ARENICOLOUS FILAMENTOUS FUNGI IN MAYAGÜEZ BAY SHORELINE, WESTERN PUERTO RICO

by

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Abstract

This study is a first attempt aimed to establish a record of identification, taxonomic characterization, and spatial distribution of arenicolous filamentous fungi in Mayagüez Bay, Western Puerto Rico. The primary goal of this study was to provide a better understanding of the fungi that is present between sand grains at certain ecological conditions at three different stations of Mayagüez Bay: Guanajibo, El Seco, and El Maní during the period of January through December, 2003. Bimonthly samplings consisted of temperature, salinity and pH measurements, and sand samples for determinations of fungi abundance. These samples were collected using conventional methods of sterilized Pyrex bottles and different medium culture for identification. Filamentous fungi grew and develop successfully in marine conditions, where they can withstand salinity variations. There were significant differences in species composition among sampling stations. The highest number of species was from El Seco sampling station, being *Aspergillus* spp. with 18.22% of representation*, A. flavus representing* 15.67%, *A. niger* (5.77%), *A. terreus* (13.39%), *Curvularia* spp. (10%), *Cladosporium* spp. (10.83%), *C. cladosporioides* (12.33%), *C. herbarum* (1.08%)*, C. oxysporum* (0.14%), *Dreschlera* spp. (0.05%), *D. biseptata* (0.03%)*, Fusarium* spp. (0.02%)*, Geotrichum* spp. *(*7.40%)*, Mucor* spp. (3.89%), *M. ramossissimus* (0.01%)*, Penicillium* spp. (1.15%), *P. rubrum* (0.001%),

Rhizopus spp.(0.001%), and *Trichoderma* spp. with 0.01% representation of the total genus identified. Salinity variations had a significant effect upon abundance of fungi, especially during the months of May thru August, when the temperatures were higher and can increase the salinity levels in sand due to seawater evaporation. These data suggest that salinity concentrations may be regulating the abundance of fungi at stations with lower salinity concentrations (35) during the coldest months of the year.

Total fungi abundance varied between 0.0 - 67.84 colonies forming units (cfu) during the dry or summer season and between 10.02 – 215.10 cfu during the rest of the year. Fungi genera were highest in amount at El Seco (215.10 cfu) in comparison with Guanajibo (165.47 cfu) and El Maní (198.07 cfu). *Aspergillus* spp. represented more than the 85% of total fungi abundance at the three stations.

Resumen

Este estudio forma parte de un esfuerzo con el fin de establecer un récord sobre la identificación, caracterización taxonómica y la distribución espacial de hongos arenícolas en la Bahía de Mayagüez, parte oeste de Puerto Rico. El objetivo principal de este estudio fue proveer un entendimiento mejor de los hongos presentes entre los granos de arena bajo ciertas condiciones ecológicas de tres diferentes lugares de muestreo: Guanajibo, El Seco y El Maní, durante los meses de enero a diciembre de 2003. Los muestreos fueron bi-mensuales y consistían en medidas de temperatura (Cº), salinidad (ssu), pH y la obtención de muestras de arena para determinar la presencia de hongos. Estas muestras fueron obtenidas utilizando métodos convencionales con botellas esterilizadas Pyrex y diferentes medios de cultivo para su identificación.

La abundancia total de hongos fluctuó entre 0.0- 67.84 unidades formadoras de colonias (ufc) durante la época de verano o seca y entre 10.02-215.10 ufc durante el resto del año. Los géneros de hongos fueron mayores en El Seco (215.10 ufc), en comparación con Guanajibo (165.47 ufc) y el Maní (198.07 ufc). *Aspergillus* spp. fue el género más representativo, formando el 85% total de la abundancia de hongos entre las tres estaciones.

Los hongos pueden crecer y desarrollarse exitosamente en

condiciones marinas y tolerar variaciones naturales de salinidad. Se encontraron diferencias significativas en la composición de especies en las estaciones de muestreo. La cantidad mayor de hongos reportada fue en la estación de El Seco, identificando a *Aspergillus* spp. con un 18.22% de representación*, A. flavus* representando un 15.67%, *A. niger* (5.77%), *A. terreus* (13.39%), *Curvularia* spp. (10%), *Cladosporium* spp. (10.83%), *C. cladosporioides* (12.33%), *C. herbarum* (1.08%)*, C. oxysporum* (0.14%), *Dreschlera* spp. (0.05%), *D. biseptata* (0.03%)*, Fusarium* spp. (0.02%)*, Geotrichum* spp. *(*7.40%)*, Mucor* spp. (3.89%), *M. ramossissimus* (0.01%)*, Penicillium* spp. (1.15%), *P. rubrum* (0.001%), *Rhizopus* spp.(0.001%), y *Trichoderma* spp con 0.01% del total de hongos identificados, Las variaciones de salinidad tuvieron un efecto significativo en la abundancia de hongos, especialmente durante los meses de mayo a agosto, cuando las temperaturas también son más altas. Estos datos sugieren que las concentraciones de salinidad pueden estar regulando la abundancia de hongos en las estaciones donde las concentraciones de salinidad disminuyen durante los meses más fríos del año. Estos patrones fueron consistentes en las tres estaciones estudiadas. También, el efecto de las diluciones de salinidad fueron de gran impacto para la abundancia de hongos en los diferentes sitios en cada una de las tres estaciones.

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Preface

This study provides a baseline characterization of temporal and spatial fungi distribution, abundance and taxonomic structure in Mayagüez Bay in relation with physical-chemical parameters and biological factors. Such relationships may be considered as criteria for infrastructure plans and water quality restoration initiative in the bay, which is heavily polluted.

This thesis is presented in a manuscript format and consists of an abstract, introduction, methods, results and discussion. This study identifies the main genera of fungi populations in Mayagüez Bay shoreline for a period of one year (January 2003-December 2003). The analysis is on the emphasis of fungi abundance relation versus biological and physico-chemical factors.

Dedication

To my parents Rosa and Honorio: for their faith, encouragement and their everyday prayers.

To my husband Wilfredo, thank you for believing in me and in my dreams. Also, thank you for sharing your time with me and for your support.

To my family and friends, with love.

Acknowledgements

First of all, thank God for giving me strength and the opportunity of fulfill my dream. I also want to express my gratitude to my parents and relatives for their emotional support at difficult and stressful times. I want to thank Dr. Maria Vargas, my comittee chairman, for her knowledge, friendship and invaluable support during the hard times and weakness moments. My gratitude also to the other members of my committee, doctors Mónica Alfaro, Baqar R. Zaidi, and Jorge R. (Reni) García for their reviews of the proposal, this manuscript and their suggestions. Your comments for the amendments and your knowledge inspired me to "run the extra mile". Special thank to doctors Omell Pagán and David González from Industrial Engineering Department for their guidance and help in the statistical analyses.

I am grateful to Dr. Fernando Gilbes, who gave me more information of the Mayagüez Bay and brought me the opportunity to have an offshore sampling to compare our findings. Thank you also to my friends from Marine Sciences Department: Ángel M. Nieves, for your friendship, your suggestions for this work and your invaluable support at stressful and disoriented moments; and Nydia J. Rodríguez for your sincere friendship.

Finally, but not less important, to my husband "Junny". You have been my strongest friend, you supported my bad temper, mood changes

and always encouraged me to never give up. My love, without your presence and cooperation this would be so hard to complete. I will always be thankful.

For you all that were part of my student life, my friends at Biology and Marine Sciences Department and all that helped me: THANKS!!!

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Introduction

Fungi are known to be present in the marine environment, where they play an important role in the degradation of organic compounds (Eriksson, 1997). The marine mycology field began about sixty years ago with Barghoorn and Linder's (1944) investigations. Some authors, like Sutherland (as cited by Kohlmeyer and Kohlmeyer, 1979), were dedicated to study higher fungi in marine habitats. Studies demonstrated that some geofungi as well as other aquatic fungi grow successfully in marine environments as halotolerant. Geofungi and marine fungi can produce toxins that affect humans, and scientists believe that marine fungi may have the same effect. Their potential toxicity has not been considered for research to this date (Sallenave-Namont, 2001).

Johnson and Sparrow (1961) established that there are many definitions that have been offered for marine fungi, based on its ability to grow on certain conditions and in seawater. It has been demonstrated that marine fungi cannot be defined by its physiology alone since other types of fungi may share the same characteristics and habitat; therefore environment has to be considered for definition. The conditions that fungi need for growth vary with the species. Many species are dominant on spores or mycelia until suitable conditions of salinity, pH, temperature, and nutrients are presented. Some fungi need enough available dissolved oxygen in order to survive (Kohlmeyer and Kohlmeyer, 2000).

When seawater, mud or sand are cultured on rich organic media, the mitosporic fungi usually dominates, but other groups such as ascomycetes could be present (Johnson and Sparrow, 1961). Geofungi and marine fungi spores can be encountered in sand, growing in natural conditions. Fungal spores and mycelia might grow among sand grains, in the sand surface and also in the interphase, where the sand and water mix together (Kohlmeyer and Kohlmeyer, 1979). The classification of imperfect fungi is mostly based in the principles presented by Saccardo in 1884 in the “Sylloge Fungorum” (Baron, 1968; Ainsworth et al., 1968).

A large number of physical, chemical, and biological factors affect marine organisms in coastal waters (García and López, 1989; García and Durbin, 1993). In Mayagüez Bay, Puerto Rico, the high sediment loading by rivers and nutrient enrichment associated with domestic and industrial effluents represent important perturbations of the marine communities (Gilbes et al., 1996). The effects of these disruptions, in comparison with commercial facilities and boating activities on marine water quality is an area of growing concern. Many accidents, such as oil spills, trash disposal, and the removal of sand for construction purposes can physically change the appearance of the Bay (Gilbes et al., 1996). Other studies relevant to Mayagüez Bay are the ones by Gilbes et al. (1996) and Parrilla (1996). Both stated that organisms are affected by the amount and composition of nutrient supply available from rainfall runoff and river discharges. Rosado (2001) found that bio-optics in Mayagüez Bay

appears to be mostly regulated by river inputs and suggested the possibility of light limitation in the bay.

Several studies have analyzed the geomorphological and circulation patterns of Mayagüez and Añasco Bays (Morelock et al., 1983; Alfonso, 1995; Capella and Grove, 2002). Since there is no physical offshore separation between the two bays and both form part of a system with common characteristics, they are usually referred to in these studies as the Mayagüez-Añasco System or MAB (Alfaro, 2002).

Mayagüez Bay is one of the most heavily polluted coastal ecosystems in Puerto Rico (Alfaro, 2002). The increased agricultural development during the 1950's resulted in massive sediment loading via tributary rivers to the marine environment (Gilbes et al., 1996). For more than 25 years, primary treated effluents from a regional sewage treatment plant (PRASA) and tuna factories (MWTC) have been discharged directly into the inner bay via shallow submarine outfalls. Sand extractions and coffee processing plant wastes are among other pressures imposed upon this shelf area (Webb et al., 1998).

