



Density and diversity of filamentous fungi in the water and sediment of Araçá bay in São Sebastião, São Paulo, Brazil

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Abstract: Araçá Bay, located in the city of São Sebastião, São Paulo, Brazil, is a protected area of substantial complexity. It represents the last remaining mangrove swamp preserve between the cities of Bertioga and Ubatuba on the northern coast of São Paulo State. This mangrove swamp has specific physical and chemical properties, and it shelters a wide variety of life, including fungi. These microorganisms are present in a variety of species with different morphophysiological features, and they have the ability to produce enzymes of biotechnological importance. The goal of this study was to quantify, isolate, and identify filamentous fungi in water and sediment samples from the Araçá Bay mangrove swamp in São Sebastião. Two samplings were performed in the summer and two were performed in the winter. The samples were collected from intertidal zones, and dissolved oxygen (DO), temperature, salinity, and pH were measured in situ. The spread plate technique was used to inoculate the samples collected on plates with a potato dextrose agar (PDA) medium. A total of 208 colonies (68 from water samples and 140 from sediment samples) were isolated, and they were identified based on their morphological characteristics. Filamentous fungus density was higher in the sediment than in the water, and the samplings performed in the winter revealed a higher density than those performed in the summer. Though some of the environmental parameters were not ideal for fungal development, a high quantity of growth was nevertheless observed. When the isolated colonies were analyzed, the greatest diversity and species richness were found in the summer samples. The genera identified in all of the samples were *Aspergillus*, *Penicillium*, *Cladosporium*, and *Fusarium*. The pathogenic species found from these genera were *Aspergillus fumigatus*, *A. terreus*, *Penicillium citrinum*, and *P. chrysogenum*. These species are also able to produce enzymes that offer a variety of applications. The fungal community described herein represents the diversity found in this mangrove swamp during the period studied. Many of the fungus species found are pathogenic and may be useful due to their ability to produce specific enzymes applicable in the biotechnological and pharmaceutical industries.

Keywords: Mangrove swamp, Environmental parameters, Mycology.

Densidade e diversidade de fungos filamentosos na água e sedimento da Baía do Araçá, São Sebastião (SP), Brasil

Resumo: A Baía do Araçá, localizada no município de São Sebastião (SP), é uma região protegida, de grande complexidade, mantendo o último remanescente preservado de manguezal entre Bertioga e Ubatuba, litoral Norte do Estado de São Paulo. Manguezal caracteriza-se com propriedades físico-químicas específicas e abriga grande diversidade de seres vivos, entre eles os fungos. Estes microrganismos apresentam grande variedade de espécies e diferentes características morfofisiológicas com capacidade de produzir algumas enzimas de importância biotecnológica. Este trabalho teve como objetivo quantificar, isolar e identificar fungos filamentosos em amostras de água e de sedimento do manguezal da Baía do Araçá, São Sebastião, (SP). Foram realizadas 2 coletas no verão e 2 no inverno na região entremarés, medidos “in situ” os parâmetros oxigênio dissolvido (OD), temperatura, salinidade e pH. Utilizou-se a Técnica “Spread Plate” para inocular os materiais coletados, em placas com meio Potato Dextrose Agar (PDA). Foram isoladas 208 colônias (68 das amostras de água e 140 das amostras de sedimento) e identificadas pelas características morfofisiológicas. As densidades de fungos filamentosos foram maiores no sedimento em relação à água e nas coletas realizadas no inverno

demonstraram maiores densidades do que no verão. Apesar de alguns parâmetros ambientais não se apresentarem ideais para o desenvolvimento dos fungos, observou-se alta quantidade de crescimento nas amostragens. Analisando as colônias isoladas, a maior diversidade e riqueza foram observadas na amostragem coletada no verão. Foram observados os gêneros *Aspergillus sp*, *Penicillium sp*, *Cladosporium sp* e *Fusarium sp* em todas as amostragens, dos quais as espécies *Aspergillus fumigatus*, *A. terreus*, *Penicillium citrinum* e *P. chrysogenum* identificados neste trabalho são considerados patogênicos mas também são espécies capazes de produzir enzimas aplicáveis em diversas atividades. A comunidade fúngica descrita apresenta a diversidade encontrada neste manguezal em relação à variedade ambiental no período estudado, sendo que muitos apresentam patogenicidade e podem ser úteis pela sua capacidade de produzir enzimas específicas aplicáveis nos estudos biotecnológicos e farmacêuticos.

Palavras-chave: Manguezal, parâmetros ambientais, micologia.

Introduction

Brazil possesses 1,225,444 hectares (approximately 12,250 km²) of mangrove swamps (MMA 2010). Of these, approximately 76 km are located on the northern coast of São Paulo State, and 2.1% are located within the city of São Sebastião (Dias-Brito et al. 2014).

