International Research Journal of Natural and Applied Sciences
Volume-2, Issue-2 (February 2015) ISSN: (2349-4077)

SEASONAL VARIATIONS OF FUNGI FOUND IN POND WATER IN THE NIGER DELTA, NIGERIA.

Douglas, S. I.,

Department of Applied & Environmental Biology, Rivers State University of Science & Technology, P.M.B. 5080, Port Harcourt, Rivers State, Nigeria.

ABSTRACT

This research was carried out to investigate the presence and occurrence of fungi in three selected pond water samples from Ogoniland, Rivers State Nigeria. Pond water samples were collected monthly for eight months covering both seasons, using Standard procedures for sample collection, isolation and identification. A total of ten fungal genera were identified, with Aspergillus sp having the highest frequency of occurrence of 38.8% and Trichoderma sp having the least frequency of occurrence of 2.1%. The population of fungal isolates were not homogenous throughout the study period and showed variations with season. The predominant fungal genera isolated were Aspergillus niger 16.3%, Fusarium sp 14.2%, Penicillum sp 8.5%, Aspergillus fumigatus 9.2%, Rhizopus sp 10.6%, Mucor sp 7.1%, Aspergillus flavus 12.8%, Trichoderma sp 2.1%, Candida sp 4.7%, Geotrichum sp 3.6% and Saccharomyces sp 3.6%, higher counts and species diversity were observed in the rainy than dry season. Higher nitrate and phosphate concentration was observed during the rainy season, acidic pH range of 3.57 to 6.87 was recorded, The Aspergillus sp was also observed throughout the study period in all sample locations. Some of these isolates Aspergillus sp and Candida sp are opportunistic pathogens. Hence, if the water from these ponds are used by the villagers without prior treatment, it may serve as a possible mode of transmission of these microorganisms to humans and may pose potential health hazard, especially to immuno-compromised individuals.

Keywords: Pond, Fungi, frequency of occurrence, pathogens

Introduction

Fungi consist of a large and diverse group of organisms in the aquatic ecosystems, which can be found in various types of water bodies, including; fresh, sea, stagnant, surface and ground

waters. Fungi are part of the biotic component of hydro-ecosystems and an important link in the food chain transformations in aquatic habitat. Most of them are saprophytic which take part in mineralization of dead organic matter, both of plant and animal origin, either produced in the water environment or carried in from outside the water. Acting as destruents, assisting in water self-purification and which naturally prevents eutrophication. Hence, aquatic fungi can be a useful tool in biological index of water purity and pollution monitoring (Godlewska et al; 2009). They have also been recovered from very diverse, remote and extreme aquatic habitats including lakes, ponds, rivers, streams, estuaries, marine environments, wastewaters, sludge, rural and urban storm water runoff, well waters, acid mine drainage and aquatic sediments (APHA, 1989; and Saju, 2011). The fungal population seen in fresh water are divided into two main groups, which includes; Hydro- fungi which require the presence of water to complete their life cycle and geo-fungi or typical soil fungi which were not normally known to aquatic existence, but they might be found in water because of adequate supply of nutrients, these were regarded as "Facultative aquatic fungi"(Saju, 2001). Pathogenic fungus are known to exists in soil as moulds and generates spores which are dispersed by physical mechanisms of gravity, wind, water and animals. Spores present in air are settled down to soil during rainfall, which are then carried by rainwater to ponds and other surface water resulting in contamination of the water body. The use of such water without treatment, can result in disease outbreak in humans and animals and plants. Aquatic fungi also play an important role in the ecosystem decomposition and nutrient recycling by breaking down leaves and woody substrates and also as symbionts in the aquatic environment (Saju, 2011, Shaista et al; 2011). Fungi are widely distributed in the environment, with more than 70,000 species of them discovered. Of these, about 300 fungal species have their habitat in human beings and about a dozen cause about 90% of all fungal infections (Bipasa et al; 2013). Some of them are plant, animal or human parasites. When conditions becomes favourable, fungi acting as saprobionts can assume pathogenic properties, being a potential source of infection (Saju, 2011,Godlewska et al; 2009). Nevertheless, waterborne fungi are associated with taste and odour problems, contamination of food and beverage preparation, and in a variety of health related effects ((Okpoko et al, 2009). The present paper deals with the study of distribution and seasonal variation of fungi in pond water.