A water quality restoration initiative was developed for Mayagüez Bay after strong pressures by local environmentalist groups and federal regulatory agencies (Alfaro, 2002). Key aspects of the restoration program included the upgrading of effluent treatment from primary to secondary level at both the regional sewage and the tuna wastewater treatment plants (Alfaro, 2002).

The Clean Water Act (CWA, Federal Water Pollution Control Act, 33 USC §1251 *et seq.*), the Coastal Zone Management Act Reauthorization Amendments (CZARA, 16 USC §1456, *et seq.*; section 6217), the Clean Vessel Act (CVA, P.L. 103-587) and the Treaty for the Prevention of Pollution at Sea (MARPOL) addressed various pollution sources, from rivers tributaries and industrial wastes. The Federal Clean Water Act, on section 305(b), requires each state and its territories a report every two years on the existing conditions on their water resources. This information could be used to compare and correlate the bioavailability of species and the environmental disturbances that may occur in the surrounding areas. Also, the findings and results obtained from this study may serve to evaluate potential impacts that may occur in the bay associated with antropogenic activities.

The specific objectives of this study were the following:

- To provide a description of the taxonomic composition of arenicolous filamentous fungi from Mayagüez Bay shoreline, western Puerto Rico.
- To compare the diversity of fungal genera found between the three sampling stations.
- To analyze the spatial taxonomic composition and abundance of fungi in Mayagüez Bay shoreline.
- To examine the potential relationships between fungi abundance with variations of physico-chemical parameters, such as salinity, pH, and temperature.

Previous work

A large number of physical, chemical, and biological factors affect marine organisms in coastal waters (Alfaro, 2002). Most marine fungi are microscopic. In ascomycetes, for example, the large ascomata occurs in *Amylocarpus encephaloides*, which does not exceed three millimeters in diameter. The marine environment does not allow fungi to develop large fruiting bodies due to waves abrasion, high salinity, and sand grains. The distributions of species vary accordingly with the climatic locations, some of them being restricted to tropical or neotropical waters (Johnson et al., 1961; Kohlmeyer, et al., 1979; Christophersen et al., 1998;). This is why Puerto Rico can be considered a rich habitat for arenicolous fungi. An arenicolous fungi is an organism that tolerates the variations of salinity concentrations, but only grows among the sand grains and they are usually environmental polluters. If the fungi are growing at marine conditions or at algae or organic material at sea, it usually is classified as halophytic, halotolerant, eurihaline, or just marine fungi (Kohlmeyer and Kohlmeyer, 1979).

The inability to quantify filamentous fungi in the environment is a major problem in marine mycology. Sea foam contains highly distinctive spores of fungi, such as *Corollospora* spp., *Lulworthia* spp., *Carbosphaerella* spp., and others (Kohlmeyer and Kohlmeyer, 1979). The irregularity of meteorological conditions also affects their “presence” on

sandy beaches (Johnson and Sparrow, 1961). The variable consistency and the salt and sand contents of foam hinder the comparison of microbial populations between samples of similar weight or volume. Dried sea foam in sand is a rich source of fungi, but it is difficult to precise their geographical origin, due to oceanic and beach extensions and wave dynamics (Kirk, 1983). Many inexpensive methods could be used to characterize these fungi and establish an ecological survey of a beach. This last statement is still a controversy, because it is known that filamentous fungi can grow in hypersaline environments, behaving as halotolerant. Kis-Papo et al. (2003) has been working on isolating filamentous fungi on the Dead Sea water. They collected a variety of filamentous fungi that can survive in 340 g/L total dissolved salts, and also tested the viability of spore formation in which such fungi can survive for prolonged periods at those saline ranges. These authors stated “the difference in survival rate between spores and mycelia of isolates of the same species, points to the existence of adapted halotolerant and/or halophilic fungi in the Dead Sea” (Kis-Papo et al., 2003).

Other investigators, such as Mejanelle (2001) had also surveyed filamentous fungi in marine environments or hypersaline waters of Secovlje salterns in Slovenia, and subsequently in the salterns of La Trinitat (Ebro Delta, Catalonia, Spain) and Bonmatí (Santa Pola, Valencian Community, Spain). Some of these fungi had been reported from hypersaline waters by as halotolerant and halophilic melanized fungi.

Some of these recent reports mentioned geophilic fungi in hypersaline environments such as *Hortaea werneckii, Alternaria alternata, Cladosporium sphaerospermum, Cladosporium* spp., and *Aureobasidium pullulans.*

The impact of studies of terrestrial fungi on ecological theory has been minimal (Seifert, 1981). Studies on the ecology of fungi have typically been relegated to mycological journals. Fungi can be studied as a part of an ecological survey and the diversity measurements could be the same that are used in nonfungal community ecology (Lloyd et al., 1968; Sanders, 1968; Poulson and Culver, 1969; Lussenhop, 1973; Abele, 1974, Seifert, 1981). However, only a few fungal taxa can be recognized *(e.g.* marine yeast, chytrids, zoospores, perennial mycelia, and basidiomycetes) as part of these ecological surveys.

An important aspect of the biology of fungi is the evolutionary and ecological importance of asexuality. Williams et al. (1981) proposed that sexuality should occur when the environment is subject to irregular perturbations whereas asexuality should be common in stable environments. There occurs a reassortment of genetic material that occurs in sexual reproduction for future unpredictable environments. This type of environment should be compared with the polluted aspects of Mayagüez Bay and it can be related with the fungi abundance that may be found.

Site description

Physical Descriptions

The Mayagüez-Añasco Bay (MAB) complex extends westward from the coastal cities of Mayagüez and Añasco. It is located on the west side of Puerto Rico, at the end of the mountain range called Cordillera Central. Mayagüez Bay covers an area of 47 km^2 of the total 100 km of the MAB complex (Alfaro, 2002).

The bay demarcations are between Punta Algarrobo and Punta Guanajibo with Añasco Bay's boundaries extending north from Punta Algarrobo to Punta Cadena, meeting at Punta Algarrobo. The insular shelf at Mayagüez Bay is 5 to 6 km wide and water depth at the shelf edge is 15 to 25 m.

The MAB basin receives a greater amount of rainfall than any other watershed in the island, caused by the convergence between the afternoon seabreeze and the easterly winds resulting in the relatively high annual rainfall of 200-205 cm (Alfonso, 1995). Most of the rainfall occurs during the months of April to October. Its lower rainfall is in May and the larger peak of rainfall is during September. The MAB receives the effluents of the Añasco, Guanajibo and Yagüez rivers by drainage areas of 473,335 km^2 and 36 km^2 respectively (Capella and Grove, 2002; Alfaro, 2002). The tide at MAB is mixed, as it includes the both diurnal tidal

component (24-hour period) and the semidiurnal tidal component (12.4-hour period), changing throughout the month (Capella and Grove, 2002; Alfaro 2002).

Materials and methods

Three different and specific stations at the coast of Mayagüez Bay where sampled bimonthly from January 2003 through December 2003. Physical-chemical parameters were considered for the study, such as salinity, pH, and temperature. These stations were chosen based on the proximity of important rivers that are nearby: Guanajibo, Yagüez, and Añasco. The establishment of these three different coastline stations was extremely important to have representative samples of different sectors among the coast. The sampling stations were fixed and recorded by using a Geographical Positioning System (GPS) and a coordinate was established to be consistent in the use of same station always.

Table 1. Geographic positions of sampling stations in Mayagüez Bay coastline.

Station	Latitude	Longitude
1 (Guanajibo)	18 º 12' N	67º 10'W
2 (El Seco)	18º 12.6' N	67º 10' W
3 (El Maní)	18º 13.4' N	67º 10'W
4 (AAA)	18º 13.4' N	67º 11' W

This table shows the specific localizations for each station sampled. Note that each station is defined by a coordinate. These coordinates were obtained by a GPS.

Another station was sampled at the “Autoridad de Acueductos y Alcantarillados” (AAA) primary sewage treatment effluent discharge in Mayagüez Bay at 600 ft of depth. This sample was taken on June 2003, at Dr. Gilbes’ offshore sampling and it was only taken for comparison of the results recorded for each site and station. For statistical representation of each station, five replicates were selected randomly at each of the stations.

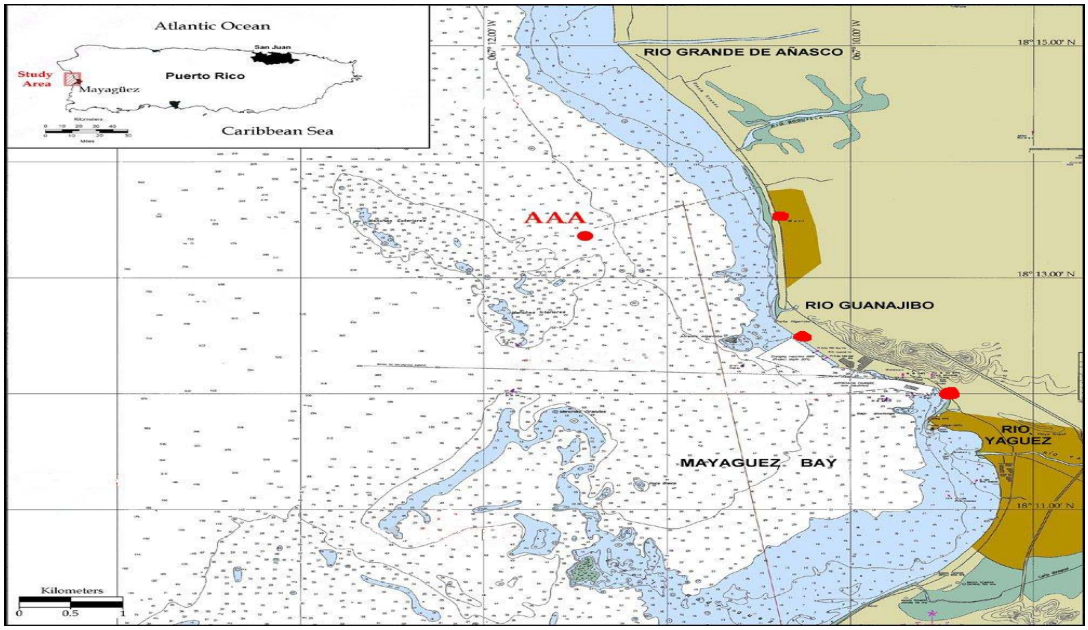


Figure 1. ***Mayagüez Bay map showing the three stations that were sampled. A fourth station was used to compare the results***.

1. Sand sampling

Since arenicolous filamentous fungal spores are found growing between sand grains, different collection sites within the Mayagüez Bay shoreline were sampled at stations fixed in Guanajibo, El Seco, and El Maní. Sand samples were collected using autoclaved 500 ml and 1000 ml Pyrex

crystal bottles. To grab the samples, a sterilized stainless steel spoon was used and the samples were taken at a depth of 1 ft. These sand samples were covered and placed in box to be analyzed at the laboratory on the same day of its collection. An Ekman sampler was used for the fourth sampling at 600 ft to grab sediment.