Araçá Bay is part of the Alcatrazes Municipal Environmental Protection Area as per Municipal Law No. 848/1992. It is also part of the Northern Coast Marine Protection Area as per State Law No. 53.525/2008 (PLDS/Araçá 2016). It represents the last remaining mangrove swamps on the São Sebastião coast, and it harbors high biological diversity (Amaral et al. 2010, PLDS/Araçá 2016). This region is considered to be relatively protected by its physical makeup and location. It includes a cove bordered by rocky walls and an extensive coastal plain with soft sand, which impedes the direct effects of tide-based hydrodynamic factors (Amaral et al. 2010, Alitto et al. 2016). The region is exposed to different types of human activity, including illegal occupation (Zanardi et al. 1999), domestic effluents (PLDS/Araçá 2016), underwater dredging (Gubitoso et al. 2008, Teodoro et al. 2011, PLDS/Araçá 2016), and activities in the nearby Port of São Sebastião (Amaral et al. 2010, PLDS/Araçá 2016).

Mangrove swamps are considered a transitory coastal ecosystem between land environments and marine environments. They are typical of tropical and subtropical regions and are effected by tides (Schaeffer-Novelli 1995, Schaeffer-Novelli 2000, Andreote et al. 2012). Mangrove swamp conditions are conducive to the feeding, protection, and reproduction habits of many animal species, and they are considered an important environment for the transformation of organic matter into nutrients (Schaeffer-Novelli 1995, Schaeffer-Novelli 2000, Sridhar 2004). Mangrove swamps are also considered an important niche repository for fungi (Paul & Clark 1989, Roitman et al. 1991, Nambiar & Raveendran 2008), which play an important role in the organic matter decomposition processes present in the ecosystem (Citrón & Schaeffer-Novelli 1983, Allsopp & Seal 1986, Jones et al. 2009).

Hawksworth (1991) estimated that fungal diversity is represented by approximately 1.5 million species around the world; however, recent studies have reported that this amount may be ten times higher: between 3.5 and 5.1 million species (O'Brien et al. 2005).

The survival of mycobiota and their diversity are influenced by a number of abiotic factors, one of the most important of which is hydrogen concentrations, which influences fungal growth capacity and composition. Temperature, salinity, dissolved oxygen (DO) levels, and the availability and diversity of substrata are factors which may also influence fungal composition in a given environment (Jaitly & Rai 1982, Jaitly 1987, Jones & Alias 1997, Tucci & Mendes 2006), as is sediment texture (Roitman et al. 1991).

The Araçá Bay mangrove swamp substratum is made of sand and mud; it is relatively compact and it includes pebbles (Amaral et al. 2010). Schaeffer-Novelli (1995) described this mangrove swamp sediment as sandy and clayey with varying features, characteristics which may result from

leaf and twig decomposition in the environment, as well as from animal matter. It may also contain the products of rock decay. Different types of rocks are brought into the environment by waves, winds, costal currents, and river flows (Schaeffer-Novelli 1995).

One study found that the fungi in sediment play an important role in processing plant-based organic matter (Carlile et al. 2001) and are active in the decomposition of organic compounds in both the sediment and the water (Harley 1971, Moore-Landecker 1996). Some characteristics of the waters in the environment favor the presence and propagation of fungi. Due to their importance in the ecosystem, these fungi must be evaluated using physical, chemical, and microbiological analyses (Jaitly & Rai 1982, Alexopoulos et al. 1996, Jones & Alias 1997, Duke et al. 1998). Jaitly & Rai (1982) isolated species of fungi from the Sunderbans mangrove swamp in India and found fungi that were thermophilic and thermotolerant. Their findings were based on specific parameters, including salinity, humidity, pH, and the amount of organic matter in the region.

Fungal species diversity in the world varies by location (latitude, longitude, and altitude), as well as by depth in the case of aquatic ecosystems. There is a tendency for greater species diversity in the tropics (Shearer et al. 2007), and most fungi isolated in the tropics and subtropics are anamorphic basidiomycetes and ascomycetes (Jones & Alias 1997, Sridhar et al. 2005). These fungi have been associated with numerous processes and functions, including organic matter decomposition (Tortora et al. 2005, Jones et al. 2009), geochemical cycles (Tortora et al. 2005), their usefulness in the pharmaceutical industry (Walsh et al. 2004), their value in the food industry (Molitoris 1995), and their utility in biotechnology (Atlas 1981, Carneiro & Gariglio 2011, Pereira & Freitas 2012, Houbraken et al. 2014), among other benefits (Tortora et al. 2005, Raghukumar 2008).

Some fungi are disease-causing pathogens responsible for mycoses and allergies. They are associated with diseases affecting humans, plants, and animals alike, and they are frequently detected in polluted areas (Walsh et al. 2004). However, they also produce enzymes with biotechnological applications. These microorganisms are being tested for their ability to produce enzymes, and the most frequently isolated genera are *Penicillium* and *Aspergillus*, the latter two of which are responsible for hydrocarbon assimilation (Atlas 1981). These species also degrade water and soil pollutants into less environmentally damaging forms (Vidali 2001, Novotny et al. 2004, Passos et al. 2009).

Aspergillus terreus was a fungus isolated from marine sediments and land habitats (Damare et al. 2006). It produces different secondary metabolites, but also causes infections such as aspergillosis and mycosis (Martins et al. 2005). Fungi are highly relevant for scientific research in the fields of biotechnology and bioremediation due to their potential ability to degrade organic compounds and contaminants, and they can also provide information on the environment, on biological and functional diversity, and on mangrove swamp ecosystems (Harms et al. 2011, Bonugli-Santos et al. 2015).

The objective of this study was to analyze the density and diversity of filamentous fungus species in water and sediment samples from the Araçá Bay mangrove swamp using molecular and morphophysiological methods, and to then correlate the findings with physical and chemical factors in the winter of 2015 and the summer of 2016.

Materials and Methods

In 2015 and 2016, samplings were performed at ten sites determined using a GPS device in the intertidal region at high tide (Figure 1). Two samplings were performed in the winter dry season (June and July 2015), and two were performed in the summer rainy season (January and February 2016). The water was collected in sterilized flasks from a depth of 15 to 30 cm, as per American Public Health Association standards (APHA, 2005). Surface sediment was collected from the same points and stored in sterile bags until processing in the Marine Microbiology Laboratory (MICROMAR), located on São Paulo State University's São Vicente campus (UNESP, São Vicente). The DO, temperature, pH, and salinity parameters were measured on site using a HORIBA U-50 multiparameter meter (Multiparameter Water Quality).

Twenty-gram sediment aliquots were conditioned in sterile flasks to which 180 mL of sterile purified water was added. The samples then underwent manual agitation for 10 minutes, a process which was repeated in order to loosen any microorganisms which had adhered to grains of sediment. Once the samples had been agitated, only the supernatant was used for the microbiological analyses (Oliveira & Pinhata 2008).

Next, 500 μ L of sample material was inoculated using spread plate technology and potato dextrose agar (PDA) plates, to which the antibiotic chloramphenicol was added. The samples were then incubated at room temperature (25°C) for 7 to 14 days. After the incubation period, the growth of filamentous fungus colonies on the plants was determined based on the total number of mold colonies on each incubated plate (APHA 2005). These values were calculated and expressed as colony-forming units (CFUs) per 100 ml (CFU 100mL⁻¹) in the case of the water samples and as

CFUs per 100 g (CFU 100g⁻¹) in the case of the sediment samples. A total of 208 colonies were isolated and re-isolated until purity was reached. Macromorphological features (color, diameter, and appearance of the colony) and micromorphological features (microstructures) were determined in order to identify the genera in a method similar to those provided by Raper & Fennell (1977), Nelson et al. (1983), Hanlin & Ulloa (1988), Pitt (1985), Domsch et al. (1993), Alexopoulos et al. (1996), Houbraken et al. (2014), and Talaiekhosani & Ponraj (2015).

Based on the most commonly observed microscopic characteristics, three physiological structure clusters were established. These three characteristics were the presence of vesicle on the conidiophore (7 colonies with *Aspergillus* features) the lack of a vesicle (11 colonies with *Penicillium* features), and a unique conidia format (2 colonies), which together represented the 20 fungi which were identified at the molecular level in the Microbiology Laboratory at the Center for Social Insect Studies at São Paulo State University's Rio Claro Campus (UNESP Rio Claro). The DNA was extracted and amplified using the polymerase chain reaction (PCR) technique. The genomic DNA was then sequenced using a modified version of the extraction technique method with cationic hexadecyl trimethyl ammonium bromide (CTAB), and two different nuclear DNA markers (LSU and ITS) were amplified for phylogenetic analysis (Möller et al. 1992). The PCR products were cleaned using a Wizard[®] SV Gel and PCR Clean-Up System (Promega). The cycle sequencing reactions were performed using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Life Technologies), and the sequences were generated using an Applied Biosystems[®] 3500 genetic analyzer (Life Technologies). The contigs were assembled in the BioEdit sequence alignment editor, version 7.1.3 (Hall 1999) and were consulted in GenBank from the National Center for Biotechnology Information (NCBI).

The statistical analysis was performed using the Past software. To determine the normality of the data, the Kruskal-Wallis test was applied at a 95% significance level ($p < 0.05$). Spearman's correlation test was used to correlate the non-parametric data on the environmental parameters and fungus density. Fungus species diversity and richness were determined using the Shannon diversity index (H) and the Chao 1 estimator, respectively.

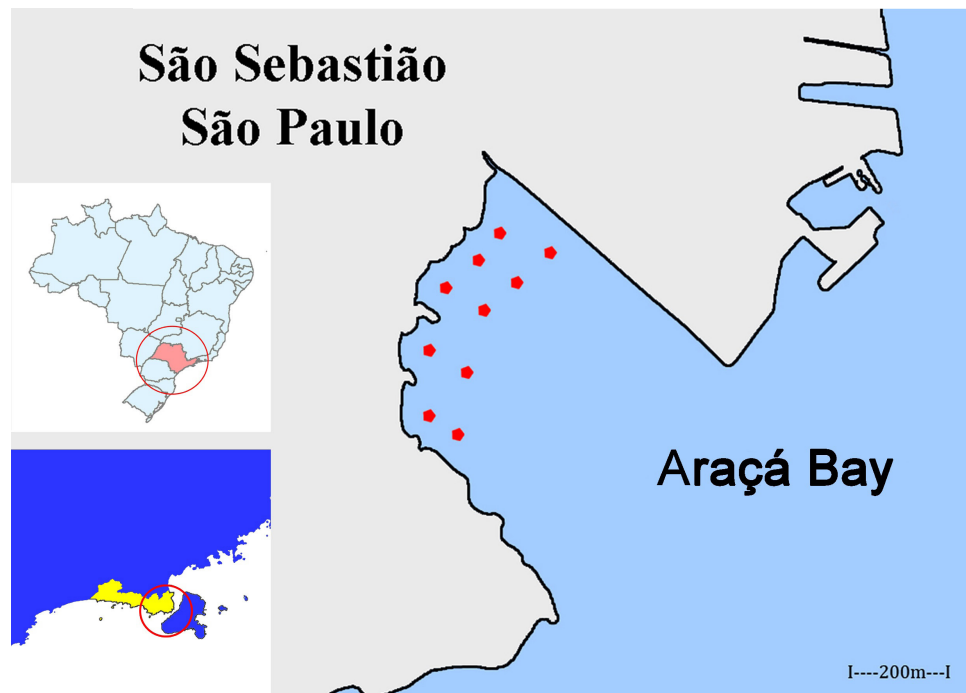


Figure 1. Map showing the research site and sampling points in the Araçá Bay mangrove swamp in the city of São Sebastião, São Paulo, Brazil.

Results and Discussion

Certain physical and chemical aspects of the environment, including temperature and pH, are important for the survival, adaptability, and growth of microorganisms (Jaitly 1987, Jones & Alias 1997, Raghukumar 2008). In this study, overall average temperature was 26°C. During the winter, this average was lower (23°C), and in the summer, it reached 27.7°C (Table 1). According to Kohlmeyer (1983) and Wong et al. (1998), the temperatures measured in this study favor fungal diversity and reproduction: these authors report that temperature affects the distribution and diversity of filamentous fungi, and that there is greater diversity in tropical areas than in temperate waters. In addition, marine fungi require temperatures between 25°C and 30°C to reproduce (Griffin 1981).

The pH levels detected herein remained slightly alkaline and ranged only from 7.7 to 8.1 during the study period (Table 1). This finding is consistent with the values reported by Sengupta & Chaudhuri (1995), who detected an alkaline pH ranging from 7.2 to 8.0 and who reported on filamentous fungi in mangrove swamp sediments from the Ganges River in India. Extracellular enzyme production and growth by marine fungi was described by Raghukumar et al. (2004), who reported a pH between 7 and 8. Jaitly (1987), Alexopoulos et al. (1996) and Raghukumar (2008) report that the best pH for the growth of different types of land fungi ranges from 4 to 7 (in the neutral to acidic range).

The salinity levels detected in this study remained below 30; the water can therefore be classified as brackish as per Brazil's National Environment Council (CONAMA) Resolution No. 357 from March 2005. The values did not vary substantially over the course of the study and ranged from 24.8 to 28.1 (Table 1). Duke et al. (1998), Bunt (1999), and Mueller & Bills (2004) report that salinity is a very important ecological parameter which is directly correlated with species distribution in mangrove swamps. Though Mueller & Bills (2004) describe salinity as a limiting factor for fungus growth, high fungal density was observed in the current study. Lorenz & Molitoris (1992) found that the ideal salinity level for the growth of certain marine fungi increases as incubation temperature increases, a finding which indicates that Araçá Bay exhibits ideal parameters for mycological development.

The DO values (mg L⁻¹) ranged from 4.0 to 6.7, with a mean value of 5.5 (Table 1). In most of the samples, dissolved oxygen values remained within the limits established by CONAMA Resolution No. 357/2005 for high-quality brackish waters. Most fungi are aerobic or microaerophilic; however, some species have been found to have a limited anaerobic metabolism, and few are capable of completely anaerobic growth (Tabak & Cooke 1968, Tortora et al. 2005). Mangrove swamps typically exhibit low levels of oxygen (Schaeffer-Novelli 1995); however, most of the samples from the Araçá Bay mangrove swamp were found to exhibit good DO quality. Because oxygen is considered an essential element for

aerobic fungi, the environment analyzed herein may be considered favorable for the development of these microorganisms.

Based on the samplings, filamentous fungus colony density was found to range from 0.1x10⁴ CFU 100 mL⁻¹ to 4.6x10⁴ CFU 100mL⁻¹ in the water and from 0.4x10⁴ CFU 100g⁻¹ to 42.5x10⁴ CFU 100g⁻¹ in the sediment. Density varied considerably between the two materials. The samplings performed in the summer produced lower values both in the water (0.2x10⁴ CFU 100mL⁻¹) and in the sediment (0.9x10⁴ CFU 100g⁻¹). In the winter, the highest density was found in a sediment sample: 42.5x10⁴ CFU 100g⁻¹ (Table 1). Pupin & Nahas (2014) analyzed dry sand from the swamp on Cardoso Island and determined the number of CFUs to be between 5.2x10⁵ g⁻¹ and 9.7x10⁵ g⁻¹, higher values than those obtained in the current study.