Materials and Methods

Sample Location

Freshwater samples were collected from three ponds namely, Kpoku, Gbenekiri, and Napaa ponds that serves as a source of drinking water in Uegwere Bo-ue community in Khana Local Government Area of Rivers State. The three ponds were designated pond A, pond B, and pond C respectively. Water samples were collected once monthly for eight months covering both dry (Dec. 2013, Jan, Feb. and March, 2014) and rainy(May, Jun, Jul and Aug. 2014) seasons, Pond water sample from Kpoku pond was labelled sample A, with coordinates of N 04^0 38.202, E $007^0$21.329, Pond water sample from Gbenekiri pond was labelled sample B, with coordinates N 04^0 38.366, E 007^0 21.410, while pond water sample from Napaa pond was labelled sample C, and with coordinates: N 040 38.578, E 007^0 21.325.

Collection of pond water samples

Composite surface water (0 - 15 cm) samples were collected and pooled together in a clean pre-sterilized 3 L container from the three ponds. The area is associated with anthropogenic pollution, soil erosion, surface run-off and other human activities. These three pond habitats are all exposed to non-point source pollutions. Samples were obtained at the areas of the ponds from where the local inhabitants usually fetched their water. This meant that those were the points where humans made direct contact with the water sources. Appropriately labelled and transported to the laboratory immediately for investigation.

Microbiological Analysis

Isolation, Characterization and Identification of fungi

Serial dilution of the pond water sample was done using physiological saline 10^{-1} to 10^{-3}. A 0.1ml aliquot was inoculated to a freshly prepared Sabourand Dextrose agar to which 0.5% Ampicillin was added to inhibit bacterial growth (Cheesebrough, 1991). Incubated at 28^0C for five days. Counts were taken and sub-cultured. The microscopic examination of the isolates was done by needle mount method(Cheesebrough), 1991. Isolates were identified on the basis of their cultural characteristics, spores and vegetative mycelium according to Barnett and Hunter(1972, Watanabe, 1994; Larone, 1995; Doggett, 2000).

Physico-chemical analyses

The water samples from each pond was examined in terms of physical and chemical contents using the standard procedures of the American Public Health Association, (APHA) for BOD, COD, nitrate, phosphate concentration and oil and grease, pH and temperatures were measured in-situ, using the pH metre (Jenway pH meter 3015 model) and mercury in glass thermometer for temperature.

Statistical analysis

Analysis of variance (ANOVA) test was used to analyze the data for fungal count.

Results

The results of this study revealed the following genera: *Aspergillus, Penicillum, Rhizopus, Mucor, Trichoderma, Cladosporium, Candida, Fusarium, Geotrchum*, and *Saccharomyces*. The predominant fungal genera isolated were *Aspergillus* niger 16.3%, *Fusarium* sp 14.2%, *Penicillum* sp 8.5%, *Aspergillus fumigatus* 9.2%, *Mucor* sp 7.1%, *Aspergillus flavus* 12.8%, *Cladosporium* sp 7.1%, *Trichoderma* sp 2.1%, *Geotrichum* sp 3.6%, *Candida* sp 4.9% and *Saccharomyces* sp 3.6%, higher counts and species were observed in the rainy than dry season. Table 1, shows the fungi genera isolated from the water samples. The fungi counts during the study period ranged from 5.7 x 10^3 to 8.0 X 10^4 Sfu/ml for Pond A, 2.2 X 10^3 to 5.2 X 10^4 Sfu/ml for pond B and 4.8 X 10^2 to 8.6 X 10^3 Sfu/ml for pond C. Pond A had more fungi population and diversity than the other two ponds, pond C had the least also for both seasons. *Candida* sp was observed only in ponds A and B, it was not seen in pond C throughout the sampling period. This organism was observed only in the dry season. *Geotrichum* sp was isolated only in the dry season, in Ponds B and only ones in Pond C. Trichoderma sp was the least frequently isolated organism, with 2.1% frequency of occurrence. *Saccharomyces* sp was not isolated isolated in sample C, during the sampling period. The temperature of the ponds ranged between 26.5 to 27.6 ^{0}C and 23.4 to 25.6 ^{0}C ± 0.6, pH of the samples ranged between 3.57 to 5.14 and 5.73 to 6.87 ± 0.5 , BOD ranged, 1.6 to 5.8 ± 0.3, and 4.8 to 10 ±0.2 mg/l, COD ranged; 1.82 to 3.32 ± 0.4 mg/l, and 2.24 to 12.4 ± 0.11, nitrate concentration ranged between 0.18 to 1.59 ± 0.7 mg/l, and 1.18 – 2.65 ± 11 mg/l, phosphate concentration ranged 0.03 to 0.21 ± 0.1mg/l and 0.32 – 0.79 ± 0.3 mg/l for

