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Marine fungal community composition and diversity across a polluted site in the south Mediterranean coast: the Monastir Bay, Tunisia

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ABSTRACT

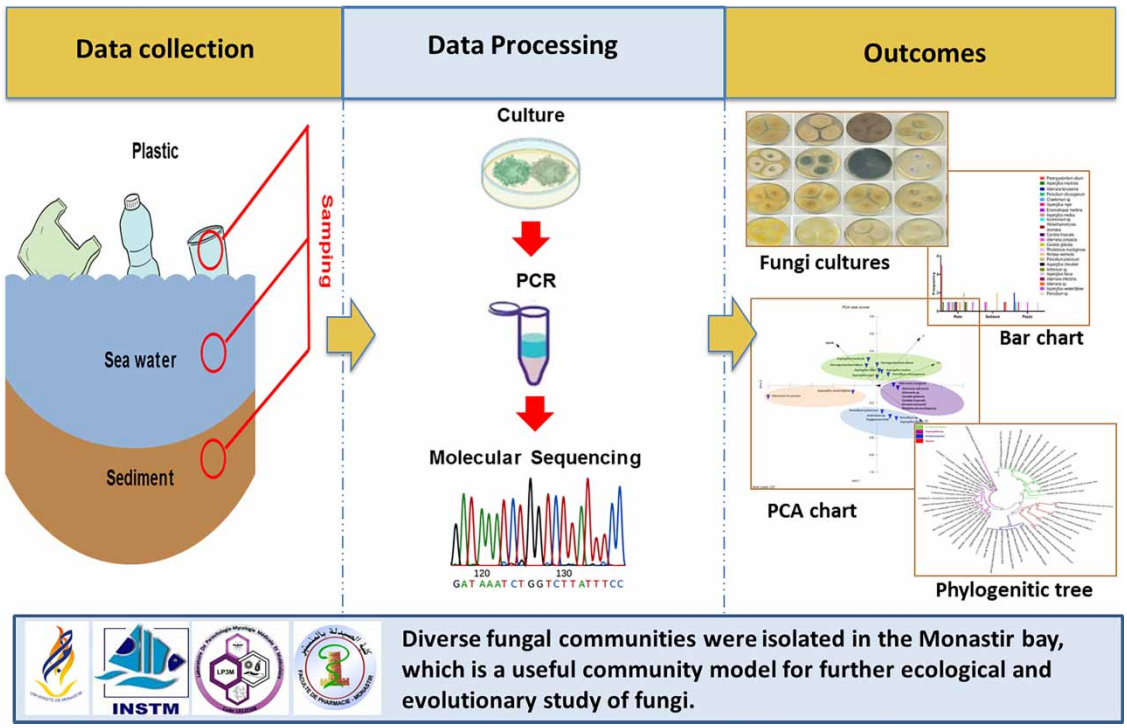
Marine fungi communities play a crucial role in the recycling of nutrients, restoration of biological systems, and the overall functioning of ecosystems. While aquatic fungal communities do react to pollution, there is a significant lack of information regarding the changes in the fungal community's structure, caused by marine pollution. In this study, we aim to address this gap in knowledge by investigating the range and makeup of fungal species present in marine environments in a polluted bay in Tunisia, spanning a biodiversity hotspot (Monastir Bay). Sequence analysis of the internal transcribed spacer region from culturable mycobiome and physicochemical parameters were investigated at seven sites in the bay. A total of 32 fungal taxa were identified at the genus and/or species levels and were assigned to four major groups (Aspergillacae 37.5%, Dothiomyceta 21.87%, Sordariamyceta 28.12%, and Yeasts 12.5%) with a remarkable predominance of *Aspergillus* genus. Assessment of the Shannon–Wiener diversity index and the Simpson dominance index revealed that the highest species diversity index (0.84) was recorded at the Kheniss site. Our results suggest the existence of diverse fungal communities, can be considered a useful community model for further ecological and evolutionary study of fungi in the bay.

Key words: *Aspergillus*, biodiversity, fungal community, marine pollution, water quality

HIGHLIGHTS

- The biodiversity of marine fungi and their ecological roles in the coastal environment of Tunisia are deeply unknown.
- A total of 32 fungal taxa were assigned to four major groups (Aspergillacae 37.5%, Dothiomyceta 21.87%, Sordariamyceta 28.12%, and Yeasts 12.5%).
- Remarkable predominance of *Aspergillus* genus is observed.
- The highest fungal taxonomic biodiversity was found in organically rich waters.

GRAPHICAL ABSTRACT



1. INTRODUCTION

The enormous biodiversity of marine fungal communities has recently attracted considerable interest. There may be many reasons for investigating the extent of marine fungal biodiversity. In fact, they have an important role as a source of biologically active secondary metabolites (Hasan *et al.* 2015). They have the ability to establish themselves and adapt to various living and non-living substrates such as algae, sediments, invertebrates, and driftwood (Raghukumar 2017; Bovio *et al.* 2018). According to the website marinefungi.org, there are currently roughly 1,900 species of marine fungi that fall within seven phyla (Azoanthellae, Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Mucoromycota, and Microsporidia), 22 classes, 88 orders, 226 families, and 769 genera. With 141 species in 59 genera, the Halosphaeriaceae family of marine fungi is the biggest family (Jones *et al.* 2015). It is worth noting that the documented number of marine fungal species (1,900 species) is significantly lower than the estimated 10,000 species (Sen *et al.* 2022), indicating that the oceans possess a rich fungal diversity that is yet to be fully explored.