Filamentous fungus density was found to be much higher a total mean in the sediment samples and relative to the winter in the case of both the water samples and the sediment samples (Figure 2). Based on Spearman's correlation test, the statistical analysis did not reveal any significant correlations between the environmental parameters and fungal density in the water (p>0.05); however, the amount of fungi in the sediment was found to be significantly and positively correlated with salinity and temperature (p=0.032 and p=0.024, respectively). In this study, filamentous fungus colony density in the sediment was significantly higher than in the water (p<0.05; non-parametric Kruskal-Wallis test=9.8x10⁻⁸).

Similar findings were reported by Gomes et al. (2011), who analyzed filamentous fungi isolated from sediment collected from a mangrove swamp in Barra das Jangadas, Pernambuco, Brazil. The authors reported a higher incidence of fungi during the dry season (77%) relative to the rainy season (23%) and found that the species *Aspergillus terreus*, *A. niger*, *Penicillium sp.*, and *Trichoderma sp.* were common in both seasons (Gomes et al. 2011). Pinto et al. (1992) also found that rainfall significantly increases the amount of isolated fungi in both land and aquatic ecosystems.

The colonies isolated were first identified based on macromorphological and micromorphological features (Figure 3). A total of 208 filamentous fungus colonies with different morphological features were isolated, 68 of which were from water samples and 140 of which were from sediment samples. In addition to anamorphs, a total of 15 genera were found: *Aspergillus*, *Aureobasidium*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Geotrichum*, *Mucor*, *Paecilomyces*, *Penicillium*, *Rhizopus*, *Sporothrix*, *Talaromyces*, *Trichophyton*, *Trichoderma*.

Of the fungi isolated, *Aspergillus* and *Penicillium* were the most commonly observed genera overall (38.9% and 35.0%, respectively). In the water, the genus *Aspergillus* was the most common (39.7%), while in the sediment, *Penicillium* was the most common (42.8%), followed by the other genera at lower frequencies (Figure 4). The colonies that were found in the asexual reproductive stage or for which microscopically differentiating features could not be detected were treated as anamorphs.

Table 1. Environmental parameters obtained from mangrove swamp samplings from Araçá Bay on the northern coast of São Paulo State, Brazil. Temp. (Temperature in °C), pH, salinity, dissolved oxygen (DO mg L⁻¹). Mean filamentous fungus density in water (expressed as CFU 100mL⁻¹) and in sediment (expressed as CFU 100g⁻¹). Min - Minimum value; Max = maximum value; x - mean; s - Standard deviation.

samples	Environmental parameters	Total		Summer		Winter	
		min - max	x ± s	min - max	x ± s	min - max	x ± s
water	Temp. (°C)	22.6 - 31.6	26.2 ± 2.9	24.3 - 31.6	27.7 ± 3.0	22.6 - 25.4	23.9 ± 1.0
	pH	7.7 - 8.1	7.9 ± 0.1	7.7 - 8.0	7.9 ± 0.1	7.8 - 8.1	8.0 ± 0.1
	salinity	24.8 - 28.1	27.0 ± 0.7	24.8 - 27.5	26.6 ± 0.7	27.1 - 28.0	27.4 ± 0.3
	DO (mg L ⁻¹)	4.0 - 6.7	5.5 ± 0.8	4.0 - 6.5	5.1 ± 0.7	5.3 - 6.7	6.2 ± 0.4
	Filamentous fungus (x10 ⁴ UFC 100mL ⁻¹)	0.1 - 4.6	0.3 ± 0.8	0.1 - 1.1	0.2 ± 0.2	0.1 - 4.6	0.5 ± 1.3
sediment	Filamentous fungus (x10 ⁴ UFC 100g ⁻¹)	0.4 - 42.5	5.0 ± 10.2	0.4 - 2.0	0.9 ± 0.4	0.5 - 42.5	10.4 ± 14.1

Density and diversity of fungi in mangrove swamp

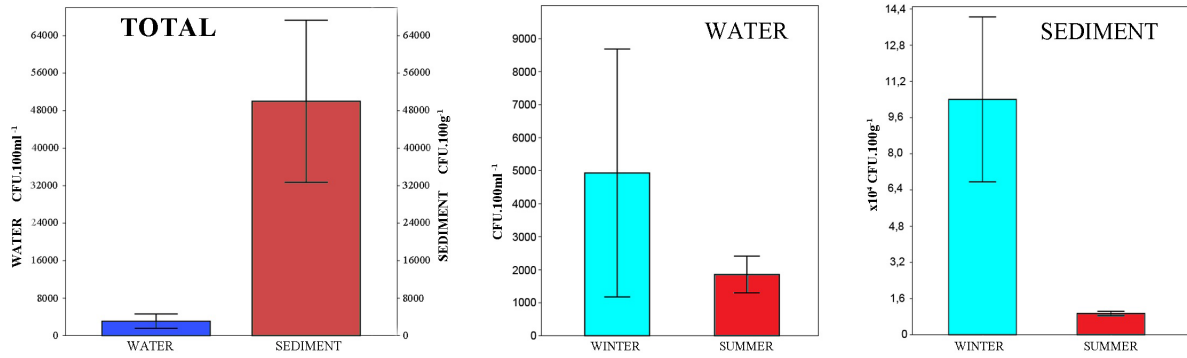


Figure 2. Mean filamentous fungus colony densities TOTAL - overall mean including the water and sediment samples; WATER (expressed as CFU 100mL⁻¹); SEDIMENT (expressed as x10⁴ CFU 100g⁻¹); comparisons between the seasons.

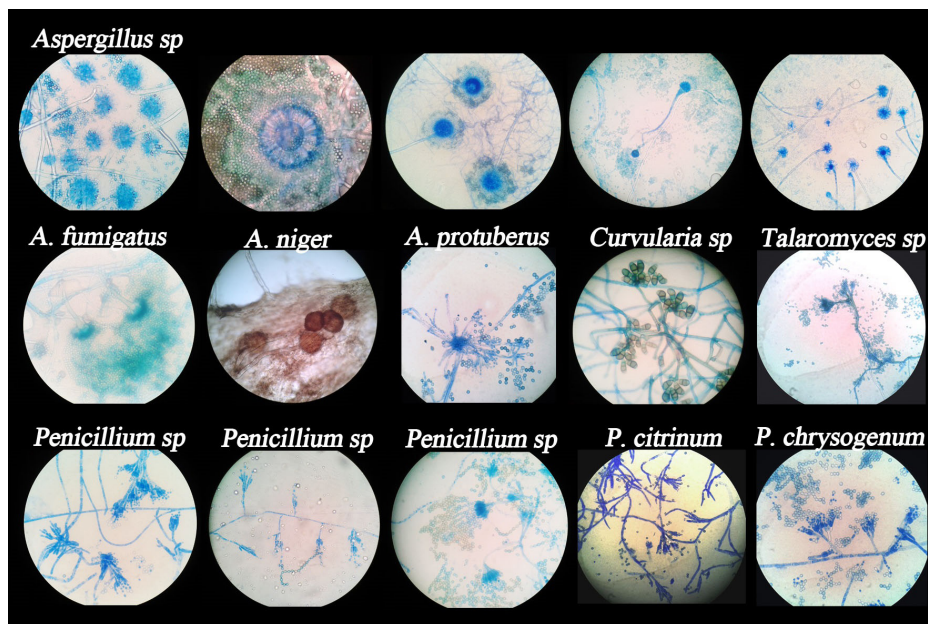


Figure 3. Isolated filamentous fungi observed microscopically.

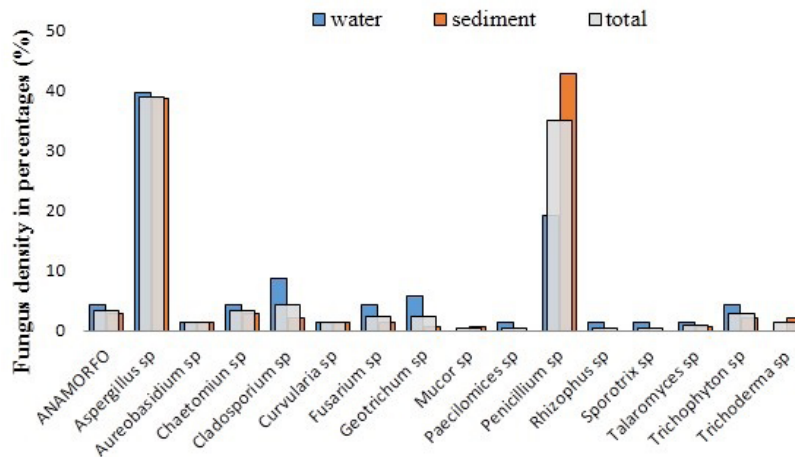


Figure 4. Percentage of filamentous fungus genera identified morphologically in samples isolated from water and sediment.

Most fungal species were observed in both materials sampled, but the genera *Paecilomyces*, *Rhizopus*, and *Sporothrix* were found only in the water samples, and *Mucor* and *Trichoderma* were found only in the sediment samples.

From the colonies which had been clustered based on micromorphological characteristics, 20 fungus samples were selected for DNA extraction, amplification, and sequencing for molecular identification. Of these 20 samples, 7 had *Aspergillus* features, 11 had *Penicillium* features, and 2 had unique conidia. Based on the sequencing and GenBank results, 2 samples were identified as *Aspergillus fumigatus*, 2 samples were identified as *A. protuberus*, 1 sample was identified as *A. terreus*, 4 samples were identified as *Penicillium citrinum*, 5 samples were identified as *P. chrysogenum*, 1 sample was identified as *Talaromyces flavus*, 1 sample was identified as *Cladosporium* sp., 2 samples were identified as *Penicillium* sp., and 2 samples were identified as *Aspergillus* sp.

In the water, the genus *Aspergillus* was dominant in the winter, representing 54.5% of the specimens found, followed by *Penicillium* at 25.0%. In the summer, 20.8% of the specimens found in the water were determined to be from the genus *Cladosporium*, and 12.5% were found to be from the genus *Aspergillus*. In the sediment, the most commonly detected genera in the winter were *Penicillium* (47.2%) and *Aspergillus* (40.8%), and the most commonly detected genera in the summer sediment were *Aspergillus* (20.0%), *Cladosporium* (13.3%), and *Trichoderma* (13.3%). The genus *Aureobasidium* was detected in both the water and the sediment and only in the summer (Figure 5).

Similar results were reported by Gomes et al. (2008), who analyzed water with high salinity and alkaline pH from Casa Caiada Beach in Orinda, Pernambuco State, Brazil. They isolated 50 species of fungi, the most common of which were from the genera *Aspergillus* and *Penicillium*. In another study by Gomes et al. (2001), *Penicillium* was the most diverse genus in the dry season sediment collected from the Barra das Jangadas swamp in Pernambuco State. Sengupta & Chaudhuri (1995) isolated fungi from the sediment and from an estuary of the Ganges River in India. The most commonly found genera were *Aspergillus* and *Penicillium*,

though the salinity levels found were lower (11 to 15) than those detected in the current study.

Gomes et al. (2008) analyzed water and sediment from a beach in Olinda, Pernambuco, in both the dry season and the rainy season. They most frequently isolated and identified *Aspergillus*, *Penicillium*, and anamorphs, followed by other genera, including *Fusarium*, *Trichoderma*, *Cladosporium*, *Curvularia*, and *Paecilomyces*. Rai et al. (1981) isolated 87 saprobic fungi from mangrove swamp wood in India. The most common ascomycetes were from the genus *Chaetomium*, the most common anamorphs were from the genus *Aspergillus*, and other frequently isolated genera included *Trichoderma*, *Pestalotiopsis*, *Curvularia*, *Fusarium*, and *Penicillium*. In southern India, water samples, sediment samples, and some aquatic organisms were collected, and different *Aspergillus* species were identified. These included *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*, and *A. terricola* (Babu et al. 2010).

Fungi from the genus *Cladosporium* are distributed worldwide and may be isolated from a variety of substrata, including water and sediment (Gomes et al. 2008), as well as from aquatic environments (Xiong et al. 2009, Silveira et al. 2013). Mbata (2008) isolated filamentous fungi from deep and highly saline marine waters. The species found included *Chaetomium globosum*, *Aspergillus versicolor*, *Hortaea wemeckii*, and *Aureobasidium pullulans*. *A. pullulans* is known to be distributed in marine environments with varying environmental conditions, and this species may be used in a variety of biotechnological applications (Liu et al. 2008) due to its ability to produce enzymes such as protease, lipase, and cellulase (Chi et al. 2009).

In Brazil, *Paecilomyces* fungi have been isolated from the sands of Ipanema Beach in Rio de Janeiro (Sarquis & Oliveira 1996), from the water and sediment on a beach in Olinda, Pernambuco (Gomes et al. 2008), and in the water from an estuary of the Patos Lagoon in the state of Rio Grande do Sul (Silveira et al. 2013). Richards et al. (2012) have described this genus as representing species of marine fungi, and Marante et al. (2012) isolated a new strain of *Paecilomyces variotti* from a marine environment, a strain which they determined was able to produce bioactive compounds.

Some of the fungi identified in the current study are known for being pathogenic. These include *Aspergillus fumigatus*, *A. terreus* (Martins et al. 2005, Walsh et al. 2008), *Penicillium chrysogenum* (Barcus et al. 2005), and

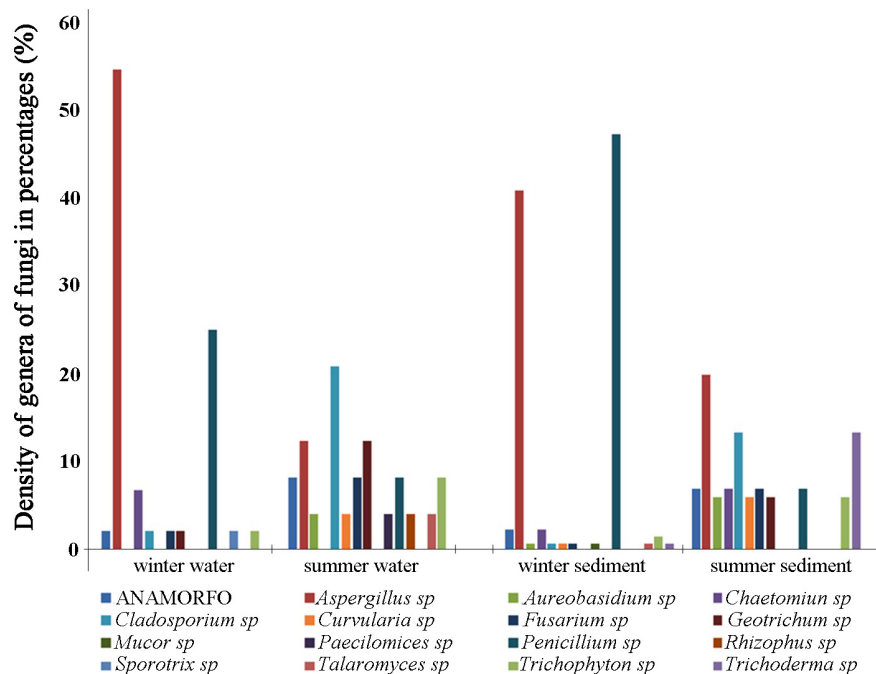


Figure 5. Filamentous fungus densities expressed as percentages (%) in water and sediment samples in the winter and summer.

Penicillium citrinum (Houbraken et al. 2010). Other studies have reported that some filamentous fungi are able to produce and assimilate enzymes for biotechnological applications. These genera include *Aspergillus* and *Penicillium*, which have proven to be important producers of enzymes such as cellulase (Zhang & Kim 2010, Castillo et al. 1994), xylanase (Zhang & Kim 2010, Shah & Madamwar 2005), lipase (Sharma et al. 2005, Zhu et al. 2009), and amylase (Prabakaran et al. 2009, Mishra & Dadhich 2010). The species *Aspergillus fumigatus* and *A. terreus* have been reported to have the potential to degrade hydrocarbon (Atlas 1981, Chaillan et al. 2004). *Penicillium chrysogenum* is able to produce amylase (Prabakaran et al. 2009, Silva et al. 2011) and alkaline protease (Zhu et al. 2009). Finally, *P. citrinum* has been found to produce both lipase (Silva et al. 2009) and cellulase (Santos et al. 2017).

In the current study, the greatest diversity was observed in the summer sediment ($H' = 2.4$), followed by the summer water ($H' = 2.1$), the winter sediment ($H' = 1.2$), and finally, the winter water, in which the lowest diversity was detected ($H' = 0.9$). According to Piedras et al. (2006), Shannon's diversity index may provide information on conservation and impacted environments. These authors also note that values between 1.0 and 3.0 reflect moderately polluted water, indicating that the region analyzed herein exhibits a good level of diversity relative to the environment. According to the Chao 1 estimator, the summer water sample exhibited the highest level of species richness among the samples (Chao 1=12). Though the summer sediment samples exhibited the highest level of diversity in this study, the water samples collected in the summer exhibited the highest amount of species richness. These findings indicate that analyses performed on summer samples may provide more information on these fungi.

Sebastianes et al. (2013) reported that the richness and diversity of endophytic fungi on leaves and branches in São Paulo swamps were higher in the summer than in the winter, results which are consistent with those of the current study. Maria & Shidhar (2003) also analyzed wood from coastal mangrove swamp in southeast India and found more fungal diversity in the rainy season than in the dry season. In the study by Yadav et al. (2016), season had a significant influence over the diversity of endophytic fungi: the highest species diversity was detected during the rainy season, while the lowest was detected during the dry season. However, their data on fungal species diversity was not found to correlate with the number of isolates found in each season.

Fungi isolated from environments with extreme variations in temperature and salinity are likely to exhibit physiological adaptations that allow them to tolerate or even affect the availability of other substances in the environment (Damare et al. 2006, Stoeck et al. 2003). Studies have noted the potential of fungi from marine ecosystems to have biotechnological applications after evaluating fungal diversity and the role of fungi in the bioremediation of pollutants in contaminated environments (Gaylard et al. 2005, Passos et al. 2009, Pereira & Freitas 2012, Bonugli-Santos et al. 2015). Filamentous fungi have been found to be very effective in removing heavy metals from aqueous solutions due to their greater resistance to these elements (Kurek et al. 1982, Collins & Stotzky 1992).

Conclusion

The isolation of filamentous fungi from habitats such as mangrove swamps, which exhibit a complex variety of physical and chemical characteristics, can aid in the determination of their biological diversity and their ability to survive under adverse conditions. This knowledge may improve our understanding of the distribution of these microorganisms and their interactions with their ecosystems, as well as the human impact on these organisms, their role in conservation, and their use in a range of preservation and remediation processes. Many of the fungi found in this study may serve as potential indicators in biotechnological processes.

The preservation of these microbial communities is beneficial for biodiversity and is an important factor in sustaining ecosystems. Furthermore, studies on diversity may help us to clarify information on native microorganisms,

on biological changes associated with environmental damage, and on how to protect plants specifically and ecosystems as a whole.

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Author Contributions

Sonia Assami Doi and Ana Julia Fernandes Cardoso de Oliveira: substantial contribution to the concept and design of the study; contribution to data collection, data analysis and interpretation; contribution to manuscript preparation and critical revision.

Aline Bartelochi Pinto: substantial contribution to the concept and design of the study; contribution to manuscript preparation and critical revision.

Maria Carolina Canali, Daiane Raquel Polezel and Roberta Alves Merguizo Chinellato: contribution to data collection, data analysis and interpretation.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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