dry and rainy seasons respectively. The oil and grease concentration was very low in all the samples.

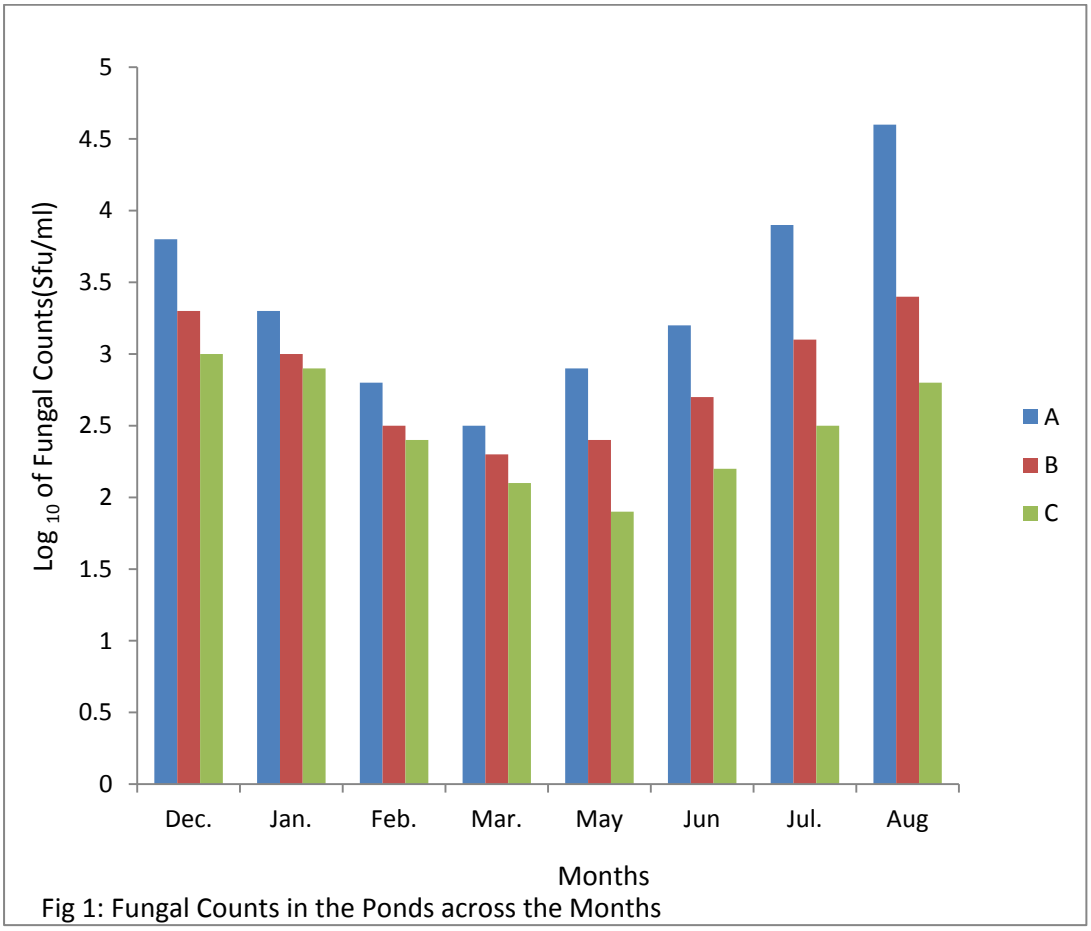

Fig 1: Fungal Counts in the Ponds across the Months

Table 1:Frequency of Occurrence

Isolates	Occurrence(n=141)	Frequency of Occurrence(%)
Aspergillus niger	23	16.3
Fusarum sp	20	14.2
Aspergillus flavus	18	12.8
Rhizopus sp	15	10.6
Aspergillus fumigatus	13	9.2
Penicillum sp	12	8.5

Mucor sp	10		7.1
Cladosporum sp	10		7.1
Candida sp	7		4.9
Geotrichum sp	5		3.6
Saccharomycetes sp	5		3.6
Trichoderma sp	3		2.1

Table 2: Distribution of Fungi in Pond A During the Dry and Rainy Seasons

Isolates	Dec.	Jan.	Feb.	Mar	May	Jun.	Jul.	Aug.
Aspergillus niger	*	*	*	*	*	*	*	*
Fusarum sp	*	*	*	Ab	*	*	*	*
Aspergillus flavus	*	*	Ab	Ab	*	*	*	*
Rhizopus sp	*	*	Ab	Ab	*	*	*	*
Aspergillus fumigatus	Ab	Ab	Ab	*	*	*	*	*
Penicillum sp	*	*	Ab	Ab	Ab	*	*	*
Mucor sp	Ab	*	*	Ab	*	*	*	*
Cladosporum sp	Ab	Ab	Ab	Ab	*	*	*	*
Candida sp	*	*	*	*	Ab	Ab	Ab	*
Geotrichum sp	*	Ab	Ab	Ab	*	*	*	*
Saccharomycetes sp	*	Ab	Ab	Ab	Ab	*	*	*
Trichoderma sp	Ab	Ab	Ab	Ab	Ab	*	*	*

Key: * Positive, Ab: absent