These organisms are important primary decomposers and live as mutualists (ectos and endosymbionts), parasites, pathogens, and sapphires. They contribute greatly to the nutrient cycle and the food web (Amend *et al.* 2019; Grossart *et al.* 2019). Marine fungi include 'marine fungi obligate species', which grow and sporulate exclusively in marine or estuarine habitats, and 'facultative species', which originate from freshwater or terrestrial environments and are able to grow and sporulate in the sea (Kohlmeyer & Kohlmeyer (1979). Due to these characteristics, many authors have proposed including these organisms in the group of bioindicators of anthropogenic alterations in the monitoring of the aquatic ecosystem ecological state and the water sanitary state (Biedunkiewicz 2007; Cudowski *et al.* 2015). In recent years, studies on the abundance and taxonomic identification of aquatic fungi in different water types, especially lakes with different trophic states, have been increasing (Ortiz-Vera *et al.* 2018; Lin *et al.* 2023). However, the impact of marine water pollution on fungal diversity and composition has not been extensively investigated. Furthermore, attempts to explain the impact of physical and chemical water parameters on planktonic biodiversity and abundance are very rare (Pietryczuk *et al.* 2018). The Monastir Bay is a semi-enclosed lagoon on the east coast of Tunisia. This region is rich in biodiversity and has important marine resources (Ben Amor *et al.* 2020). However, significant increases in industrial, urban, fishing, and aquaculture activities have had a

strong impact on environmental quality (Sassi *et al.* 1998; Khiari *et al.* 2017; Damak *et al.* 2019). The Bay of Monastir is characterized by high levels of organic pollution (up to 6% of total organic carbon) in coastal areas and severe eutrophication. This degradation appears to be closely related to: (i) the weak hydrodynamic state created by the underwater topography (Souissi *et al.* 2014), which makes it a suitable environment for contamination by plastic debris; and (ii) the evolution of the plastisphere (Tarchi *et al.* 2023). Furthermore, the practice of fish farming aquaculture has emerged as an additional means of introducing nutrients into the bay (Challouf *et al.* 2018; Damak *et al.* 2020). As a result, the Monastir Bay has been recently classified as moderately to highly polluted with sediments posing a significant risk to the ecosystem (khiari *et al.* 2021). Given this circumstance, the monitoring of biological indicators is becoming an absolute priority as they serve a vital role in describing and predicting changes within the environment. Specifically, the utilization of marine microorganisms is highly recommended for evaluating the overall quality of marine ecosystems, alongside the examination of physical and chemical parameters. This is due to the fact that marine microorganisms are ubiquitously distributed in seawater, exhibit a rapid turnover in biomass, and display prompt responses to environmental fluctuations (Giuliano 2000).

Furthermore, the biodiversity of marine fungi and their ecological roles in the southern Mediterranean are largely unknown. Therefore, the main objective of this study was to identify culturable fungal communities isolated from the coastal waters of the Bay of Monastir (Tunisia). The genetic diversity (ITS sequences) of 32 isolated fungal strains was assessed and the distribution of microorganisms was tracked using a diversity index.

2. MATERIALS AND METHODS

2.1. Site description

The coast of Tunisia, south Mediterranean region, is about 1,200 km long, accounting for about 2.5% of the entire Mediterranean coast. The Bay of Monastir extends from the city of Monastir to the city of Bekalta. It is a semi-enclosed lagoon on the east coast of Tunisia (Figure 1). The Monastir-Bekalta coast is 38 km long and lies between latitude 35°37° and 35°47′N and longitude 10°50′ and longitude 11°20′E east (Figure 1). Seawater, sediment, and plastic debris samples (three samples from each matrice) were collected from seven coastal marine points of the Monastir Bay in summer (June 2021) (Table 1, Figure 1).

2.2. Sampling strategy

Along each site, the surface water (1 l) was loaded into presterilized glass bottles with screw caps, and surface sediment samples were carried out from a depth of 0–5 cm using a GRAB-type surface sediment sampler. All sediment samples were transferred into sterile plastic bags.

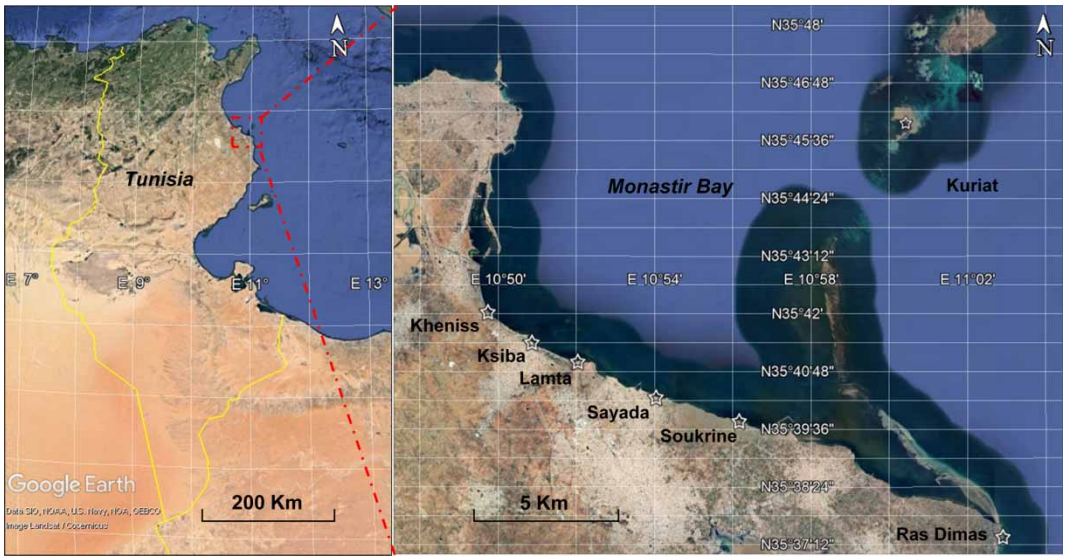


Figure 1 | Sampling sites in the coastal Mediterranean region of Monastir. (Images retrieved from Google Maps (URL: https://www.google.com/maps).)

Table 1 | Fungi isolated from the Monastir Bay by sequence comparison with BLASTn (NCBI GenBank database)

Site	Code collection of culture of microorganisms	Sampling origin	Top BLAST (number of acess on Gen Bank)	Query coverage (%)	(%) Identity	Proposed taxa (Gen-Bank acc. no.)
RAS DIMAS	MF-ITS1	Water	*Parengyodontium album MT626052*	99	100	*Parengyodontium album OQ626169*
	MF-ITS2	Water	*Parengyodontium album MN944461*	99	99.64	*Parengyodontium album OQ626170*
SOUKRINE	MF-ITS3	plastic	*Alternaria tenuissima MT453271*	100	99.60	*Alternaria tenuissima OQ626171*
	MF-ITS4	plastic	*Alternaria tenuissima MT487771.1*	100	100	*Alternaria tenuissima OQ626172*
	MF-ITS5	plastic	*Penicillium chrysogenum MK140686*	100	95.68	*Penicillium chrysogenum OQ626173*
SAYADA	MF-ITS6	Water	*Parengyodontium album ON365712*	98	99.65	*Parengyodontium album OQ626174*
	MF-ITS7	Water	*Aspergillus insulicola MT898544.1*	100	96.03	*Aspergillus insulicola OQ626175*
	MF-ITS8	Water	*Chaetomium sp. MK361149.1*	98	98.45	*Chaetomium sp. OQ626176*
	MF-ITS9	Water	*Aspergillus niger MT508805*	100	99.62	*Aspergillus niger OQ626177*
	MF-ITS27	Water	*Arthrinium sp. MH384416*	100	100	*Arthrinium sp. OQ626195*
LAMTA	MF-ITS10	Plastic	*Alternaria compacta ON790484*	99	100	*Alternaria compacta OQ626178*
	MF-ITS32	Water	*Aspergillus flavus MH793845*	99	100	*Aspergillus flavus OQ626200*
	MF-ITS26	Water	*Penicillium polonicum KF597019*	100	97.83	*Penicillium polonicum OQ626194*
	MF-ITS29	Water	*Penicillium sp.KM108340.1*	94	95.61	*Penicillium sp.OQ626197*
KSIBET	MF-ITS11	Water	*Parengyodontium album MT626052*	99	98.88	*Parengyodontium album OQ626179*
	MF-ITS12	Sediment	*Emericellopsis maritima OQ300337*	97	99.06	*Emericellopsis maritima OQ626180*
	MF-ITS25	Sediment	*Aspergillus medius ON753782*	100	100	*Aspergillus medius OQ626193*
	MF-ITS28	Water	*Penicillium polonicum MN623481*	99	99.81	*Penicillium polonicum OQ626196*
KHENISS	MF-ITS13	Water	*Parengyodontium album MT626052*	99	99.64	*Parengyodontium album OQ626181*
	MF-ITS14	Plastic	*Aspergillus niger MW282896*	98	97.57	*Aspergillus niger OQ626182*
	MF-ITS15	Water	*Acremonium sp. KR425649*	97	99.81	*Acremonium sp. OQ626183*
	MF-ITS16	Water	*Wickerhamomyces anomalus MN783635*	100	100	*Wickerhamomyces anomalus OQ626184*
	MF-ITS23	Water	*Aspergillus medius ON753782*	100	100	*Aspergillus medius OQ626191*
	MF-ITS24	Water	*Aspergillus chevalieri OK189597*	74	75.39	*Aspergillus chevalieri OQ626192*
	MF-ITS31	Water	*Aspergillus westerdijkiae KP689263.1*	100	100	*Aspergillus westerdijkiae OQ626199*
KURIAT	MF-ITS17	Water	*Candida tropicalis MT490211*	100	100	*Candida tropicalis OQ626185*
	MF-ITS18	Sediment	*Alternaria infectoria MT561399*	100	99.81	*Alternaria infectoria OQ626186*
	MF-ITS30	Sediment	*Alternaria sp. KP749178*	100	98.44	*Alternaria sp. OQ626198*
	MF-ITS19	Water	*Candida glabrata LC389261*	100	100	*Candida glabrata OQ626187*

(*Continued.*)

Table 1 | Continued

Site	Code collection of culture of microorganisms	Sampling origin	Top BLAST (number of acess on Gen Bank)	Query coverage (%)	(%) Identity	Proposed taxa (Gen-Bank acc. no.)
	MF-ITS20	Water	*Rhodotorula mucilaginosa KY104848*	99	100	*Rhodotorula mucilaginosa OQ626188*
	MF-ITS21	Sediment	*Hortaea werneckii MK157015*	94	99.60	*Hortaea werneckii OQ626189*
	MF-ITS22	Sediment	*Hortaea werneckii MZ736071*	96	99.60	*Hortaea werneckii OQ626190*

Plastic debris was picked up on the shore at the Monastir Bay. Plastic fragments floated on the surface or were found as deep as 20 cm. Collected samples of water, sediment, and plastic waste were transported in freezers kept at 4 °C and processed immediately in the laboratory. *In situ* analyses of physicochemical parameters (temperature, pH, salinity, and dissolved oxygen) were also performed on the seawater at each site.

2.3. Isolation and identification of marine fungi

Fungi from the seawater, sediment, and plastic debris samples were isolated on solid media plates of Sabouraud dextrose agar (SDA) (Thermo Fisher Scientific, Waltham, Massachusetts, USA) supplemented with antibiotics (0.5% chloramphenicol) (Thermo Fisher Scientific) to suppress bacterial growth. To proceed, 1 g of sediment/or 1 mL of sampled seawater was suspended in 10 mL of sterile seawater (the seawater was collected from the bay and sterilized by filtration in 0.45 and autoclaved before using it in media preparation). This solution was diluted to 10^{-1}, 10^{-2}, and 10^{-3}, and 1 mL of each dilution was poured into a 90-mm Petri dish with SDA media. The plastic pieces were collected with a pair of presterilized tweezers, and a 1 cm^2 piece was cut with a pair of scissors. Three pieces were then placed on a 90 mm Petri dish with SDA media. Experiments were performed in triplicate for each sample. The plates were incubated at 28 °C for 2–6 days and examined daily for the growth of fungi. Fungal colonies were sub-cultured onto fresh SDA plates for pure, single colony isolation, and identification. Filamentous fungi were identified in terms of macroscopic and microscopic morphological features (Kirk *et al.* 2008). Apparently, monomorphic cultures obtained after at least two transfers onto fresh agar plates were further authenticated using molecular tools to check the strain identity.

2.4. DNA isolation, PCR, and sequencing

A plug of the mycelium for filamentous fungi and 1–5 yeast colonies were suspended in a lysis buffer. Genomic DNA was isolated using the Genomic DNA Purification Kit (Pure Link™ Genomic DNA Purification Kit) following the manufacturer's instructions. The DNA concentration was estimated at 260 nm using a NanoDrop™ 2000 (Thermo Fisher Scientific, Wilmington, USA). Forty nanograms of DNA were used as the template in a PCR to span the entire sequence of the internal transcribed spacer region (ITS1-5.8S-ITS2), and the primers used for amplification were ITS5 (5′-GGAAGTAAAAGTCG TAACAAGG-3′) and ITS4 (5′-TCCT-CCGCTTATTG ATATGC-3′). PCR was performed in a final volume of 50 μL containing 1× PCR buffer, 3 mM $MgCl_2$, 250 mM deoxynucleotides (dNTPs), 0.4 μM of each primer, and two units of Dream Taq DNA polymerase. The amplification program consisted of an initial denaturation for 2 min at 95 °C, followed by 35 cycles of 1 min at 95 °C for denaturation, 45 s at 55 °C for annealing, and 1.5 min at 72 °C for extension and ended with an extension for 5 min at 72 °C. Amplified fragments were visualized on a 1% agarose gel (GENAXXON bioscience) and sequenced by RAN BioLinks SARL using the Sanger approach. To deduce the taxonomy of the fungal isolates, the obtained sequences were edited using BioEdit software version 7.2 and then compared with data available in the public GenBank database using the BLASTn sequence match algorithm (Altschul *et al.* 1997) (http://www.ncbi.nlm.nih.gov/BLAST). Sequences from the current study were submitted to GenBank (accession numbers are given in Table 1).

2.5. Sequence alignment and phylogenetic analyses

The sequences were aligned by the CLUSTAL W program (Thompson *et al.* 1994) using the BioEdit package. Phylogenetic and molecular evolutionary analyses were performed using MEGA X (Kumar *et al.* 2018). The phylogenetic tree was constructed using the neighbor-joining algorithm (Gascuel 1997) with bootstrap values calculated from 1,000 replicates.

2.6. Statistical analysis

The frequency of each taxon was determined by dividing the total number of sets of each taxon encountered by the total number of samples examined. The online software GraphPad Prism 8.4.3 was used to visualize the data distribution through a bar graph design. Fungi diversity at each collection site was assessed using the Shannon–Wiener diversity index. The Simpson dominance index and species evenness (H/H_{max}) were also determined (MVSP version 3.22). Significant differences in seawater fungal communities and their physicochemical parameters between study sites were analyzed using one-way ANOVA (R version 4.3.0), with significance set at p-value <0.05. Principal compound analysis was guided on biological and physiological parameters and the fungi distribution from different sampling regions using MVSP version 3.22. Indeed, the Eigen analysis was set to 1E-007, data were centered and normalized, no data transformation was applied and axes were extracted using the Jolliffe rule.

3. RESULTS

This cross-sectional study evaluated the variety and distribution of fungi in the Monastir Bay over the duration of the investigation. Thirty-two pure cultures of fungi were obtained from the seven studied sites along the Monastir Bay (Table 1). Twenty strains were obtained from water matrices and six strains were obtained from both sediment and plastic matrices. The number of strains isolated in water was higher than that of sediment and plastic strains, with the Simpson index (D) and Shannon–Wiener index being equal to 0.89 and 1.09, respectively (Figure 2, Table 2). In addition, a significant difference between the two types of samples was verified with an f-ratio value of 5.002 and a p-value = 0.009 (one-way ANOVA). Three fungi genera were associated with plastic: *Aspergillus, Alternaria*, and *Penicillium*.

In Table 3, the Shannon–Wiener diversity index, Simpson's dominance index, and species evenness of fungal species isolated in the Monastir Bay are presented for the assessed sites. The highest species diversity index of 0.84 (Shannon–Wiener) and dominance index of 0.86 (Simpson's) were recorded at the Kheniss collection site, while the Ras Dimas collection site had the lowest species diversity index of 0.00 and lowest species evenness of 0.00. The Kheniss site also had the highest

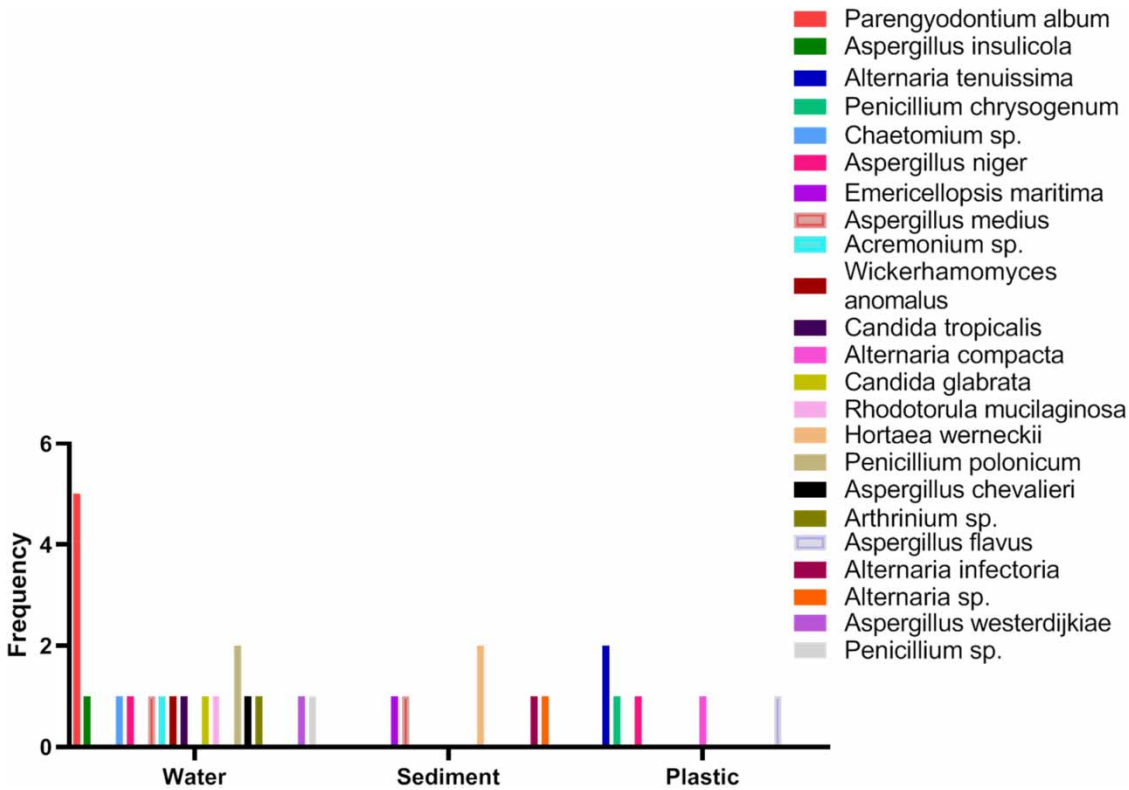


Figure 2 | Distribution of fungal strains according to the matrices.