Figure 2. Ekman sampler. This instrument was used to take samples of sand at 600 ft.

Sand samples were processed by weighting 40 g of sand and then added into a 125 ml Erlenmeyer flask containing 10 ml of sterile seawater, in a microbiological cabinet or hood. These samples were placed in a shaker with magnetic stirrers to homogenize for 30 minutes.

2. Culture medium

Fractions of the homogenized samples were transferred to a Fisherbrand Petri dish containing GPYA medium. This medium was prepared by using 1000 ml of aged- filtered seawater, 1.0 g of dextrose, 0.1 g of yeast extract, 0.1 g of peptone, and 16.0 g of agar. It also contained antibiotic solution to avoid bacterial growth. A control from this

medium was prepared with the same amounts of dextrose, yeast extract, peptone, and agar, but changing the aged-filtered seawater for distilled water. The stock of antibiotic was prepared with 10 ml of distilled water, 0.1 g of Penicillin G, and 0.1 g Streptomycin. The Petri dishes with the samples were sealed with paraffin and kept in dark at environmental temperature for one week. After this period, the fungi colonies were transferred individually to a smaller Petri dish of 60 mm x 15 mm with GPYA medium for the isolation of the colonies. To ensure the veracity of the results, other medium were prepared for culturing fungi. These media were Marine Agar (MA), Malt Extract Agar (MEA), and Rose Bengal Agar (RBA), following the instructions on each of the media package. The different mediums were prepared in two groups: one with distilled water and the others with aged-filtered seawater with fixed salinity of 35-40 and antibiotic solution.

3. Identification of isolates

After one week, the colonies were transferred to a slide culture. A tease mount was observed on the conventional Olympus model microscope at 60 x. *Practical Mycology: Manual for Identification of Fungi* by F. Sigurd (1961), *The Genera of Hyphomycetes in Soil* by G.L. Barron (1968), *Medically Important Fungi- A Guide to Identification* by D.H. Larone (1995), and *The Atlas of Clinical Fungi* by G.S. de Hoog (2000) were used for taxonomic identification.

4. Water collection and sediment quality analysis:

The water was collected and sediment samples were analyzed according to the following methods:

- **Seawater collection:** Aged seawater was used to prepare medium. A sterilized aluminum covered Pyrex crystal bottle of 500 ml and 100 ml were used to collect the water. This water was filtered with a Nalgene 0.45 µ micropore filter to prepare each medium.
- **Salinity:** the salinity measures were obtained by using an Olympus model refractometer.
- **pH:** This range was estimated by colorimetric assays.
- **Temperature:** seawater and air temperature measurements were obtained with a mercury thermometer.

For the AAA station, these measurements were taken with CTD/rosette instrument (figure 3). Also, the water samples were taken with a Bailer sampler.

Figure 3. CTD/rosette instrument to measure temperature, conductivity, fluorescence, transmissivity, and water samples for pH, salinity, and dissolved oxygen.

Figure 4. Bailer sampler used for water collection at 10 ft.

5. Statistical analysis

An analysis of variance (ANOVA) was performed to test the alternative hypothesis that there are significant differences in mean quantities of fungal genus between different sampling stations in Mayagüez Bay shoreline, western Puerto Rico. The statistical model used was a nested ANOVA. This model was used to test each physico-chemical parameter and the mean abundance of fungi especially at the five sites selected randomly at each station. The type I error was set at $\alpha = 0.05$, and only this range were considered significant. The statistical program used was Minitab.

6. Pictures

Pictures were taken by using an Olympus digital camera model Camedia.

Results

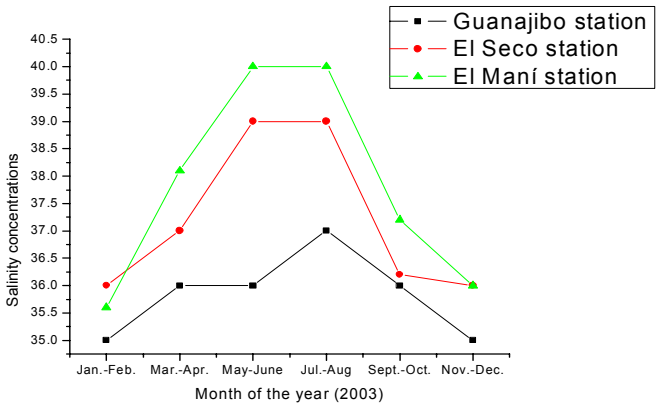
Salinity

Maximum bimonthly concentration of salinity was consistently higher during summer at the three stations, with peaks of 37 at Guanajibo station, 39 at El Seco, and 40 at El Maní. Highest salinity concentrations were found at El Maní station, near commercial facilities and ports. Salinity diminutions were measured during the coldest months of the year (January - February 2003; October - December 2003) with lowest salinity concentrations of 35 at Guanajibo station, 36 at El Seco station, and 35.6 at El Maní station (Table 2, Figure 5).

Table 2. Salinity concentrations at the sampling stations during the year 2003.

Month of the year (2003)	Salinity concentrations-Guanajibo station	Salinity concentrations-El Seco station	Salinity concentrations-El Maní station
Jan.-Feb.	35	36	35.6
Mar.-Apr.	36	37	38.1
May-June	36	39	40
Jul.-Aug	37	39	40
Sept.-Oct.	36	36.2	37.2
Nov.-Dec.	35	36	36

Figure 5. Bimonthly readings of salinity concentrations at sampling stations in Mayagüez Bay during 2003.



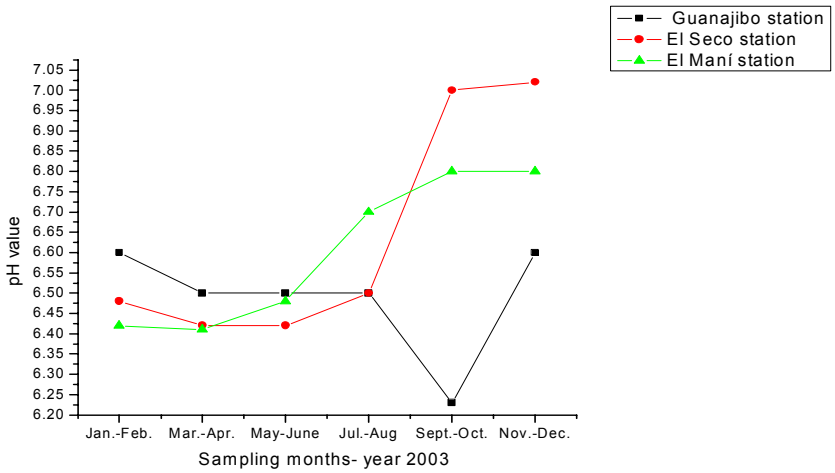
pH

Maximum bimonthly values for pH were consistently higher for El Seco station, with peaks of 7.02 during the months of November - December, 2003. pH values diminutions were measured at El Maní and Guanajibo stations. This last station remained with the lowest average pH value of 6.23 during the months of September-October, 2003; in comparison with three stations. The results for pH varied from 6.23 – 7.02 for each of the sampling stations (Table 3, Figure 6).

Table 3. Average pH values for each sampling station during the year 2003.

Month of the year (2003)	Average pH values- Guanajibo station	Average pH values- El Seco station	Average pH values- El Maní station
Jan.-Feb.	6.6	6.48	6.42
Mar.-Apr.	6.5	6.42	6.41
May-June	6.5	6.42	6.48
Jul.-Aug	6.5	6.5	6.7
Sept.-Oct.	6.23	7.0	6.8
Nov.-Dec.	6.6	7.02	6.8

Figure 6. Bimonthly readings of average pH values at sampling stations in Mayagüez Bay during year 2003.



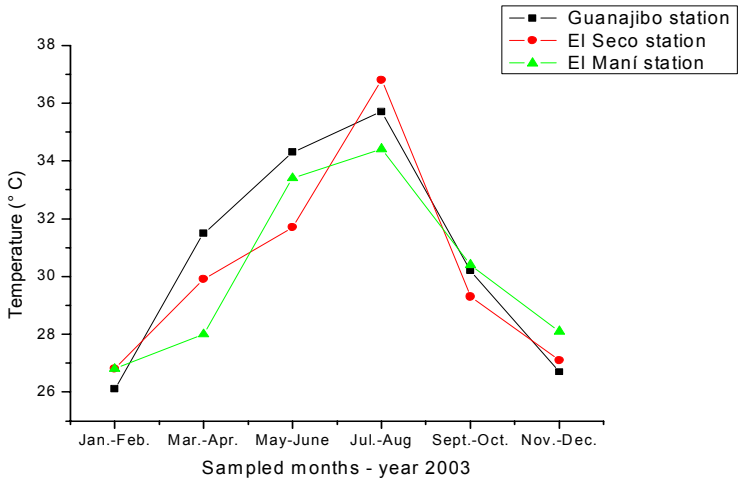
Temperature

Temperature readings were obtained through the sampling months of year 2003. The readings varied from 26.1 ºC in January – February to 36.8 ºC during July – August 2003, at each station. Temperature readings increased substantially during May – August 2003, with peaks of 35.7 °C at Guanajibo station, 36.8 °C at El Seco station, and 34.4 °C at El Maní station. The lowest temperature value was read for Guanajibo station, with 26.1 °C during January – February 2003 (Table 4, Figure 7).

Table 4. Temperature readings (Cº) at the sampling stations during the year 2003.

Month of the year (2003)	Temperature readings (˚C)- Guanajibo station	Temperature readings (˚C)- El Seco station	Temperature readings (˚C)- El Maní station
Jan.-Feb.	26.1	26.8	26.8
Mar.-Apr.	31.5	29.9	28
May-June	34.3	31.7	33.4
Jul.-Aug	35.7	36.8	34.4
Sept.-Oct.	30.2	29.3	30.4
Nov.-Dec.	26.7	27.1	28.1

Figure 7. Bimonthly readings of temperature (°C) at sampling stations in Mayagüez Bay during year 2003.



Fungi

There were different patterns of mean abundance of fungal genus between the three stations: Guanajibo, El Seco, and El Maní. The fungal genus identified or present in this study among the sampling stations were: *Aspergillus* spp. with 16.18% of representation*, A. flavus* representing 12.33%, *A. niger* (3.67%), *A. terreus* (11.35%), *Alternaria* spp. (12.54%), *Curvularia* spp. (9%), *Cladosporium* spp. (9.83%), *C. cladosporioides* (11.33%), *C. herbarum* (1.08%)*, C. oxysporum* (0.14%), *Dreschlera* spp. (0.05%), *D. biseptata* (0.03%)*, Fusarium* spp. (0.02%)*, Geotrichum* spp. *(*7.40%)*, Mucor* spp. (3.89%), *M. ramossissimus* (0.01%)*, Penicillium* spp. (1.15%), *P. rubrum* (0.001%), *Rhizopus* spp.(0.001%), and *Trichoderma* spp with 0.01% representation of the total genus identified. The phylum identified for the sampling stations were mostly ascomycetes, which represents 85% of the total, in which some of the species are mitosporic fungi. Other phylum identified was ascomycetes, with a 15 % of total representation (Table 5).