Table 3: Distribution of Fungi in Pond B During the Dry and Rainy Seasons

Isolates	Dec.	Jan.	Feb.	Mar	May	Jun.	Jul.	Aug.
Aspergillus niger	*	*	Ab	*	*	*	*	*
Fusarum sp	*	*	Ab	Ab	*	*	*	*
Aspergillus flavus	*	Ab	Ab	*	*	*	*	Ab
Rhizopus sp	Ab	Ab	*	Ab	Ab	*	*	*
Aspergillus fumigatus	Ab	*	*	*	*	*	Ab	Ab
Penicillum sp	Ab	Ab	Ab	*	*	*	*	*
Mucor sp	*	Ab	Ab	Ab	Ab	*	*	*
Cladosporum sp	Ab	Ab	Ab	Ab	*	*	*	*
Candida sp	*	*	*	Ab	Ab	Ab	Ab	Ab
Geotrichum sp	Ab	Ab	Ab	Ab	*	*	Ab	*
Saccharomycetes sp	*	Ab	Ab	Ab	*	*	*	*
Trichoderma sp	*	Ab	Ab	Ab	Ab	Ab	*	*

Table 4: Distribution of Fungi in Pond C During the Dry and Rainy Seasons

Isolates	Dec.	Jan.	Feb.	Mar	May	Jun.	Jul.	Aug.
Aspergillus niger	Ab	*	*	Ab	*	*	*	*
Fusarum sp	Ab	Ab	Ab	*	*	Ab	*	*
Aspergillus flavus	*	Ab	Ab	*	*	Ab	Ab	*
Rhizopus sp	Ab	*	Ab	*	*	Ab	*	Ab
Aspergillus fumigatus	Ab	*	*	Ab	*	Ab	*	Ab
Penicillum sp	*	Ab	Ab	*	*	Ab	*	*
Mucor sp	Ab	Ab	Ab	Ab	*	*	Ab	*
Cladosporum sp	Ab	Ab	Ab	*	*	*	Ab	Ab
Candida sp	*	*	Ab	Ab	Ab	Ab	Ab	*
Geotrichum sp	*	Ab	Ab	Ab	Ab	*	*	*
Saccharomycetes sp	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Trichoderma sp	Ab	Ab	Ab	Ab	*	*	Ab	Ab

Table 5: Physicochemical Characteristics of Seasonal Changes in the Ponds

Parameters	**Dry**	**Rainy**
Temp(ºC)	26.3 - 27.2 ± 1.5	23.4 - 25.6 ± 0.8
pH	3.57 - 5.14 ± 0.6	5.73 - 6.87 ±0.5
BOD(mg/l)	1.6 - 5.8 ± 0.3	4.8 - 10 ± 0.2
COD(mg/l)	1.82 - 3.32 ± 0.4	2.42 - 12.4 ± 0.1
Nitrate(mg/l)	0.18 - 1.59 ±0.7	1.18- 2.65± 0.11
Phosphate(mg/l)	0.03 - 0.21 ±0.1	0.32 - 0.79 ±0.3
Oil & Grease(mg/L)	0.001	0.001

Discussion

The *Aspergillus* genera was the most frequently isolated and had the highest frequency of occurrence of 38.3%, which was also observed by other researchers, it was observed throughout the study period, while *Trichoderma* sp had the least frequency of occurrence of 2.1%. Pond A had the highest fungal population and diversity while Pond C had the least throughout the study period. Rainy season had more fungal diversity and population than what was observed during the dry season. Higher fungal counts observed during the rainy and dry seasons are statistically significant at $P \leq 0.05$ observed between seasons regarding the fungal counts. The lower counts during the dry season might be due to high temperature and lack of rainfall. The higher counts observed may be as a result of frequent rainfall, which leads to surface run offs, carrying both nutrients and soil microbes into the water body. Odokuma and Okpokwasili, 1997 and Ogbonna, 2010, also observed higher microbial counts in the rainy than dry season. Higher fungal population during the rainy season may also be due to favourable physicochemical parameters like; temperature, pH, nitrate concentration and phosphate concentration. These higher nutrient concentration may also be from the surrounding, since these substances are components of agricultural fertilizers commonly used by farmers in the communities, as the ponds are close to farmlands.

The genus *Apergillus* was the most frequently isolated in our investigation. The results of other researchers; Okpako, *et al*; 2009, Bipasa *et al*, 2013 are consistent with our findings that *Aspergillus* sp is the most commonly isolated genera in water. This organism is an opportunistic pathogen in plants and animals. That this organism is ubiquitous in nature with

strong adaptive behaviour forming complex plant polymer in food stuff even with acidic pH, dried foods and those with high concentration of sugars such as jams, jellies. These organisms produce toxins called aflatoxins (B1, B2, G1 and G2), which is the most toxic and potent hepatocarcinogenic, natural compounds ever characterized (Okpako *et al*, 2009 Bipasa *et al*, 2013 Saju, 2011). Resulting in a wide range of diseases in man, ranging from hypersensitivity reactions to invasive infections associated with angiinvasions. A. flavus was also frequently isolated in this study, particularly during the rainy season. This species is known to be the second leading cause of invasive and non-invasive aspergillosis (Saju, 2022). Also, *A. niger* is a known allergen and may cause opportunistic invasive infections in hospitalized immunized patients (Okpako *et al*; 2009). Also isolated in high number is *Fusarium* sp found in several samples in this study, is a known agent of superficial infections (keratitis and cutaneous infections, onychomycosis and infections of wounds and burns) (Okpako, *et al* 2009). *Penicillium* species were also isolated in this work, they are known to cause allergy, asthmatic attack and some respiratory problems, if susceptible host are exposed. Also observe was *Rhizopus* sp, organisms are known to cause diseases in immunocompromised patients. *Mucor* was also isolated, which is known to be a major cause of thrombosis, infarction, nasal or paranasal sinus infection and GI disorders. *Trichoderma* species are soil borne organisms which might have been introduced into the ponds as a result of surface runoff, and they are characterized by rapidly growing colonies that have a great potential for spore production (Okpako *et al*; 2009). Some known species have been reported to cause mycosis and allergy in humans. In this study, *Trichoderma* sp had the least frequency of occurrence. This may suggest that *Trichoderma* sp is not a common inhabitant of the pond water system. Although it is unlikely that concentrations as low as those reported in our study can cause fungal infection in healthy people, immunosuppressed persons are at risk of infection. I want to recommend that routine microbiological investigations should be done in hosptals or institutions where immunosuppressed individuals are treated.. [Okpoka *et al*; 2009]. *Candida* sp were also isolated from this study, although their frequency of occurrence was low. Their presence especially during the dry season may be as a result frequent visits made to the ponds by the villagers and the dislodgement of the organism from those abhorring them . Bathing around in and around the pond by the villagers.

Conclusion

The presence of these large variety of fungal species in the pond water habitat indicates a dynamic and diverse fungal community. Most of the species identified from the water seems to be well adapted for survival in this environment and highly influenced by external environmental conditions. The use of Immunosuppressive agents and other medical practices have created an ecological platform for *Aspergillus* sp to establish growth on immunocompromised host. Therefore, Invasive aspergillosis is increasing in case of organ transplantation and bone marrow recipients. If this water is not treated before use, can cause problems for human health if used for drinking and swimming activities.

References

APHA (American Public Health Association) (1989). Standard Methods for the Examination of Water and Wastewater. 17th Ed. Washington DC, USA: American Public Health Association; 1989.

Bipasa, D. I., S. Bhattacharya and S. Das (2013)Burden of Candida spp. and Aspergillus spp. in pond water in and around South Kolkata, India. *Int.Journal.Curr.Microbiol.App.Sci*. 2(11): 256-260

Barnett, H. L. and Hunter, B. B. 1972. Illustration of Generaof Fungi Imperfecti. 3rd edition, Burgess Publication Co. Minneapolis.

Cheesebrough, M.2000 District Laboratory Practice in Tropical Countres, part 2. Cambridge University Press, London 143 – 156.

Doggett MS (2000). Characterization of fungal biofilms within a municipal water distribution system. Appl. Environ. Microbiol. 66(3): 1249-1251.

Godlewska, A., B. Kiziewicz, E. Muszynska, and B. Mazalska (2009) Fungi and Straminiplous Organisms Found in Ponds in Bialystok. *Polish J. of Environ. Stud*.18(3): 369-376

Larone DH (1995). Medically important fungi: a guide to identification. ASM press, Washington D.C.

Odokuma, L. O. and G. C. Okpokwasili (1997) Seasonal Influences of organic Pollution monitoring of the New Calabar River, Nigeria. *Environmental Monitoring and Assessment 45:43-56*

Ogbonda, D. N. (2010) Seasonal Dynamics of Microbial Population and Physicochemical Characteristics of A Water Body Receiving Industrial Pollutants In Port Harcourt, Nigeria. *Agric. Bio. J. N. Am*. 1(6):1333 – 1339.

Okpako, E. C., A. N. Osuagwu, A. E. Duke and V. O. Ntui (2009)Prevalence and significance of fungi in sachet and borehole drinking water in Calabar, Nigeria. *Afr. J. of Microbiol. Res*. 3(2) 56 – 61

Saju, S. D.(2011) Occurrence of Fungi in Pond water (Dumaratarai Talab) of Raipur City, C. G, India. *Journal of Phytology*, 3(4): 30 – 34.

Shaista, P. I., S. Lanjewar, K. Sharma and U. Kutti (2011) Isolation of Fungi from the surface water of River. Journal of Experimental Science, 2(10): 58 - 59

Shekha, Y. A., Hero, M., