmaceutical and personal care products and are classified as Emerging Organic Contaminants (EOCs) [21] that can decrease water quality more than any other pollutants because it cannot be removed by traditional wastewater treatment plants (WWTPs). They can enter the water as effluent from houses, industries and hospitals and they may exist in drinking water [22]. Ibuprofen [2-(4-isobutylphenyl)-propionic acid] is a type of non-steroidal anti-inflammatory drug (NSAID) used over-the-counter (OTC), as a potent analgesic to treat pain, inflammation and fever [23]. Ibuprofen was detected in water systems used for irrigation [24-27] and municipal drinking water supplies [28]. About 15% of ibuprofen was found in human urine as an unchanged molecule [29]. and the medication is still found in the aquatic environment at low to trace amounts [30]. Because of the continuous consumption of ibuprofen, its toxicity in the aquatic environment is also increased by time due to the cumulative effects and its elimination cannot be achieved by the traditional methods [8]. Consequently, ibuprofen exhibited harmful effects on the aquatic environment and the microbial biomass of some communities [31], as detected for microbial diversity in aquatic mesocosms [32]. Ibuprofen has toxic effects on several aquatic creatures, such as the Japanese rice fish, zebrafish, and planktonic crustaceans, were found to be negatively impacted by ibuprofen's which effects on their reproduction [33]. Ibuprofen (0.1 and 0.3 mg/L) had an adverse effect on the development of the microalgal diatom *Phaeodactylum tricorutum* [34]. Plant poisoning is a result of ibuprofen exposure which decreased the length of shoots and roots, leaf area, carotenoid, chlorophyll a and b, glutathione reductase, total chlorophyll, mineral (K and Mg) and soluble protein contents in cowpea (*Vigna unguiculata*), while simultaneously increasing the contents of Ca and Mn, sodium

translocation from roots to shoots, H₂O₂, malondialdehyde, superoxide dismutase, catalase, and ascorbate peroxidase [35]. Recently, many studies were focused on the ability of ibuprofen degradation by physical, chemical and biological methods [36]. Furthermore, the degradation of different organic wastes including municipal solid waste (MSW) containing diverse types of EOCs, pesticides, Pharmaceuticals and personal care products (PPCPs), and xenobiotics can be evaluated by microorganisms because various microorganisms have ability to degrade ibuprofen in different ways [37]. Compared to traditional approaches, the biodegradation methods have certain advantages because it is safe, nontoxic a natural process, economical, and has a wide range of favourable conditions. The way by which microorganisms metabolize ibuprofen is not well understood. However, several organisms could degrade the drug producing hydroxyl- and carboxylated-ibuprofen [38-42]. In this study, we investigated the effect of ibuprofen, as it is an emerging pollutant, on fungal diversity as well the fungal community of Nile River water using a microcosm experiment.

MATERIALS AND METHODS

Experimental design.

About 10 L of Nile water was collected from the Nile River in Asyut Governorate, one of the largest cities of modern Egypt, into sterilized bottles and transported immediately to the laboratory and kept in the dark at *in situ* temperatures. In the microcosm experiment, 100 mg of commercial ibuprofen were added to 1000 ml Nile River water in 1 L sterilized glass bottles, and 2 g of fresh Nile River mud (rich with microbial communities and nutrients) were added and the microcosm experiment was incubated at the room temperature with gentle shaking. As well as, the control microcosm experiment was performed without the addition of ibuprofen. The microcosm experiment was performed in three replicates. The impacts of ibuprofen on fungal composition and their function dynamics were measured at different incubation time intervals (0, 15, 30, 45, 60, 75 and 90 days).

Physico-chemical analysis of water samples

Physical characteristics of the water sample collected from the microcosm experiment such as pH value, total dissolved solids (T.D.S), and conductivity were measured *ex-situ* using pH/conductivity meter (Jenway Model 3450 conductivity/pH meter), total dissolved organic carbon (TDOC) and total dissolved organic nitrogen (TDON) were measured using CHNS analyser in the central laboratory at Faculty of Science, Assiut University. The total dissolved phosphorus (TDP) was measured in the central laboratory for chemical analysis at the Faculty of Agriculture Assiut University. All these parameters were measured at zero time and after the 1st, 2nd, and 3rd months of cultivation of previous treatments.

Isolation and Identification of fungi

Fungi were isolated from water samples collected from each treatment (control and ibuprofen treated water) of the microcosm experiment. 1 mL of water sample from each treatment was cultivated on PDA (Potato dextrose agar) Petri dishes and then incubated for 7 days at 28°C ±2, the growing fungal isolates were purified and identified and then preserved on PDA agar slopes and stored at 5°C for further experiments. Potato dextrose agar was made by adding 20 g of glucose, filtrate of 200-gram potato, 30 mg of Rose-Bengal and 18 g of agar with 0.25 g of chloramphenicol to prepare 1 L of medium. This process was repeated every 15 days until 90 days for isolation of fungi from water sample at different incubation time intervals. The identification of isolated fungi was mainly based on their macroscopic and microscopic characteristics according to the relevant taxonomical keys [43-47].

Analysis of fungal diversity and their correlation with the physical properties and time of isolation

Using PAleontological STatistics (PAST, Version 4.03, USA), fungal diversity was calculated, as well Canonical correspondence analysis (CCA) was employed to determine the relationship between the measured physical parameters and a certain species' occurrence and period of isolation. Analysis of canonical correspondence [48] is an analysis of a site/species matrix where each site has given values for one or more environmental variables.

RESULTS

Physicochemical characteristics of the experimented water samples

The results of the physicochemical characteristics of the water samples from the microcosm experiment are summarized in Table (1). We observed that pH was increased with time as a result of activities and functionalities of fungal communities and consequently increasing eutrophication with high levels of nutrients (nitrogen and phosphorus level) for both control and ibuprofen treatment. The electrical conductivity and organic matter (C, N and P) were increased after the treatment with ibuprofen. Interestingly, the obtained results revealed that the concentration of carbon and nitrogen and phosphorus was decreased by increasing time incubation in the ibuprofen treated microcosm. This might be brought on by the breakdown of ibuprofen and other polymeric compounds, which might also have an impact on the variety of recovered fungi.

Table 1: Physicochemical properties of water sample collected from microcosm experiment in the control and ibuprofen treatment.

Fungi	Control						Ibuprofen					
	pH	Conduct (μS/cm)	T.D.S (mg/L)	C (%)	N (%)	P (mg/dl)	pH	Conduct (μS/cm)	T.D.S (mg/L)	C (%)	N (%)	P (mg/dl)
0 time	7.3	254.3	0.4	6.35	0.44	6.9	6.9	304.7	0.4	20.2	0.76	0
1st month	6.7	297.7	0.4	6.08	0.23	9.8	6.7	360.7	0.4	8.54	0.504	17.56
2nd month	7.9	269	0.4	6.03	0.23	18.2	7.9	324.7	0.4	7.51	0.503	25.77
3rd month	8.2	314.3	0.4	5.97	0.24	26.6	8.0	348	0.4	6.48	0.501	33.99

The variety of fungi and the composition of the community in control and ibuprofen treated water

The data in Table (2) showed that totally 26 fungal species from 16 fungal genera were obtained from the microcosm experiment. Eleven genera and 19 fungal species were recovered from the control microcosm while 12 fungal genera and 18 fungal species were estimated from ibuprofen treated water. The highest occurrence of fungal species from the control microcosm was recorded for *Trichoderma* sp. whereas the highest occurrence was recorded for *A. niger* recovered from ibuprofen microcosm experiment. As well, the obtained results showed that fungal diversity, occurrence, and species composition in control ibuprofen water treatments are different. Two fungal species namely, *Aspergillus*

niger and *Trichoderma* sp. were the most prevalent in both experimental treatments. For *Aspergillus niger* it was estimated similarly in both the control and ibuprofen microcosm experiments (NCI=7), whereas *Trichoderma* sp. estimated the highest occurrence (NCI=9) for the control microcosm experiment but the occurrence of *Trichoderma* sp. decreased in ibuprofen (NCI =3). *Aspergillus sydowi* was the most predominant occurrence in ibuprofen (27%) compared with control (6%). Interestingly, 7 fungal species were recorded in control microcosm only such as *Acremonium strictum*, *Aspergillus fumigatus*, *Epicoccum* sp., *Fusarium* sp., *Penicillium globosum*, *Trichoderma harzianum* and *Neosartorya* sp. As well as, in the ibuprofen microcosm experiment, there are 7 fungal species were not recorded in the control microcosm experiment namely, *Acremonium* sp., *Chaetomium elatum*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *Microascus alveolaris*, *Stachybotrys* sp. and *Acrostalagmus luteoalbus*. in the other hand, both of these fungi are present in control (unpolluted) water and ibuprofen treated water including *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus sydowi*, *Cladosporium sphaerospermum*, *Curvularia* sp., *Penicillium duclauxii*, *Penicillium islandicum*, *Penicillium purpurogenum*, and *Trichoderma* sp. as shown in Table 2.

Fungi \ Treatment	Control			Ibuprofen		
	TC	TC (%)	NCI	TC	TC (%)	NCI
<i>Acremonium</i>						
<i>Acremonium strictum</i>	8	13	4	0	0	0
<i>Acremonium</i> sp.	0	0	0	4	6	2
<i>Acrostalagmus luteoalbus</i>	0	0	0	1	2	1
<i>Alternaria</i> sp.	2	3	1	0	0	0
<i>Aspergillus flavus</i>	2	3	2	1	2	1
<i>A. fumigatus</i>	2	3	1	0	0	0
<i>A. niger</i>	9	14	7	12	19	7
<i>A. terreus</i>	4	6	3	4	6	4
<i>A. sydowi</i>	4	6	2	17	27	4
<i>Chaetomium elatum</i>	0	0	0	1	2	1
<i>Cladosporium cladosporioides</i>	0	0	0	2	3	1
<i>C. sphaerospermum</i>	1	2	1	2	3	2
<i>Curvularia</i> sp.	1	2	1	2	3	2

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<i>Epicoccum sp.</i>	2	3	1	0	0	0
<i>Eurotium</i>						
<i>Eurotium chevalieri</i>	0	0	0	1	2	1
<i>Fusarium sp.</i>	2	3	2	0	0	0
<i>Microascus alveolaris</i>	0	0	0	3	5	1
<i>Neosartorya sp.</i>	1	2	1	0	0	0
<i>Penicillium duclauxii</i>	4	6	3	3	5	1
<i>Penicillium globosum</i>	1	2	1	0	0	0
<i>Penicillium islandicum</i>	1	2	1	1	2	1
<i>Penicillium purpurogenum</i>	3	5	2	1	2	1
<i>Phoma sp.</i>	1	2	1	2	3	1
<i>Stachybotrys sp.</i>	0	0	0	1	2	1
<i>Trichoderma harzianum</i>	1	2	1	0	0	0
<i>Trichoderma sp.</i>	11	17	9	8	13	3
White mycelia	4	6	4	0	0	0

Table 2: Total count (TC), Total count percent (TC%), No. of case isolation (NCI) of fungi isolated from control and ibuprofen treated water microcosm experiment.

The Simpson index shows the evenness of the aquatic fungal community, the Shannon index is the diversity index that estimates the number of fungal individuals taking into account the number of fungal individuals, dominance (d) determining the dominance of fungal taxa in a specific site, and fungal taxa (s) which indicates the number of isolated fungal species from collected samples. So, the presented data in Table (3) showed that the highest fungal taxa were recorded for control microcosm at 0 time, 45, 60 and 90 days recording 6 fungal taxa, but the highest fungal taxa in ibuprofen treated water was estimated after 45 days estimating 9 fungal taxa. As well as, the highest fungal individuals were recorded for control at 45 days recording 13 fungal individuals but, in the ibuprofen, treated experiment the highest fungal individuals were estimated at 75, 90 days recording 16 fungal individuals. The highest fungal dominance was recorded for control recording 0.36 after 75 days of incubation while in ibuprofen treated water the highest fungal dominance was recorded after 90 days of incubation recording 0.38. The highest Simpson and Shannon diversity indexes were estimated for control samples recording 0.80 and 1.73, respectively, but in ibuprofen treated water, the highest Simpson and Shannon diversity indexes recorded 0.86, and 2.08, respectively. These diversity indexes illustrate the highest evenness of fungal community in ibuprofen after 0 time, 15

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days and 30 days of incubation compared with control at 15 days only. The obtained result illustrated that fungal diversity, occurrence and species composition in control and after treatment with ibuprofen were different.

Table3: Fungal diversity of freshwater fungi with control and water treated with ibuprofen for 3 months of incubation

	Control							Ibuprofen						
	0 time	15 days	30 days	45 days	60 days	75 days	90 days	0 time	15 days	30 days	45 days	60 days	75 days	90 days
Taxa (S)	6	4	5	6	6	4	6	3	1	4	9	3	7	5
Individuals	9	4	9	13	8	10	11	3	1	4	15	8	16	16
Dominance (D)	0.21	0.25	0.23	0.19	0.18	0.36	0.19	0.33	1	0.25	0.13	0.46	0.17	0.38
Simpson (1-D)	0.79	0.75	0.76	0.80	0.81	0.64	0.80	0.66	0	0.75	0.86	0.59	0.82	0.63
Shannon (H)	1.68	1.38	1.52	1.71	1.73	1.16	1.72	1.09	0	1.38	2.08	0.97	1.84	1.24
Evenness e^{H/S}	0.89	1	0.91	0.92	0.94	0.80	0.93	1	1	1	0.89	0.88	0.90	0.69

Alpha diversity (α -diversity) is the mean diversity of species in a community according to Whittaker[49], it is also illustrated that in the ibuprofen microcosm experiment the fungal diversity decreased significantly after 60 and 90 days of incubation compared with the control microcosm experiment as shown in Figure (1), this results fit with fungal diversity indices as illustrated in table (3) as for Simpson, Shannon (H) and evenness which they provide vital information regarding the rarity and abundance of species in a community, we observed a significant decrease in values in the treatment of ibuprofen when compared with control (un-polluted water).

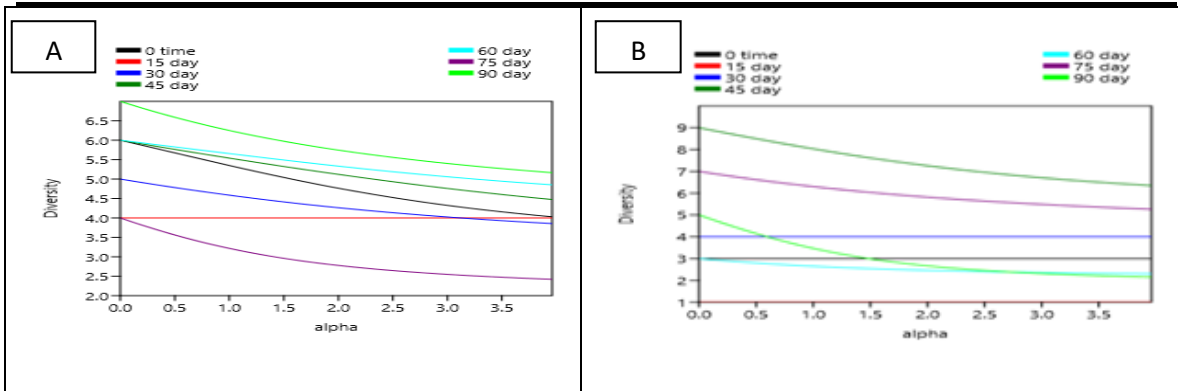


Figure (1): the alpha diversity of fungal species recovered from the microcosm experiment for control (A) and ibuprofen treated experiment (B) at different time intervals.

Correlation between abiotic factors of water treatments and species occurrence

Canonical correspondence analysis demonstrated that, the measured physicochemical properties remarkably affected the occurrence of aquatic fungal taxa in microcosm treatments (Figs 2 A, B). The physicochemical parameters of the control microcosm illustrated that the total dissolved organic carbon and total dissolved organic nitrogen had influence on the occurrence of *Cladosporium sphaerospermum*, *Aspergillus fumigatus*, *Trichoderma* sp., *Penicillium islandicum*, *Penicillium globosum*, *Penicillium duclauxii*. Whereas the pH value and total dissolved organic phosphorus showed obvious interaction with *Acremonium strictum* and *Epicoccum* sp. On the other hand, the conductivity did not exhibit any interaction with the recovered fungi. Interestingly, the relationship between the physicochemical parameters of the water treated with ibuprofen and the occurrence of recovered fungal species showed that pH and total dissolved organic phosphorus (TDOP) had influence on the occurrence of *A. sydowi*, *Penicillium duclauxii*, *Aspergillus terreus*, *Microascus alveolaris*, *Acrostalagmus leuteoalbus*, *Aspergillus niger*, *Trichoderma* sp. Furthermore, the organic matter such as total dissolved organic carbon (TDOP), and total dissolved organic nitrogen (TDON) interacted with *Penicillium purpurogenum* and *Curvularia* sp. and conductivity related to the occurrence of *Chaetomium* sp., *Alternaria* sp. and *Cladosporium sphaerospermum* as shown in Figure (2).

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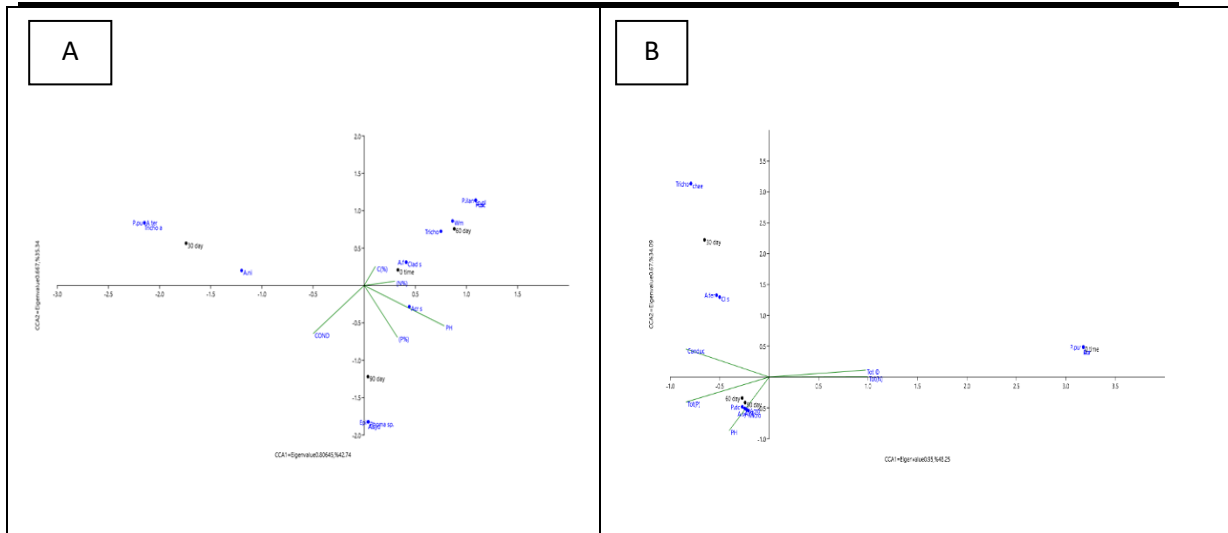
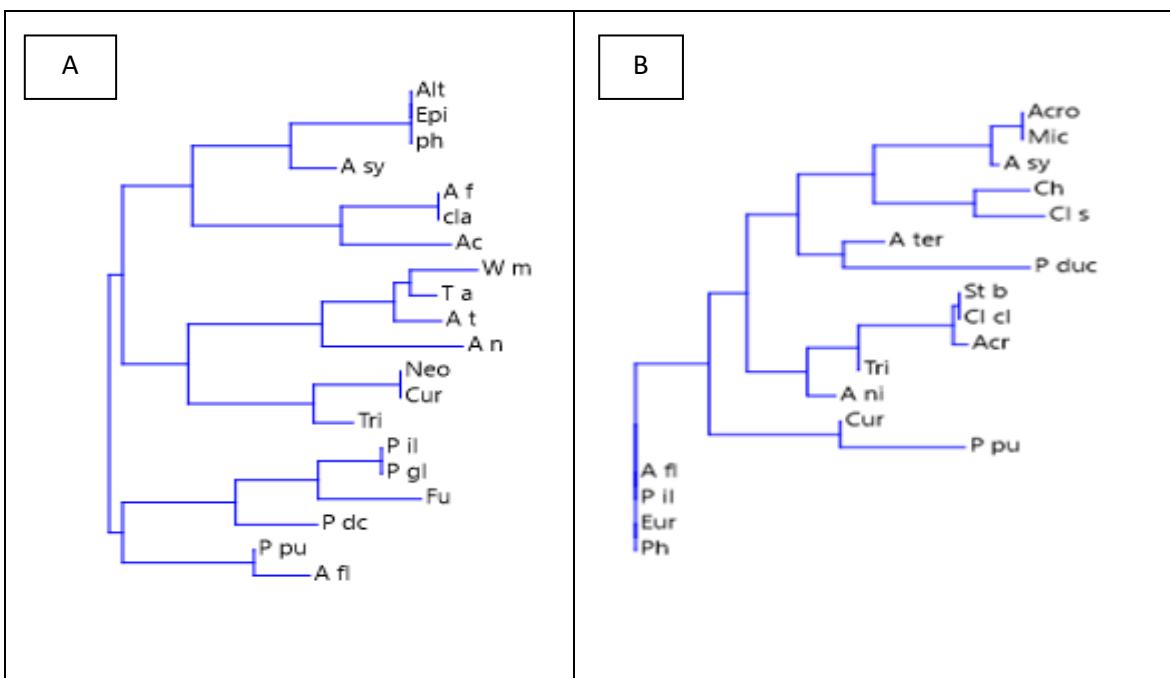


Figure 2: The interaction of physicochemical properties with fungi recovered from control microcosm (A) and ibuprofen treated microcosm (B). Acr s = *Acremonium strictum* , A f = *Aspergillus fumigatus* , A fl = *Aspergillus flavus* , A n = *Aspergillus niger* , A t = *Aspergillus terreus* , A sy = *Aspergillus sydowi* , Epi = *Epicoccum* sp., P.pu = *Penicillium purpurogenum* , P il = *Penicillium islandicum* , P dc = *Penicillium dauclauxii* , Tricho a = *Trichoderma harzianum* , Tricho = *Trichoderma* sp., Wm = White mycelia, Ph = *Phoma* sp., P gl = *Penicillium globosum* , Alt = *Alternaria* sp., Fus = *Fusarium* sp. Acr = *Acremonium* sp., A ni = *Aspergillus niger*, A syd = *Asydowi*, A ter = *Aspergillus terreus*, Cld s = *Cladosporium sphaerospermum* , Chae = *Chaetomium* sp., Cur = *Curvularia* sp., Mic = *Microascus alveolaris* , P.dc = *Penicillium dauclauxii*, P.pur = *Penicillium purpurogenum*, Tri = *Trichoderma* sp. , Acro = *Acrostalagmus leuteoalbus*

Cluster analysis of the recovered fungi

Data in Fig. (A, B) illustrated the cluster analysis of the recovered fungi for control and ibuprofen treated microcosm showing an obvious grouping of the fungal communities. In control (Un-polluted water) there are three separated fungal grouping, the first group includes 7 fungal species namely, *Acremonium strictum*, *Aspergillus fumigatus*, *Aspergillus sydowi*, *Cladosporium sphaerospermum*, *Epicoccum* sp., *Phoma* sp., *Alternaria* sp., the second group includes *Aspergillus niger*, *Aspergillus terreus*, *Trichoderma harzianum*, *Trichoderma* sp., White mycelia, *Curvularia* sp., *Neosartorya* sp. and the third group includes 5 fungal species namely *Penicillium purpurogenum*, *Penicillium islandicum*, *Penicillium dauclauxii*, *Penicillium globosum*, *Fusarium* sp. Whereas in ibuprofen treated water it was found that, there are two separated fungal groups, the first group include 6 fungal species such as *Aspergillus sydowi*, *Aspergillus terreus*, *Cladosporium sphaerospermum*, *Chaetomium* sp., *Microascus alveolaris* , *Penicillium dauclauxii*, the second group includes 7 fungal species namely, *Acremonium*



sp., *Aspergillus niger*, *Curvularia* sp., *Penicillium purpurogenum*, *Trichoderma* sp., *Cladosporium cladosporidies*, *Stachybotrys* sp. Also, there are fungal species not found in a group such as *Aspergillus flavus*, *Penicillium islandicum*, *Eurotium chevalieri*, *Phoma* sp. which indicates that the different microcosm treatments may showed a variation in fungal community structure.

Figure (3) Cluster analysis showing the fungal communities estimated from the control (A) and ibuprofen treated microcosm

(B). (A) Ac = *Acremonium strictum*, A f = *Aspergillus fumigatus*, A fl = *Aspergillus flavus*, A n = *Aspergillus niger*, A t = *Aspergillus terreus*, A sy = *Aspergillus sydowi*, Cla = *Cladosporium sphaerospermum*, Epi = *Epicoccum* sp., P.pu = *Penicillium purpurogenum*, P il = *Penicillium islandicum*, P dc = *Penicillium duclauxii*, T a = *Trichoderma harzianum*, Tri = *Trichoderma* sp., Wm = White mycelia, Ph = *Phoma* sp., P gl = *Penicillium globosum*, Cu = *Curvularia* sp., Neo = *Neosartorya* sp., Alt = *Alternaria* sp., Fu = *Fusarium* sp., (B) Acr = *Acremonium* sp., A ni = *Aspergillus niger*, A sy = *Aspergillus sydowi*, A ter = *Aspergillus terreus*, A fl = *Aspergillus flavus*, Cl s = *Cladosporium sphaerospermum*, Ch = *Chaetomium* sp., Cur = *Curvularia* sp., Mic = *Microascus alveolaris*, P duc = *Penicillium duclauxii*, P.pu = *Penicillium purpurogenum*, P il = *Penicillium islandicum*, Tri = *Trichoderma* sp., Acro = *Acrostalgnus luteoalbus*, Eur = *Eurotium chevalieri*, Cl cl = *Cladosporium cladosporioides*, St b = *Stachybotrys* sp., Ph = *Phoma* sp.

Discussion

Fungi represent one of the most important organisms for eliminating different pharmaceuticals in water through different mechanisms but the impact of increasing urbanization led to obvious effects on the diversity of fungi and a decline in water quality

[3, 10], The result of this study showed that ibuprofen (one of the most emerging fresh water contaminant) affected on fungal diversity and composition in River Nile water in microcosm design [50-52]. Physicochemical characteristics of the water samples from the microcosm experiment showed that pH was decreased after treatment of the water sample with ibuprofen due to the acidic nature of ibuprofen [53]. Whereas the electrical conductivity and organic matters (C, N) were increased after treatment and the concentration of carbon and nitrogen was decreased by increasing the incubation time of ibuprofen-treated water due to the fungal activities in the microcosm experiment. on the other hand, the electrical conductivity of ibuprofen microcosm experiment was increased by increasing incubation time this may be attributed to the mineralization process of organic matter and other polymeric compounds in water and fungal activities and consequently pH levels of water were elevated in the microcosm experiment by increasing incubation time [54]. Ortiz-Vera et al.[55] stated that lower pH is known to increase acid-tolerant fungi and decrease overall fungal diversity. The obtained results stated that, 11 fungal genera and 19 fungal species were recovered from the control microcosm whereas, 18 fungal species related to 12 genera were recovered from an ibuprofen microcosm experiment. *Aspergillus niger* and *Trichoderma* sp. were the most prevalent fungal species. Fungal species namely, *Acremonium strictum*, *Aspergillus fumigatus*, *Epicoccum* sp., *Fusarium* sp., *Penicillium globosum*, *Trichoderma harzianum* and *Neosartorya* sp. were estimated in control microcosm only; whereas, in the ibuprofen microcosm experiment, 7 fungal species were not recorded in the control microcosm experiment namely, *Acremonium* sp., *Chaetomium elatum*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *Microascus alveolaris*, *Stachybotrys* sp. and *Acrostalagmus luteoalbus*. Interestingly, Pietryczuk [56] revealed that water pollution usually leads to a decrease in the taxonomic diversity of aquatic fungi, including the complete disappearance of some species such as *Acremonium strictum*, *Penicillium globosum*, *Aspergillus fumigatus* and *Trichoderma harzianum*. and the appearance of some other fungal species results from treatment namely, *Microascus alveolaris*, *Acrostalagmus leuteoalbus*, *Cladosporium cladosporidies*, *Chaetomium elatum*, *Eurotium chevalieri*, *Stachybotrys* sp. Water quality and the diversity of planktonic fungal communities have been found to be substantially associated with the level of

anthropogenic activities [57], therefore, fungal communities in aquatic environments can act as bioindicators. Furthermore, the obtained data revealed that the highest Simpson (0.80) and Shannon diversity (1.73) indexes were estimated for control samples, on the other hand, in ibuprofen treated water, the highest Simpson (0.86) and Shannon diversity (2.08) indexes. These diversity indexes illustrate the highest evenness of fungal community in ibuprofen compared with control microcosm experiment. Interestingly, Khalil et al., [58] stated that The highest Simpson and Shannon diversity indexes of hyphomycetous fungi were estimated for the highest polluted water samples. Whereas, aquatic fungi are not only found in environments with clean water; they are also prevalent and actively involved in the decomposition of organic matter in harsh environments like aquatic ecosystems contaminated with emergent pollutants [59]. The function and health of aquatic ecosystems can be impacted by the disposal of untreated or imperfectly treated effluents, which can have an impact on the microbial diversity of natural environments [60, 61].

On the other hand, the relationship between the physicochemical parameters of the water sample treated with ibuprofen and the occurrence of recovered fungal species showed that pH and total dissolved organic phosphorus (TDOP) had influence on the occurrence of fungi recorded in ibuprofen treated microcosm when compared with control (un-polluted water). Accordingly, Bai [57] reported that, the organic matter, total nitrogen, water temperature, and pH were the main determinants of fungal community composition. As well, According to Abdel-Raheem [62], the pH value in particular is significant in defining the diversity and composition of fungal species. Comparing the ibuprofen-treated fungal communities to the control ones, the fungal communities displayed a different structure [63]. The obtained data stated that, in control (Un-polluted water) microcosm, three separated fungal grouping were obtained whereas in ibuprofen treated water only two separated fungal groups were recorded. Furthermore, there are number of separated fungal species that are not found in grouping which indicates that the different microcosm treatments may showed a variation and changes in fungal community structure So, the level of urbanization in the River Nile and the influence of anthropogenic activities in the water, even though to a smaller amount of contaminants drove a significant effect on microbial diversity, structure and function in aquatic

ecosystem. Interestingly, Khalil et al., [58] concluded that, compared to other less polluted areas, the prevalence of aquatic fungi was noticeably declining in stressed water sites. As well, isolating some aquatic fungi from these polluted water environments is significant and interesting since these water habitats have relatively higher levels of organic matter, water conductivity, cations, and anions.

CONCLUSION

Pharmaceutical substances like ibuprofen that are released into the water by various anthropogenic activities, could have an impact on the fungal diversity and communities in the Nile River. It seems that some aquatic fungus species can serve as an excellent indicator of the water quality, cleanliness, and sanitary safety of surface waters, especially in environmentally significant areas. The obtained results suggested that aquatic fungi are potential candidates as bioindicators for emerged pollutants in the aquatic environments. As well as the isolation of fungal species from the contaminated water may provide valuable understandings concerning the fungal adaptation, and their functional role as bioindicators and pollutants degraders of these emergent compounds.

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