Table 5. Abundance of fungi (cfu) determined from the three sampling stations: Guanajibo, El Seco, and El Maní during year 2003

Fungi genera	*Guanajibo station*	*El Seco station*	*El Maní station*
Aspergillus spp.	***386.95***	***408.62***	***371.46***
A. flavus	***283.51***	***311.33***	***294.32***
A. niger	***255.04***	***0.02***	***10.13***
A. terreus	***227.93***	***282.75***	***308.69***
Cladosporium spp.	***236.58***	***306.72***	***361.52***
C. cladosporioides	***221.15***	***114.62***	***313.6***
C. herbarum	***228.91***	***225.14***	***255.06***
Curvularia spp.	***266.38***	***220.21***	***329.17***
Dreschlera spp.	***51.19***	***26.47***	***0.15***
Fusarium spp.	***7.71***	***1.81***	***0.23***
Geotrichum spp.	***4.31***	***0.41***	***0.32***
Mucor spp.	***0.5***	***0.38***	***0***
M. ramossissimus	***0***	***0.67***	***0***
Penicillium spp.	***247.77***	***87.86***	***197.79***
P. rubrum	***1.66***	***279.14***	***0***
Rhizopus spp.	***0.5***	***0.20***	***0.34***
Trichoderma spp.	***28.19***	***53.18***	***1.47***

1. Aspergillus species

This is a common environmental pollutant and a fungal opportunist. It was described by Micheli ex Link in 1904 (Larone, 1995). Macroscoscopically, *Aspergillus spp.* have a velvety texture (Kern et al., 1997), and can present different colors due to dense production of conidia. Some species may be blue, green, yellowish, white, and black, such as *A. niger*. While examining it microscopically, the mycelium is septate and have unbranched, rough or smooth conodiophores with a foot cell as an appendix at their base supporting a large vesicle at the tip (Kern et al, 1997; Lozada et al, 1999; and Gene, 2001). This vesicle supports the phialides, which produces the phialoconidias.

A. flavus

Macroscopically, the colony is olive green to parrot green; exudate absent; reverse uncolored to pale buff; soluble pigment absent. Colonies on MEA 50-70 mm in diameter; conidia olive; no exudate or soluble pigment; reverse uncolored to pale buff. Its microscopic characteristics include hyaline stipes, walls finely rough to rough. Vesicles 30-35 um wide, hyaline, globose, subglobose to pyriform in shape. Aspergilli uniseriate or biseriate. Conidia 3.8-5 um in diameter, globose to subglobose, surface texture finely rough to rough at 60X.

A. niger

While observing the colony, it can be described as powdery, black colored, dark colored reverse, soluble pigment absent. Its microscopic characteristics include dark colored stipes, walls finely rough to rough. Vesicles are globose in shape. Conidia are rough at 60 x.

A. terreus

Colony diameter after seven days incubation on MEA at 25°C 30-45 mm; conidia cinnamon brown, brownish orange or caramel; exudate hyaline, brown or absent; reverse yellow, brownish orange or brown; soluble pigment absent or colored as reverse. Microscopically, Stipes 100-280 um long, smooth-walled, hyaline. Vesicles 11-1 5 um wide, pyriform to

subglobose in shape, hyaline in color. Aspergilli biseriate. Conidia 1.6-2.5 um long, globose to sub-globose, smooth-walled.

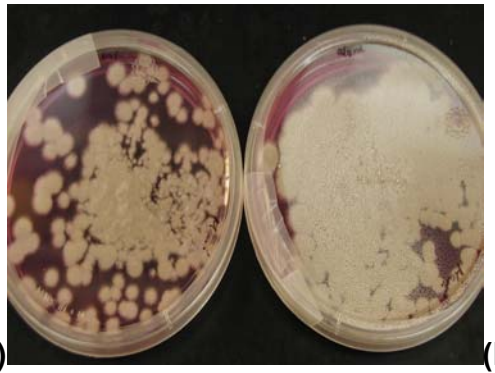
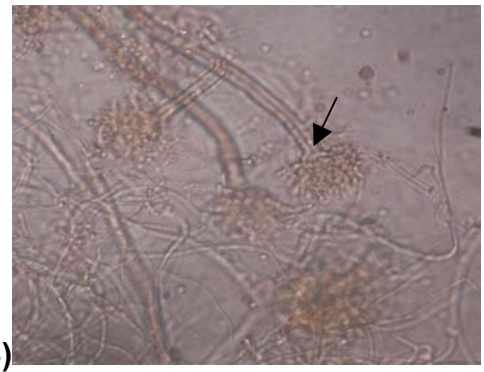
(A)  **(B)** 

Figure 8. (A) Aspergillus terreus growing on Rose Bengal Agar. Note the differences in coloration while being growth on a medium with aged-filtered seawater (dish 1) and distilled water (dish 2). (B) Aspergillus spp. observed at 60x on an Olympus model microscope. Note the vesicle supporting the phialides and phialoconidia.

2. Cladosporium species

The genus *Cladosporium* includes over 30 species. The most common ones include *Cladosporium elatum*, *Cladosporium herbarum*, *Cladosporium sphaerospermum*, and *Cladosporium cladosporioides*. It was first described by Link ex Gray in 1821 (De Hoog, 1995; Gene et al., 2000). This colony is powdery or velvety and dark gray-green with a black reverse (Sutton et al. 1998). It has septate hyphae with dark-colored short chains of blastoconidia (Lozada et al., 1999). Each conidia carry a distinctive scar at each point of attachment.

C. cladosporioides

Colony is velvety, brownish, without exudates. On the reverse, the colony is brown or black. Microscopically, the conidia are single-celled, brown, and dark-colored short chains of blastoconidia. Each conidia carry the distinctive scar of the specie.

C.herbarum

Colony is velvety, green, without exudates. On the reverse, the colony is olive-green. Microscopically, the conidia are large single-celled, brown, and dark-colored short chains of blastoconidia. Each conidia carry the distinctive scar of the specie.

C. cladosporioides

Colony is velvety, brownish, without exudates. On the reverse, the colony is brown or black. Microscopically, the conidia are single-celled, brown, and dark-colored short chains of blastoconidia. Each conidia carry the distinctive scar of the specie.

C. herbarum

Colony is velvety, green, without exudates. On the reverse, the colony is olive-green. Microscopically, the conidia are large single-celled, brown, and dark-colored short chains of blastoconidia. Each conidia carry the distinctive scar of the specie.

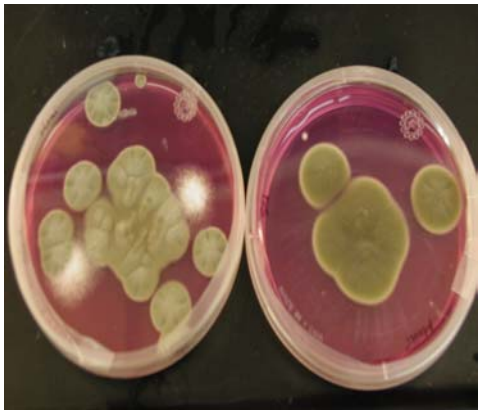
(A)  (B) 

Figure 9. (A) Cladosporium spp. Note the dark colored septate hyphae and the conidia. (B) Cladosporium herbarum growing on Rose Bengal Agar (RBA). Note the differences in coloration while being growth on a medium with aged-filtered seawater (dish 1) and distilled water (dish 2).

3. Curvularia species

This genus was described by Boedijn in 1933 (Larone, 1995). The colony exhibits a variation of colors among the species: light pink, orange, or green, with a brown reverse (Sutton et al. 1981). The septate mycelium is dark and has large poroconidia that are septate into four to five cells. This conidia bent over the conidiophore, which is commonly referred as a phragmoconidia.

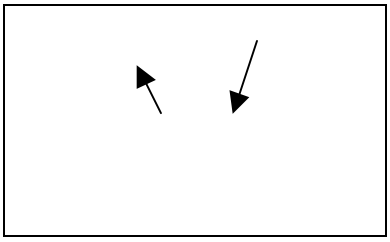


Figure 10. Curvularia spp. observed in an Olympus model microscope at 60x magnification. Each conidia is bended and centrally distended owing a over-enlarged central cell.

4. Fusarium species

The *Fusarium* species exhibits a rapid-growing colony that is white at first, changing to lavender, yellow or orange, depending on the species. *Fusarium spp*. had been described first by Link ex Gray in 1821. The mycelium is septate and the conidiophores are single or branching, with micro and macrophialoconidia. Its macrophialoconidia is septated.

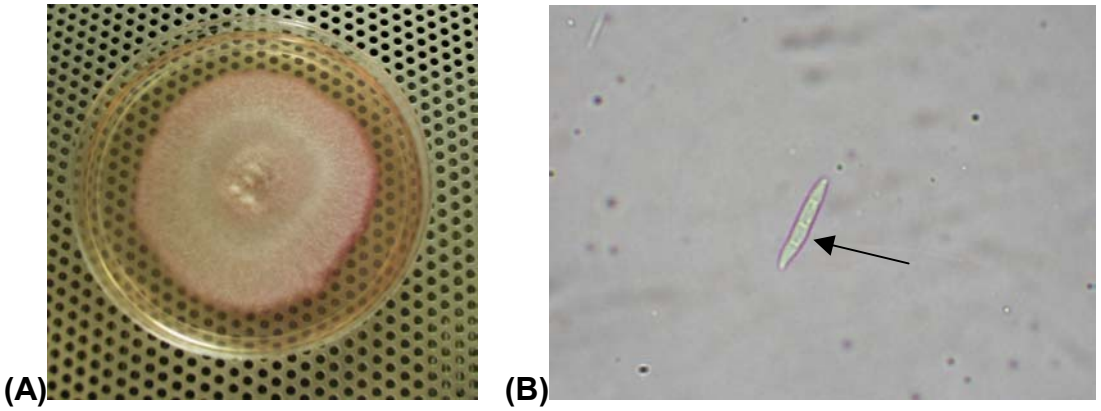


Figure 11. (A) Fusarium spp. growing on Rose Bengal Agar. Note the change of color in the media. (B) Macroconidia of Fusarium spp.

5. Geotrichum species

Geotrichum spp. was first described by Libk ex Persoon in 1822 (Gene et al., 2000). This genus includes several species, being *G. candidum* the most common. Its reproduction consists in the fragmentation of the mycelia, which is also called the arthrospore. *Geotrichum spp.* colony appearance is white to cream and yeast-like. The conidia are absent.

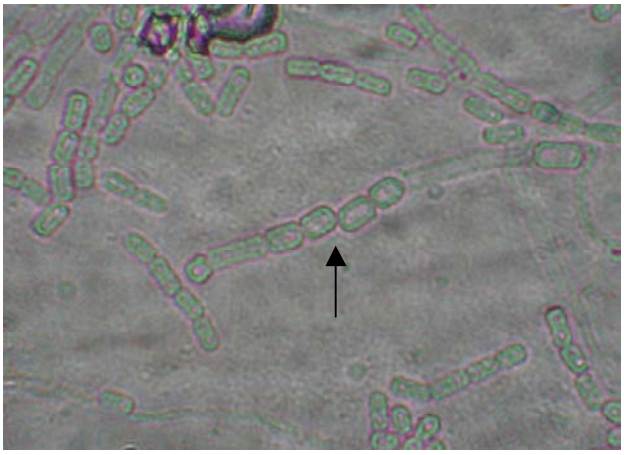


Figure 12. Geotrichum spp. Note the fragmentation of the mycelia or the arthrospore at 60 x in an Olympus model microscope.

6. Mucor species

This genus was first described by Micheli ex Saint-Amans in 1821 (Larone, 2001). *Mucor spp*. is a filamentous fungi that is morphologically alike the genus *Rhizopus spp*. It can be velvety to cottony in appearance, with grey color and hyaline reverse. Microscopically, the sponrangiophore is hyaline to light brown colored, globose, without apophysis or rhizoids.

7. *Penicillium* species

This genus was described by Link in 1809 (Gene et al., 2000). This genus is principally filamentous, with exception of *P. marnefei*, which is a thermal dimorphic. The genus *Penicillium* spp. has several species. Macroscopically, the colony is blue to green, with exudates, and a green colored reverse. The mycelium is septate and bear a flask-shaped phialides which support chains of round phialoconidias that are green colored.

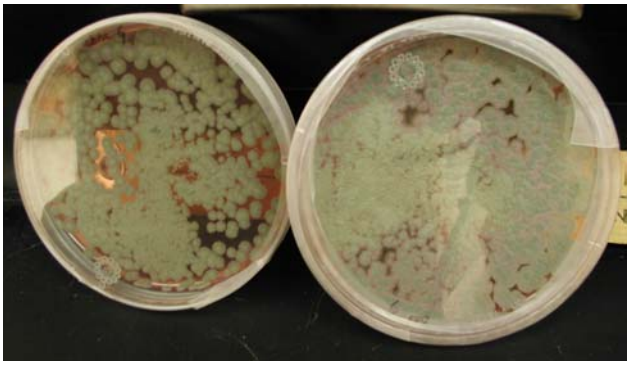


Figure 13. (A) Penicillium spp. growing on Rose Bengal Agar. Note the differences in coloration while being growth on a medium with aged-filtered seawater (dish 1) and distilled water (dish 2).

8. Rhizopus species

Rhizopus spp. was first described by Ehrenberg ex Corda in 1838. The genus *Rhizopus* contains several species. Morphologically, *Rhizopus* species have some features, such as the length of rhizoids and sporangiophores, the diameter of sporangia, the shape of columellae, and the size, shape and surface texture of sporangiospores. (See appendix 2 for drawing illustration).

9. Trichoderma species

Trichoderma spp. was described by Persoon ex Gray in 1801 (Sigurd, 1961; Lozada et al., 1999. Macroscopically, the colony is scattered blue-green and turns yellow-green patches when mature. These patches may sometimes form concentric rings. They are more readily visible on potato dextrose agar compared to malt extract agar. Reverse is pale, tan, or yellowish.

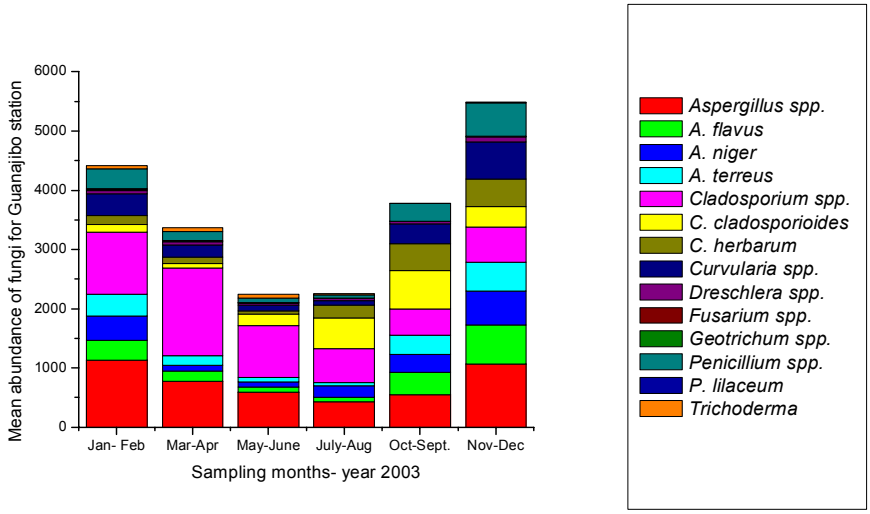
Abundance of fungi identified at each station

Guanajibo station showed decreased amount in fungi species, in comparison with El Seco station. This sampling station repeated the patterns of fungi abundance similar to El Maní station, in which the mean abundance of fungi genera was decreased at each sampling. These patterns of decreasing abundance of these microorganisms were observed particularly during the months of May- August 2003, when the temperatures were arisen and the precipitation or rainfall season were at its peak (Table 6, Figure 14).

Table 6. Abundance of fungi (cfu) determined from station 1: Guanajibo during year 2003

Fungi genera	Jan.-Feb.	Mar.-Apr.	May-June	Jul.-Aug.	Sept.-Oct.	Nov.-Dec.
Aspergillus spp.	513.78	174.18	390.68	229.07	548.63	465.38
A. flavus	326.74	179.00	87.11	72.37	373.18	662.65
A. niger	416.24	94.74	83.72	60.53	304.72	570.27
A. terreus	359.41	60.54	83.72	54.07	327.62	482.23
Cladosporium spp.	105.40	147.94	66.37	65.37	441.17	593.24
C. cladosporioides	128.59	75.48	69.85	58.07	648.72	346.17
C. herbarum	156.96	112.45	55.94	124.69	456.46	466.93
Curvularia spp.	364.83	100.24	99.58	72.64	330.94	630.07
Dreschlera spp.	56.40	51.81	30.58	41.81	43.68	82.88
Fusarium spp.	20.07	12.52	6.19	2.91	0.31	4.23
Geotrichum spp.	8.92	7.7	4.75	0.25	0.61	3.62
Mucor spp.	0	1.0	0	0	2.0	0
M. ramossissimus	0	0	0	0	0	0
Penicillium spp.	338.56	155.78	64.61	52.95	304.72	570.02
P. rubrum	0	0	0	0	0	9.98
Rhizopus spp.	1.0	0	0	0	2.0	0
Trichoderma spp.	54.16	49.97	38.56	22.86	3.59	0.01

Figure 14. Taxonomic composition of the most abundant fungal genera at Guanajibo sampling station in Mayagüez Bay (January-December 2003).

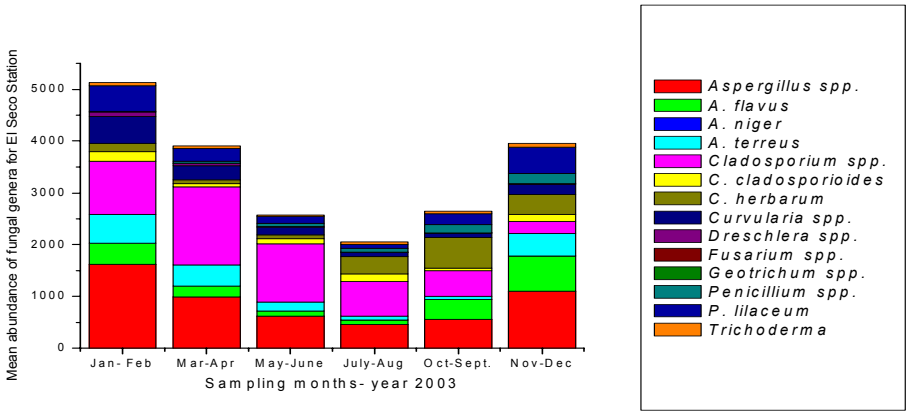


El Seco station showed an increased abundance in fungi species. The genera and species that were identified in this station were: *Aspergillus* spp., *A. flavus, A. terreus, Cladosporium* spp., *C. cladosporioides, C. herbarum, Curvularia* spp, *C. herbarum, Dreschlera* spp., *Fusarium* spp., *and Penicillium* spp. This station showed a decreased amount of fungi genera during the months of May-August, 2003. The mean abundance of fungal genera was higher and the fungal genera identified at this station were not the same as the other present in Guanajibo and El Maní station (Table 7, Figure 15).

Table 7. ***Abundance of fungi (cfu) determined from station 2: El Seco during year 2003***

Fungi genera	Jan.-Feb.	Mar.-Apr.	May-June	Jul.-Aug.	Sept.-Oct.	Nov.-Dec.
Aspergillus spp.	961.83	250.34	116.58	58.16	557.73	507.05
A. flavus	412.71	205.82	96.79	84.18	389.97	678.53
A. niger	0	0	0	0.01	0.01	0.1
A. terreus	554.34	404.37	175.8	74.61	52.95	434.40
Cladosporium spp.	102.24	510.53	134.08	370.86	491.57	231.05
C. cladosporioides	190.63	66.32	92.78	145.35	59.37	133.27
C. herbarum	152.97	66.32	69.88	96.68	582.20	382.80
Curvularia spp.	527.22	271.26	148.19	87.10	89.83	197.63
Dreschlera spp.	88.08	51.27	10.28	0.02	0.55	8.62
Fusarium spp.	1.67	0.03	0	0	1.69	7.45
Geotrichum spp.	0	0	1.0	0.95	0.50	0
Mucor spp.	0	1.0	0	0	0	1.3
M. ramossissimus	0	0	0	1.0	1.0	2.0
Penicillium spp.	0	33.22	63.12	74.12	156.38	200.3
P. rubrum	503.96	254.28	136.61	75.31	211.42	493.28
Rhizopus spp.	0	0.2	0.02	0	0	1.0
Trichoderma spp.	62.08	50.83	25.85	41.81	52.18	86.34

Figure 15. Taxonomic composition of the most abundant fungal genera at El Seco sampling station in Mayagüez Bay (January- December 2003).



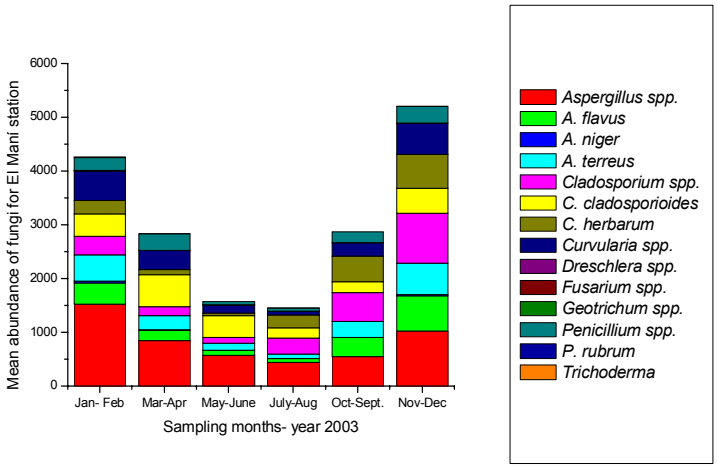
El Maní station also showed a decreased abundance in fungi species, being the same pattern as Guanajibo station. The genera and species that were identified in this station were: *Aspergillus* spp., *A. flavus, A.niger, Cladosporium spp., C. cladosporioides, C. herbarum, Curvularia* spp., *Dreschlera* spp., *Fusarium* spp., *Geotrichum* spp., *Penicillium* spp,

and *Trichoderma* spp. This station showed a decreased amount of fungal genera during the months of May-August, 2003 (Table 8, Figure 16).

Table 8. ***Abundance of fungi (cfu) determined from station 3: El Maní during year 2003***

Fungi genera	Jan.-Feb.	Mar.-Apr.	May-June	Jul.-Aug.	Sept.-Oct.	Nov.-Dec.
Aspergillus spp.	523.71	444.39	375.05	239.31	544.40	101.90
A. flavus	395.49	188.57	88.30	72.93	360.02	660.63
A. niger	26.17	17.77	0.08	0.05	0.27	16.45
A. terreus	494.29	252.64	133.04	86.14	295.59	590.42
Cladosporium spp.	342.96	175.52	99.93	92.06	540.28	918.36
C. cladosporioides	419.25	393.32	204.31	188.57	201.14	475.01
C. herbarum	251.90	87.57	54.96	43.65	466.81	625.47
Curvularia spp.	550.00	366.88	154.90	66.22	257.16	579.88
Dreschlera spp.	0.70	0.43	0.01	0.15	0.31	0
Fusarium spp.	0.10	0.44	0.42	0.20	0.01	0.22
Geotrichum spp.	0.10	0.74	0.13	0	0	0.97
Mucor spp.	0	0	0	0	0	0
M. ramossissimus	0	0	0	0	0	0
Penicillium spp.	246.32	301.59	62.13	66.32	193.90	316.47
P. rubrum	0	0	0	0	0	0
Rhizopus spp.	1	1	0	0	0	0.01
Trichoderma spp.	5.25	2.40	0	0.28	0	0.91

Figure 16. Taxonomic composition of the most abundant fungal genera at El Maní sampling station in Mayagüez Bay (January- December 2003).



On the 600 m sampling at AAA, only few organisms were identified. These genera were *Aspergillus* spp., *Cladosporium* spp, *Rhizopus* spp., and *Penicillium* spp. The colony forming units were less than 1.0 for each genus. This sampling station could be used as a control to compare the findings at Guanajibo, El Seco, and El Maní stations. Because this was only a one-time sampling used like a control, no further studies were performed this station.

Statistical analyses

Nested ANOVA showed significances between the interactions of salinity with month and station per month. Some relations were found for station and site at each station for salinity concentrations (Table 9). Also, these interactions were also demonstrated by nested ANOVA for temperature values for station and site at each station and the interactions with month and station per month (Table 10).

Table 9. Analysis of Variance for Salinity (ssu), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Station	2	23.878	23.849	11.925	4.60	0.033
Site(Station)	12	31.033	31.086	2.590	2.01	0.027
Month	5	1505.583	1502.463	300.493	233.41	0.000
Station*Month	10	113.638	113.638	11.364	8.83	0.000
Error	150	193.112	193.112	1.287		
Total	179	1867.244				
S = 1.13464	**R-Sq= 89.66%**	**R-Sq(adj)= 87.66%**				

Table 10. Analysis of Variance for Temperature (°C), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Station	2	24.560	24.640	12.320	3.48	0.064
Site(Station)	12	42.410	42.473	3.539	2.61	0.004
Month	5	1430.797	1430.711	286.142	210.79	0.000
Station*Month	10	90.334	90.334	9.033	6.65	0.000
Error	150	203.621	203.621	1.357		
Total	179	1791.723				
S = 1.16511	**R-Sq = 88.64%**	**R-Sq(adj) = 86.44%**				

When the physico-chemical parameters were tested by ANOVA, only temperature and salinity were found significant for fungi development. There was found significant differences for fungi abundance when compared with salinity readings; particularly with *Aspergillus* spp*., A. flavus, A. terreus, Curvularia* spp., and *Penicillium* spp. (Table 11).

Table 11. One-Way Analysis of Variance (ANOVA) testing for differences in abundance of fungal genera at different salinity.

Fungi genera	Source	DF	SS	MS	F	P
***Aspergillus* spp.**	Salinity (ppt)	13	159803	12293	22.50	0.000
	Error	166	90699	546		
A. flavus	Salinity (ppt)	13	33061	2543	9.05	0.000
	Error	166	46625	281		
A. terreus	Salinity (ppt)	13	35812	2755	13.14	0.000
	Error	166	4798	210		
***Curvularia* spp.**	Salinity (ppt)	13	36215	2786	12.63	0.000
	Error	166	36603	221		
***Penicillium* spp.**	Salinity (ppt)	13	25676	1975	13.23	0.000
	Error	166	24783	149		

Further ANOVA statistical analysis with <0.05 revealed significant effects of temperature on growth and development for genus: *Aspergillus* spp., *A. flavus*, *A. terreus*, *Curvularia* spp., and *Penicillium* spp. (Table 12).

Table 12. One-Way Analysis of Variance (ANOVA) testing for differences in abundance of fungal genera at different temperatures

Fungi genera	Source	DF	SS	MS	F	P
***Aspergillus* spp.**	Temp (ºC)	73	184404	2526	4.05	0.000
	Error	106	66098	624		
Aspergillus flavus	Temp (ºC)	73	56211	770	3.48	0.000
	Error	106	23475	221		
Aspergillus terreus	Temp (ºC)	73	51772	709	3.99	0.000
	Error	106	18838	178		
***Curvularia* spp.**	Temp (ºC)	73	51043	699	3.40	0.000
	Error	106	21776	205		
***Penicillium* spp.**	Temp (ºC)	73	34945	479	3.27	0.000
	Error	106	15514	146		

In a final ANOVA test (Table 13), the genus *Aspergillus niger, Cladosporium* spp*., C. cladosporioides, Dreschlera spp., Fusarium* spp., *Geotrichum* spp., *and Penicillium rubrum*, resulted in a higher abundance at the stations. For this test, the presence of these fungi genus is statistically significant to the sampling stations chosen.

Table 13. Two-Way Analysis of Variance (ANOVA) testing for differences in abundance of fungal genera at different stations

Fungi genera	Source	DF	SS	MS	F	P
Aspergillus niger	Station	2	30743	15372	102.80	0.000
	Error	177	26468	150		
***Cladosporium* spp.**	Station	2	81875	40938	27.17	0.000
	Error	177	266641	1506		
C. cladosporioides	Station	2	22799	11399	38.59	0.000
	Error	177	52290	295		
***Dreschlera* spp.**	Station	2	829.71	414.86	66.50	0.000
	Error	177	1104.21	6.24		
***Fusarium* spp.**	Station	2	21.096	10.548	40.04	0.000
	Error	177	46.626	0.263		
***Geotrichum* spp.**	Station	2	7.3190	3.6595	70.36	0.000
	Error	177	9.2055	0.0520		
Penicillium rubrum	Station	2	1093.86	546.93	176.66	
	0.000					
	Error	177	547.99	3.10		

Discussion

Previous findings in studies at the Caribbean Sea have shown that there are some natural disturbances that can affect coasts and its dynamic populations (García and López, 1989; Gilbes et al., 1996). The coastal areas of Mayagüez Bay had been disrupted by these natural events and from antropogenic activities. Some of the natural disturbances include rainfalls and river run-off discharges, that may carry dissolved organic material and massive sediment into the marine environment (Gilbes et al., 1996). In other hand, antropogenic disturbances had also an important impact on the organism composition and appearance of coast. Sand extractions and movements from one place to another is one of the pressures imposed upon this shelf area (Webb et al., 1998). In El Seco for example, sand movement took place in order to be used as a filling for residential area. This lead to changes in the appearance of the costal Bay area and the type of sand grains, in comparison with Guanajibo and El Maní. In these areas, the sand grains are more firmer, rough, rounder and lighter in color than in El Seco, where the sand grains appear to be smoother, and brown colored with earth appearance (Morelock et al., 1983).

Since this is the first attempt of identifying filamentous fungi at coastal areas of Mayagüez Bay, we correlated our results for each site in the stations of Guanajibo, El Seco, and El Maní with parameters of salinity, temperature, and pH by testing them via ANOVA.

Mean abundance of fungi genera was first related with salinity. Monthly variations of salinity (ssu) measured at each sampling during the study year coincided with seasonal patterns of rainfall and river discharges in this part of the Island. The first result of ANOVA showed significance of salinity for each station in relation with some fungal genera growth or abundance. Higher abundance of fungi genera found at each station was associated to low salinity concentrations at the coast during the coldest months of the year, from January to February and from September through December, when there was large rainfall events occurring during these last months. Seasonal rainfall river discharges and currents may explain, in part, the monthly variation in mean abundance reported for each station by increasing in amount when the salinity levels were lower. Gilbes et al. (1996) reported salinity reductions regulated by seasonal rains and river discharge at all the sampling stations fixed in their study at Mayagüez Bay, with a reduction on salinity (31 to 33 ssu) during October. Since coastal areas received the impact of river discharges, Parrilla (1996) observed a low salinity layer from Añasco River during the rainfall season. Also, the dissolved organic materials and detritus dissolved in water may have an important impact of the organisms patterns found at the coastal areas. It has been found out, that contamination of marine and river waters and soil with nitrates significantly influence the viability of microscopic fungi living there. This influence was positive when the nitrates content was moderate (from 0.2 to 2 g/l), extremely high

concentrations of nitrates (20 g/l) lead to the inhibition of fungi growth and to their death (Alton, 1991). These results may be possibly associated also to the intrusion of freshwater plumes form Amazon and Orinoco rivers (Corredor and Morell, 2001; Alfaro, 2002). The findings of Rosado (2001) about the detritus importance for biological processes in the bay during the rainy season suggest that during strong rainfall events, water circulation transport nutrient inputs throughout the bay and introduce other factors that may affect the composition of the coastal areas (Alfaro, 2002). On the other hand, when the salinity levels were higher, especially during the hottest months of the year (May though August), the mean abundance of fungal genera decreased for each of the stations. Differences among sampling stations may be related with water changes processes or river discharges that creates a mosaic or a taxonomic arrangement for fungi genus.

Evaporation of water in sand at higher temperatures may explain the monthly variation of fungi during these months by increasing the amount of salinity levels. When the temperatures rise, there is an increase in evaporation, leading to concentrations of salt in sand. This is important, especially for the proliferation of some fungal genera that appears to be sensitive to salt concentrations variations, such as: *Aspergillus* spp., *A. flavus, A. terreus, Curvularia* spp., and *Penicillium* spp.

When the fungi cultures were inoculated at laboratory conditions, it presented the same patterns with the salinity levels. The colonies that

were transferred to any of the medium that were used (Malt Extract Agar, Potato Dextrose Agar or Rose Bengal Agar) prepared with distilled freshwater exhibits a complete medium utilization and grown with a different appearance than those inoculated in the same medium prepared with filtered seawater. These results suggest that there is a close relationship between the salinity concentrations and the fungal development, rather than the culture medium used.

pH variation among sampling stations were consistent during the whole year of study, with minimal variation among the sampling stations. It showed not to be significant, when tested by ANOVA analysis. The fungal genera showed to be tolerant to pH variations between 6.0 to7.02, which is a normal parameter for marine water ecosystems, but reported fungi tolerance of pH is about 5.5 (Larone, 1995). The results presented showed some fungal genus that may be tolerant to higher or basic pH levels, rather than the tolerance limits.

Another possible factor influencing the mean abundance of fungi genera may be the temperature. Temperature fluctuation appears to be more decisive for mean abundance of fungal genera. When temperatures arise, sand grains became hot and this might be detrimental for the development, protection, and the maintenance of fungi, by affecting its metabolic rate and structure formation (St-Germain et al.,1996). Some fungal genera showed no resistance to temperature variations as showed in ANOVA test (Table 12).

It is important also to highlight that some of the fungi genus identified at the sampling stations are extremely related with some mycotic diseases. For example, *Aspergillus* spp. is a common environmental pollutant and a fungal opportunist. It belongs to the Fungi Kingdom, Phylum Ascomycota, Order Eurotiales, and Family Thichocomaceae (de Hoog et al., 2000). Some of the *Aspergillus* species are common oportunistic pathogens, being *A. fumigatus* the most pathogen of the genus (Kern et al., 1997). It may cause disseminated aspergillosis, pulmonary disease, allergic bronchopulmonary disease, keratomycosis, otomycosis, and infections of nasal sinuses (Lozada et al., 1999). *A. flavus* is a potential aflatoxin producer, which is detrimental to animals and may cause their death. Aflatoxins are well recognized as a cause of liver cancer, but they have additional important toxic effects (Williams et al., 2004). In human cases, aflatoxins are especially toxic if consumed. Williams et al. (2004), showed that in farm and laboratory animals, chronic exposure to aflatoxins compromises immunity and interferes with protein metabolism and multiple micronutrients that are critical to health. The identification of this genus and specie of fungi is important at Mayagüez Bay, because some of the people that lives nearby consume sea products that may be infected with this toxin, although it is illegal to fishing or swimming at Mayagüez Bay.

Other fungal species that are a potential risk for humans includes: *Curvularia* spp., *Fusarium* spp, and *Mucor* spp., although every fungi may

be an opportunistic pathogen. *Curvularia spp*. belongs to the Fungi Kingdom, Phylum Ascomycota, Class Euascomycetes, Order Pleosporales, and Family Pleosporaceae (Larone et al., 1995). This fungi species usually causes keratomycosis and occasionally may produce mycetoma and it can infect any person that may be walking around with naked feet. *Fusarium* spp. belongs to the Fungi Kingdom, Phylum Ascomycota, Order Hypocreales, Family Hipocreaceae (de Hoog et al., 2000). This genus is one of the principal causes for keratomycosis and onychomycosis (Guarro et al., 1995). *Fusarium* spp. is also associated with infected agricultural products, maybe discharged by river and entering into the estuarine parts of the bay. *Mucor* spp. belongs to the Fungi Kingdom, Phylum Zygomycetes, Order Mucorales, Family Mucoraceae (Larone, 1995; Sutton et al., 1998). *Mucor* genus contains several species. The most common ones are*: M. amphibiorum, M. circinelloides, M. hiernalis, M. indicus, M. racemosus* and *M. ramosissimus* (de Hoog et al., 2000). This genus may be related with mucormycosis infections. *Rhizopus* spp. belongs to the Fungi Kingdom, Phylum Zygomycetes, Order Mucorales and Family Mucoraceae (de Hoog et al., 2000). This genus may lead to zigomycosis illness and is morphologically alike *Mucor* spp, but this genus contains rhizoids.

Other genus identified in this study was *Trichoderma* spp. This genus belongs to the fungi Kingdom, Phylum Ascomycota, Class Euascomycetes, Order Hypocreales, and Family Hipocreaceae (Sigurd,

1961; Larone, 1995; and Eriksson, 1997) All of these fungi genus may potentially increase health-related risks that include illness and in severity cases may lead to death.

Finally, these results indicate that other factors introduce substantial variability along spatial stations and sites at Mayagüez Bay. These factors are mainly the impact of Tuna Factories or WTC during the 90's decade (Alfaro 2002). The discharges of industrial wastes to the bay polluted it and the waves dynamic bring it on the coast, contaminating it with a large amount of phosphates. Singh et al. (1980), demonstrated that there was solubilization of rock phosphate in semi-solid lignocellulose medium by *Aspergillus fumigatus*. This pattern may be actually repeating by fungi identified at the stations.

These fungi cannot be precisely defined as endemic for each station, due to wave dynamics, air circulations patterns and river discharges. However, identification and genus patterns for sampling stations were very different from each other.

There are combined effects of the processes operating at Mayagüez Bay have resulted in large-scale alterations of marine community structure (Alfaro, 2002). There is chronic degradation of coral reefs in the inner bay, low oxygen concentrations and some other stress indicators of a distorted ecosystem. These results should be also considered to correlate our findings with further research that demonstrate

the mean abundance of fungal genera has to be with the ecological disturbances at Mayagüez Bay.

When the results were compared to the one-time sampling obtained from the AAA station in June 2003, only few fungi genera were reported for this sampling, and in some cases the numbers does not exceed 1.0 cfu and it was mostly *Aspergillus* spp. Nieves-Rivera (personal comment, 2003) reported that sea fans (*Gorgonia ventalina*) are being affected by *Aspergillus* spp. in P.R. and in Mona Island, causing a disease and eventual death. The main reason for this small abundance may be related to the depth, salinity concentrations, or in most cases wave abrasion dynamics and pollution. It is necessary to correlate on future investigations the findings with other physico-chemical the mean abundance of fungal genera. Also, it is necessary to evaluate the physico-chemical parameters that may be interacting in the coast an altering the chemistry of the sand itself.

Conclusions

1. Fungal communities in coastal areas of Mayagüez Bay were apparently influenced by the interaction of physico-chemical parameters such as temperature, salinity, and anthropogenic activities. It was evident that during rainfall season, the amount of fungi was higher at the three sampling stations.

2. Increased salinity concentrations during the hottest months of the year may be interfering with the fungi development by affecting its metabolism. There must be a closed relationship with the evaporation of water in sand and the increased salinity levels with the decreased abundance of fungal genus.

3. The lack of possible relations with pH parameters suggests that this physico-chemical parameter is not interfering with the development of fungal colonization in certain areas of the bay coastal area, and that these fungal genus may be adapted to tolerate pH, higher than its optimal developmental pH.

4. Anthropogenic activities such as sand extraction, mostly used for construction purposes affected not only the aesthetics, but also the taxonomic composition of the coastal area. Many of the fungi identified in

this study are not allochthonous to the place where they were found, especially in El Seco area, which was filled with sand transported from other places a long time ago.

5. Fungi are abundant in massive pollution, especially where organic matter is abundant, and nutrient loading via tributary rivers have a direct impact on fungal development. Other fungal genus not reported in this study does not tolerate these disruptions from antropogenic activities.

Literature cited

Abele, L.G. 1974. Species diversity of decapod crustaceans in marine habitats. Ecology 55: 156-161.

Acevedo, C. 1991. Hongos de arena, mangle y madera de La Parguera, P.R. M.S. Tesis, Universidad de Puerto Rico, Mayagüez, Puerto Rico. 40 pp.

Alfaro, M. 2002. Oceanografic features and zooplankton community structure at Mayagüez Bay, Puerto Rico. Ph.D. Thesis. University of Puerto Rico, Mayagüez, Puerto Rico. 147 pp.

Alfonso, E. 1995. The coastal current regime in Añasco Bay during a one-year period. M.S. Thesis. University of Puerto Rico, Mayagüez, Puerto Rico. 116 pp.

Alton, L.V. 1991. The viability of species of microscopic fungi in nitrate polluted water. Gig. Sanit. Nov(11):27-9.

Anaissie, E., G. P. Bodey, H. Kantarjian, J. Ro, S. E. Vartivarian, R. Hopfer, J. Hoy, and K. Rolston. 1989. New spectrum of fungal infections in patients with cancer. Rev. Infect. Dis. 11:369-378.

Anaissie, E. J., G. P. Bodey, and M. G. Rinaldi. 1989. Emerging fungal pathogens. Eur. J. Clin. Microbiol. Infect. Dis. 8:323-330.

Barron, G.L. 1968. The Genera of hyphomycetes in soil. Krieger publishing Co. 339 pp.

Barghoorn E.S, and Linder D.H. 1944. Marine fungi: their taxonomy and biology. Farlowia. 1:395-467 .

Capella, J.E. and K.A. Grove. 2002. A review of oceanographic data from the Mayagüez and Añasco Bay system. Final Project Report. University of Puerto Rico, Mayagüez, Puerto Rico. 48 pp.

Cogentrix of Mayagüez, Inc. 1990. NDPES permit application, technical support document for a proposed 300MW cogeneration facility. Submitted to the U.S. Environmental Protection Agency (EPA), Washington, D.C. 270 pp.

Collier, L., A. Balows, and M. Sussman. 1998. Topley & Wilson's Microbiology and Microbial Infections, 9th ed, vol. 4. Arnold London, Sydney, Auckland, New York. 156 pp.

Corredor, J.E., J.M. Morell, and A. Mendez. 1984. Dissolved nitrogen, phytoplankton biomass and island mass effects in the northeastern Caribbean sea. Carib. J. Sci. 20 (3-4).

Christophersen C, O. Crescente, J.C. Frisvad, L. Gram, J. Nelsen, P.H. Nelsen and L. Rahbaek. 1998. Antibacterial activity of marine-derived fungi. Mycopathologia. 1999; 143:135-138.

de Hoog, G. S., J. Guarro, J. Gene, and M. J. Figueras. 2000. Atlas of Clinical Fungi, 2nd ed, vol. 1. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Del Poeta, M., W. A. Schell, and J. R. Perfect. 1997. In vitro antifungal activity of pneumocandin L-743,872 against a variety of clinically important molds. Antimicrob. Agents Chemother. 41:1835-1836.

de Shazo, R. D., and R. E. Swain. 1995. Diagnostic criteria for allergic fungal sinusitis. Journal of Allergy & Clinical Immunology. 96:24-35.

Dixon, D. M., and A. Polak-Wyss. 1991. The medically important dematiaceous fungi and their identification. Mycoses. 34:1-18.

Ebright, J. R., P. H. Chandrasekar, S. Marks, M. R. Fairfax, A. Aneziokoro, and M. R. McGinnis. 1999. Invasive sinusitis and cerebritis due to *Curvularia clavata* in an immunocompetent adult. Clin Infect Dis. 28:687-689.

Eriksson, OE. 1997. Supraordinal taxa of Ascomycota. Myconet. 1:1-16.

Fernandez, M., D. E. Noyola, S. N. Rossmann, and M. S. Edwards. 1999. Cutaneous phaeohyphomycosis caused by *Curvularia lunata* and a review of C*urvularia spp.* infections in pediatrics. Pediat Inf Dis J. 18:727-731.

Fischer, A. G. 1960. Latitudinal variations in organic diversity. Evolution 14: 64-81.

Fung-Tomc, J. C., B. Minassian, E. Huczko, B. Kolek, D. P. Bonner, and R. E. Kessler. 1995. In vitro antifungal and fungicidal spectra of a new pradimicin derivative, BMS-181184. Antimicrob. Agents Chemother. 39:295-300.

Gilbes, F., J.M. López, and P.M.Yoshioka.1996. Spatial and temporal variations of phytoplankton chlorophyll a and suspended particulate matter in Mayagüez Bay, Puerto Rico. J. Plankton Research. Vol 18(1):29-43.

Guarro, J., and J. Gene. 1995. Opportunistic fusarial infections in humans. Eur.J. Clin. Microb. Infect. Dis. 14:741-754.

Guarro, J., T. Akiti, R. Almada-Horta, L. A. M. Leite, J. Gene, S. Ferreira-Gomes, C. Aguilar, and M. Ortoneda. 1999. Mycotic keratitis due to *Curvularia senegalensis* and in vitro antifungal susceptibilities of *Curvularia spp.* J.Clin Microbiol. 37:4170-4173.

Groll, A. H., and T. J. Walsh. 2001. Uncommon opportunistic fungi: new nosocomial threats. Clin Microbiol Infect. 7:8-24.

Hwang, S.C, C.S. Lin, I.M. Chen; J.M. Chen, L.Y. Liu; and W.K. Dodds.2004. Removal of multiple nitrogenous wastes by *Aspergillus niger* in a continuous fixed-slab reactor. Bioresour Technol. June;93(2):131-8.

Johnson, T.W., Jr. and F.K. Sparrow, Jr. 1961. Fungi in oceans and estuaries. J. Cramer, Weinheim: I-XXIV, 1-668.

Kappe, R., and A. Schulze-Berge. 1993. New cause for false-positive results with the Pastorex *Aspergillus spp.* antigen latex agglutination test. J. Clin. Microbiol. 31:2489-2490.

Kern, M.E and K. S. Blevins. 1997. Medical Mycology: A self instructional text. Second edition. F.A. Davis Company. 241 pp.

Kis-Papo T., A. Oren, S.P. Wasser, and E. Nevo. Fungi in hypersaline Dead Sea water. Microb. Ecol. Feb., 45(2):183-90.

Kirk, P.W. Jr. 1983. Direct enumeration of marine arenicolous fungi. Mycologia. 75(4): 670-682.

Kohlmeyer J., and Kohlmeyer E.1979. Marine mycology: the higher fungi. New York: Academic Press.

Kohlmeyer J., and Kohlmeyer, E. 2000. Lulworthiales, a new order of marine Ascomycota. Mycologia. 92(3). pp. 453-458.

Kohlmeyer J., Volkmann-Kohlmeyer B.1991. Illustrated key of the filamentous higher marine fungi. Bot Mar. 34:1-61.

Kuhn, F. A., and A. R. Javer. 2000. Allergic fungal rhinosinusitis – Perioperative management, prevention of recurrence, and role of steroids and antifungal agents. Otolaryngol Clin N Amer. 33:419-432,XI,NIL_5.

Lake, F. R., J. H. Froudist, R. McAleer, R. L. Gillon, A. E. Tribe, and P. J. Thompson. 1991. Allergic bronchopulmonary fungal disease caused by *Bipolaris spp.* and *Curvularia spp.*. Australian & New Zealand Journal of Medicine. 21:871-4.

Larone, D. H. 1995. Medically Important Fungi - A Guide to Identification, 3rd ed. ASM Press, Washington, D.C.

Manning, S. C., S. D. Schaefer, L. G. Close, and F. Vuitch. 1991. Culture-positive allergic fungal sinusitis. Archives of Otolaryngology -- Head & Neck Surgery. 117:174-178.

McGinnis, M. R., and L. Pasarell. 1998. In vitro testing of susceptibilities of filamentous ascomycetes to voriconazole, itraconazole, and amphotericin B, with consideration of phylogenetic implication. Antimicrob. Agents Chemother. 36:2353-2355.

Mejanelle L., J.F. Lopez, N. Guinde-Cimerman, J.O. Grimalt. 2001. Ergosterol biosynthesis in novel melanized fungi from hypersaline environments. J.Lipid. Res. 42(3):352-358.

Moore R.T. and S.P. Meyers. 1959. Thalassiomycetes I. Principles of delimitation of the marine mycota with the description of a new aquatically adapted Deuteromycetes genus. Mycologia. 51:871-876.

Moss, ST. 1986. The biology of marine fungi. Cambridge: Cambridge University Press.

Morelock, J., K. Grove, and M.L. Hernandez.1983. Oceanographic and patterns of shelf sediments of Mayagüez, Puerto Rico. J. Sed. Petrol., 53:371-381.

Parrilla, D. 1996. Distribution of chlorophyll-a, suspended particulate matter and nutrients in the vicinity of the Añasco River outlet. M.S. Thesis. University of Puerto Rico. Mayagüez, Puerto Rico. 87 pp.

Pitt, J. I. 2000. Toxigenic fungi: which are important? Med Mycol. 38:17-22.

Pitt, J. I., J. C. Basilico, M. L. Abarca, and C. Lopez. 2000. Mycotoxins and toxigenic fungi. Med Mycol. 38:41-46.

Pritchard, R. C., and D. B. Muir. 1987. Black fungi: a survey of dematiaceous hyphomycetes from clinical specimens identified over a five year period in a reference laboratory. Pathology. 19:281-4.

Rinaldi, M. G., P. Phillips, J. G. Schwartz, R. E. Winn, G. R. Holt, F. W. Shagets, J. Elrod, G. Nishioka, and T. B. Aufdemorte. 1987. Human *Curvularia spp.* infections. Report of five cases and review of the literature. Diagn. Microbiol. Infect. Dis. 6:27-39.

Rosado, M. 2001. Variability in the bio-optical properties of Mayagüez Bay. M. S. Thesis. Universidad de Puerto Rico, Mayagüez, Puerto Rico. 61 pp.

Sallenave-Namont C. 2001. Toxigenic saprophytic fungi in marine shellfish farming areas. Mycopathologia. 149: 21-25.

Seifert, R.P. 1981. Applications of a mycological database to principles and concepts of population and community ecology. Ecology. George Washington University. Chapter 1:11-22.

Segura-Torres, W. 2002. Low frequency transport components in Mona passage. Ph.D. Thesis. Universidad de Puerto Rico, Mayagüez, Puerto Rico. 317pp.

Sigurd, Funder. 1961. Practical Mycology: Manual for identification of fungi. A Broggers, Oslo, Norway. 144 pp.

Singh, C.P., MM. Mishra, and K.S. Yadav.1980. Solubilization of insoluble phosphates by thermophilic fungi. Ann Microbiol. Nov-Dec;131B(3):289-96.

St-Germain, G., and R. Summerbell. 1996. Identifying Filamentous Fungi A Clinical Laboratory Handbook, 1st ed. Star Publishing Company, Belmont, California.

Sutton, D. A., A. W. Fothergill, and M. G. Rinaldi (ed.). 1998. Guide to Clinically Significant Fungi, 1st ed. Williams & Wilkins, Baltimore.

U.S. EPA. 1997. Water quality assessment: a screening procedure for toxic and conventional pollutants. EPA/600/6-85/002a, b. U.S. EPA, Athens, GA.

Wicklow, D. and Carroll, G. 1981. Fungal Community: It's organization and role in the ecosystem. Dekker Editorial.

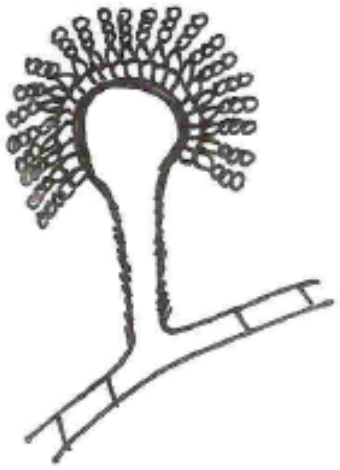
Williams, J.H., T.D Phillips, P.E. Jolly, J.K Stiles and D. Agga. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr.Nov;80(5):1106-1122.

Yu, D.S.; Peng Y.Z; and K. Zhang. 2004. Effects of seawater salinity on nitrite accumulation in short-range nitrification to nitrite as end product. J Environ Sci. 16(2):247-51.

Appendix 1a. A partial view of the Añasco Bay Shoreline.

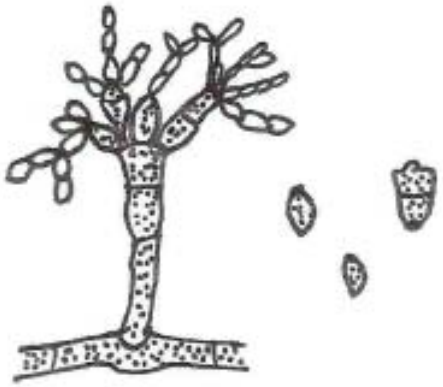
Appendix 1b. A partial view of the El Maní area (Mayagüez Bay shoreline)

Appendix 2. Most abundant fungal genera for the study



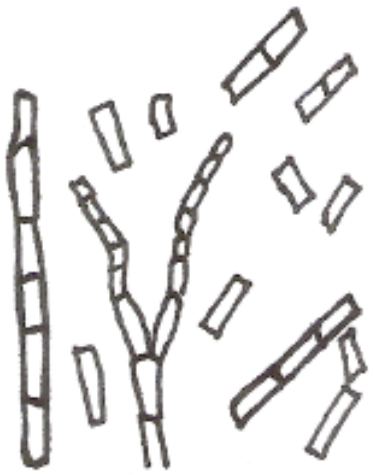
***Aspergillus* spp.**

***A. flavus* spp.**



Cladosporium spp.

Curvularia spp.



***Geotrichum* spp.**

***Mucor* spp.**