Table 2 | Fungal species diversity, dominance, and evenness of distribution in three matrices (water, sediment, and plastic)

Matrice	Simpson's dominance index (*D*)	Shannon–Wiener index (*H*)	Evenness	Num. Spec.
Water	0.89	1.09	0.93	15
Sediment	0.77	0.67	0.97	5
Plastic	0.77	0.67	0.97	5

Table 3 | Fungal species diversity, dominance, and evenness of distribution in the Monastir Bay

Site	Simpson's dominance index (*D*)	Shannon–Wiener index (*H*)	Evenness	Num. Spec.
RAS DIMAS	0.00	0.00	0.00	1
SOUKRINE	0.44	0.28	0.92	2
SAYADA	0.80	0.70	1.00	5
LAMTA	0.75	0.60	1.00	4
KSIBET	0.75	0.60	1.00	4
KHENISS	0.86	0.84	1.00	7
KURIAT	0. 82	0.76	0.98	6

species dominance index of 0.86 (Simpson's), whereas the Ras Dimas site had the lowest species dominance. These findings are illustrated in Figure 3 and Table 3. Although there were variations in species diversity, dominance, and evenness among the different examined sites, no statistically significant differences were observed (one-way ANOVA, f-ratio = 0.75, $p = 0.60$). Ascomycetes were the dominant fungal community.

To investigate the relationships between environmental and biological data, principal component analysis (PCA) was carried out (Figure 4). PC1 (axis 1), with a percentage of 59.16%, was supported by oxygen ($r = 0.48$), temperature ($r = 0.46$), pH ($r = 0.61$), and salinity ($r = -0.42$). PC2 (axis 2), with a percentage of 30.14%, was supported by oxygen ($r = -0.48$), temperature ($r = 0.63$), pH ($r = 0.28$) and salinity ($r = 0.55$). We classified species into groups according to

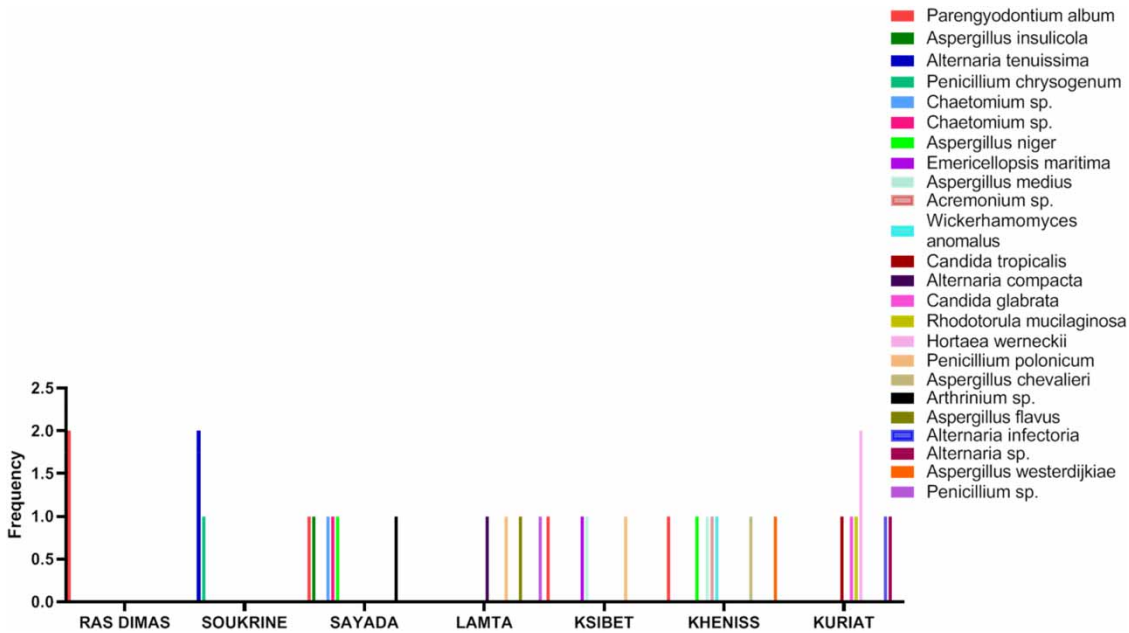


Figure 3 | Distribution of fungal strains according to the site.

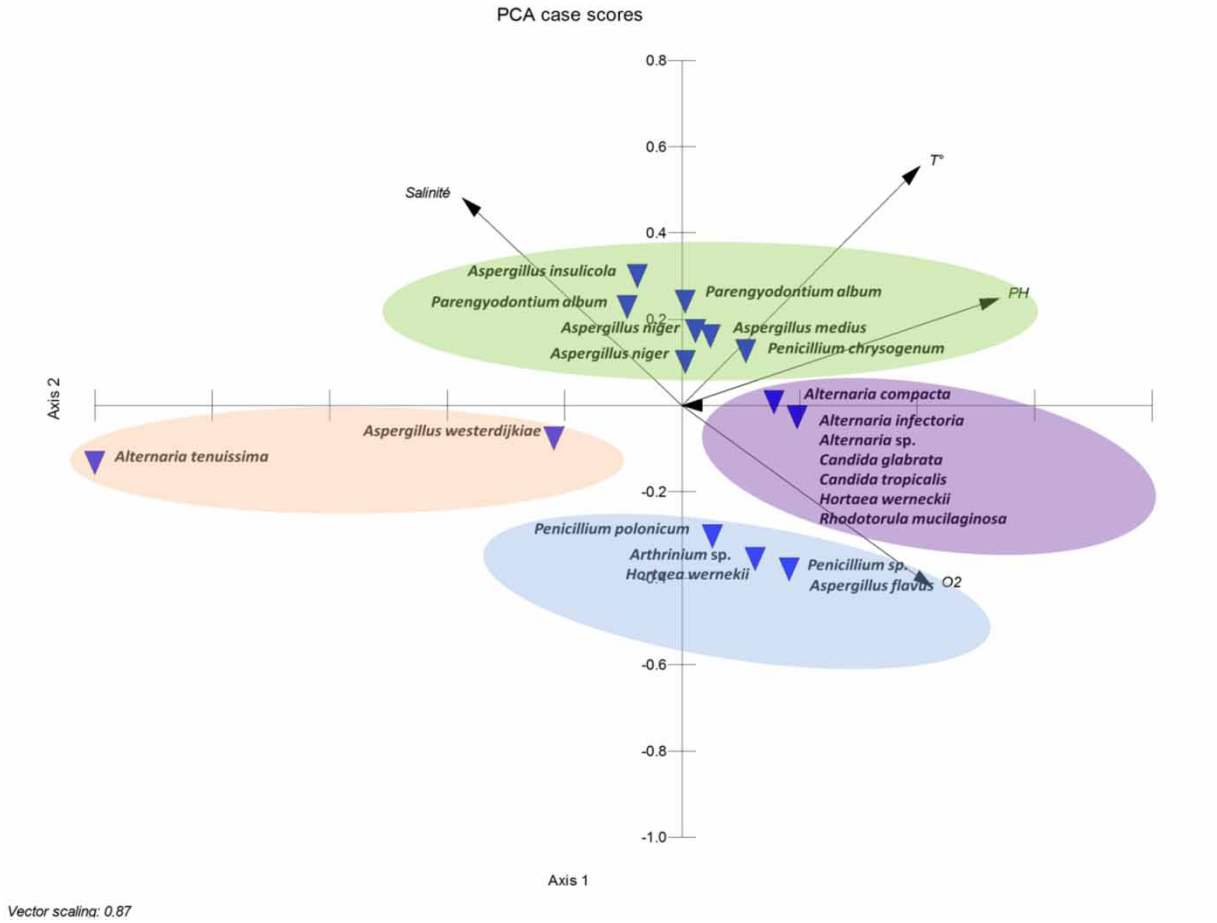


Figure 4 | PCA chart inferred from data corresponding to species distribution according to biological and physiological parameters. Green ellipse refers to the O–T + pH + S + phenotype, purple ellipse refers to O + T + pH + S– phenotype, blue ellipse refers to (O + T–pH–S–) phenotype, orange ellipse correspond to O–T–pH–S + phenotype.

'Oxygen-Temperature-pH-Salinity' (O/T/pH/S) phenotypes. The first group containing *Parengyodontium album* and *Aspergillus spp. (A. niger, A. medius* and *A. insulicola*) and *Penicillium chrysogenum* was characterized by the O–T + pH + S + phenotype, species belonging to this group seem to not tolerate high oxygen concentrations ($r = 0.12$–0.30). A second group demonstrated salinity tolerance (O–T–pH–S+), containing *Alternaria tenuissima* and *Aspergillus westerdijkiae* ($r = -0.22$–0.98). A third group was characterized by an O + T + pH + S– phenotype containing yeasts (*Candida* sp., *Rhodotorula mucilaginosa*, and *Hortaea werneckii)* and *Alternaria* spp. ($r = 0.15$–0.20), which seems to not tolerate high salinity concentrations. The last group was oxygen-tolerant (O + T–pH–S–), and this group contained *Penicillium* sp., *Arthrinium* sp., *Aspergillus flavus*, and *Hortaea werneckii* ($r = 0.30$–0.38).

The genetic link between our acquired sequences and the GenBank reference sequences was confirmed using phylogenetic analysis. The phylogenetic tree's topology revealed a distinct separation into clades with strong support, which corresponded to the recognized taxa belonging to the Aspergillacae (37.5%), Dothiomyceta (21.87%), Sordariamyceta (28.12%), and Yeasts (12.5%) (Figure 5). Aspergillacae demonstrated two distinct clads: the *Aspergillus* genera (bootstrap value = 72), which demonstrated a more diversified structure compared to *Penicillium* spp. (bootstrap value = 98). Sordariamyceta was mostly represented by *Parengyodontium album*, which was a ubiquitous species detected in almost all sites, and the genetic analysis demonstrated no variability among these isolates with a bootstrap value of 100%. Similarly, Dothiomyceta was represented by *Alternaria* species with a bootstrap value of 100%, and *A. tenuissimia* and *A. compacta* were the predominant species. The yeast group was very diversified (87%), and distinct branches were shown for each genus with a predominance of *Hotrtea* and *Candida*.

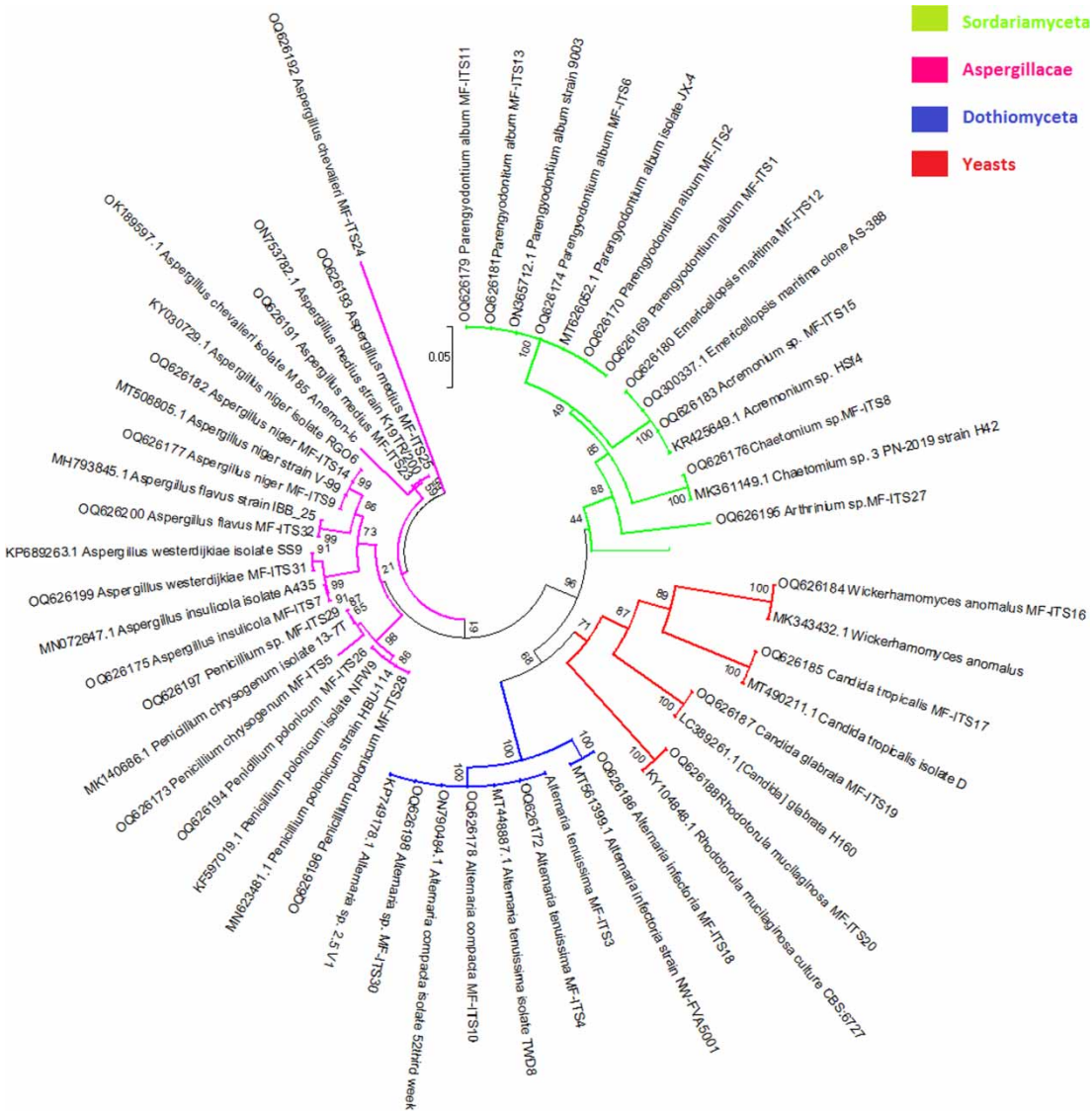


Figure 5 | Phylogenetic analysis by the neighbor-joining algorithm using MEGA 11 software based on the ITS1-5.8S-ITS2 region. The numbers above the branches indicate the bootstrap sampling percentages.

4. DISCUSSION

This is the first study that thoroughly examines fungal diversity in the coastal marine environments of Tunisia's Monastir Bay utilizing a culture-dependent methodology. Rapid anthropogenic disruptions are occurring in this ecosystem due to development, tourism, navigation, and the growth of aquaculture. In this study, 32 fungal taxa were identified in seven different sites from the Monastir Bay. Interestingly, Ascomycota was the dominant phylum in samples from the bay. A similar result (Abdel-Wahab *et al.* 2019) has been observed in the Red Sea mangroves in Saudi Arabia and in the coastline of Alexandria, Egypt (Gad *et al.* 2020). Ascomycota is the largest group of the Kingdom Mycetae with more than 32,000 species, of which more than 500 species are of obligate marine origin (Raghukumar 2017). Our isolated strains were assigned to four major groups (Aspergillacae 37.5%, Dothiomyceta 21.87%, Sordariamyceta 28.12%, and Yeasts 12.5%). In Ascomycota, members of Sordariomycetes, Dothideomycetes, and Eurotiomycetes have been frequently found in marine environments (Wang *et al.* 2019b).

Aquatic contamination has been extensively monitored over the past 10 years using molecular, biochemical, and cellular markers (Boulajfene *et al.* 2021). Therefore, marine fungi may one day allow this group of species to be used as bioindicators

of ecological status and indicators of the quality of the water (Pietryczuk *et al.* 2018). Disposing of untreated effluents can have an impact on the function and health of aquatic ecosystems, which can also have an impact on the microbial diversity of aquatic environments. Studies have shown that biotic interactions and local environmental variation can affect how microbial communities in the Monastir Bay are organized. However, water contaminants can also have an impact on microbial diversity and community composition, making them sensitive indicators of ecosystems (Ortiz-Vera *et al.* 2018). Our results showed that the contaminated site Kheniss presented the highest fungal diversity. Geochemical studies have demonstrated that metal and organic pollutants had an impact on this shoreline (Sassi *et al.* 1998; Sahnoun 2000; Sahnoun *et al.* 2003; Nouira *et al.* 2013). Because the hydrodynamics in the bay are weak, nutrient-rich wastewater discharged at various locations along the shoreline has gradually caused eutrophication and is the main cause of the decreasing nature of surface sediments (Sassi *et al.* 1998).

Furthermore, Nouira *et al.* (2013) stated that the highest concentrations of additives in pesticides (polychlorinated biphenyl (PCBs)) were detected in front of the Drain of Kheniss and near Teboulba city, which has known rapid industrialization and socio-economic development for the last 20 years. Also, metal analyses performed in this littoral (Sassi *et al.* 1998; Sahnoun *et al.* 2003; Tarchi *et al.* 2023) have shown that higher levels of Pb, Cr, and Zn were found in front of Kheniss City and Lamta-Sayada agglomeration. (Sassi *et al.* 1998; Sahnoun *et al.* 2003; Tarchi *et al.* 2023). The high fungal species diversity observed at the kheniss site can be explained by the diversity of pollutants. We noted that *Aspergillus* was the dominant fungal genus in the bay (21.87%). This genus is widely distributed in a variety of ecosystems around the world. Researchers have identified the culturable diversity of marine fungi mostly in nutrient-rich sediments using culture-based approaches (Sen *et al.* 2022). *Aspergillus*, *Trichoderma*, *Arthrinium*, *Cladosporium*, *Penicillium*, *Cystobasidium*, *Exophiala*, *Graphium*, *Lecanicillium*, *Purpureocillium*, *Acremonium*, *Coniothyrium*, *Simplicillium*, and *Mucor* species were the most frequently observed filamentous fungi and molds in previous studies, and a significant portion of the culturable diversity in the water implying that environmental conditions may affect the organization of the fungus population, which in turn may affect how ecologically diverse coastal sediments (Wu *et al.* 2023). Metagenomic techniques have been used in India to identify various fungal species as prospective biomarkers for nutrient pollution or eutrophication in the confluence zone of the Ganges and Yamuna rivers. These genera include *Penicillium*, *Kluyveromyces*, *Nakaseomyces*, *Aspergillus*, and *Lodderomyces*. According to Al-Nasrawi (2012) and Amend *et al.* (2019), filamentous fungi like *Aspergillus*, *Cunninghamella*, *Penicillium*, *Cladosporium*, *Mucor*, and *Fusarium* contribute to the breakdown of aliphatic and aromatic hydrocarbons in marine settings. In both freshwater and marine water ecosystems, certain filamentous fungi, including *Aspergillus*, *Penicillium*, *Trichoderma*, *Mucor* (*M. hiemalis*), and *Mortierella*, can accumulate toxic heavy metals in their biomass and are used as an efficient tool for biomonitoring and bioremediation of heavy metals (Hoque & Fritscher 2019). The presence of *Candida* yeasts and two significant genera of Basidiomycota yeasts, including *Rhodotorula* and *Rhodosporidium*, were also shown by a molecular diversity investigation of marine fungus across 130 environmental samples from Europe (Richards *et al.* 2015). According to a recent study conducted in South Africa, the prevalence of yeast variety in surface water poses a risk to water users and may be a sign of numerous types of pollution (Monapathi *et al.* 2021). Eutrophication's excess carbon allowed for thicker biofilms, which increased the amount of organic matter in the ocean according to Misic *et al.* (2022).

A significant issue is the buildup of plastic waste and trace elements in terrestrial and aquatic habitats (Bradney *et al.* 2019). Few studies have so far specifically targeted microeukaryotic communities, and more precisely fungal communities, associated with plastic debris (Amend *et al.* 2019). While playing a vital role as decomposers in the environment, fungi comprise only about 3% of all eukaryotic organisms in the plastisphere (Rogers *et al.* 2020). *Aspergillus* sp., and *Candida* sp. are eukaryotic pathogens interacting with different plastic polymers in environmental and nosocomial studies (Ormsby *et al.* 2023). Though fungi are common plastic colonizers in the ocean only two species, *Zalerion maritimum* and *Alternaria alternata*, have been identified as polyethylene degraders in the marine realm (Vaksmaa *et al.* 2023). Several fungal taxa including *Phaeophleospora eucalypticola*, *Alternaria* sp., *Aureobasidium* sp., and *Cladosporium* sp. were demonstrated to have strong plastic-degrading activity (Kim *et al.* 2022).

In the present study, the composition of fungal community showed a close relation to most measured environmental factors, that is, temperature, salinity, pH, and dissolved oxygen. The current study demonstrated that yeast community (*Candida* sp., *Rhodotorula mucilaginosa*, and *Hortaea werneckii*) to have important concentrations of oxygen, pH, and temperature; in contrast the filamentous fungi were ubiquitous. Our findings are in agreement with previous reports, highlighting the influence of water, temperature, and oxygen concentration as major environmental factors influencing the assembly of fungal communities in coastal ecosystems (Wang *et al.* 2018, 2019a, 2019b; Rojas-Jimenez *et al.* 2019). The study reported by

Xu *et al.* (2023) assessed the spatiotemporal distribution of fungal communities in Dongshan Bay, Southern China (a semi-enclosed bay with mariculture activity) using high-throughput sequencing techniques of both DNA and RNA showed that total and active fungal communities were related to several environmental factors, such as temperature, pH, dissolved oxygen, total dissolved solids, NH_4^+, NO_2, NO_3, and PO_3^-. Nevertheless, Rojas-Jimenez *et al.* (2020) were unable to detect a strong effect of depth and the overlying water temperature, salinity, dissolved oxygen, and pH on the composition of fungal communities in several marine sediments in the Eastern Tropical Pacific of Costa Rica.

Additional studies across all seasons are needed to evaluate the diversification and distribution of fungal communities across space and time, which would profoundly enhance our understanding of the possible seasonal differences in fungal community composition and offer insights into eco-evolutionary processes along a gradient in anthropogenic impacts in the Monastir Bay.

5. CONCLUSIONS

Fungal communities interact with the environment and can serve as bioindicators of human activity in aquatic ecosystems. Several studies have examined the impact of human activities on fungal communities, finding community diversity and composition to be affected by such activities. Here, we sequenced the internal transcribed spacers of fungi to explore the diversity of fungal communities at seven sites in the Monastir Bay. The results of the study showed that the highest fungal taxonomic diversity was found in the site with a high pollution degree. In addition, factors affecting the structure of fungi community including water pH, salinity, and dissolved oxygen concentration were investigated. Findings presented here appear to provide another important reason to include data on the abundance and diversity of fungal species when assessing the ecological and sanitary status of marine waters.

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AUTHOR CONTRIBUTIONS

A.N. developed the original idea, contributed to interpreting the data, supervised the project and revised the manuscript. R.C.B. was responsible for developing the protocol, conducting the experiments, analyzing the data, and drafting the initial manuscript. R.B.D. assisted in the experiments and sample collection, while K.G. contributed to the development of the protocol, assisted in the experiments, and provided revisions to the manuscript. S.B. and R.C. both assisted with the experiments and made contributions to the manuscript. N.H. participated in both the drafting and revision of the manuscript. H.B. reviewed and revised the manuscript, as well as providing guidance throughout the project.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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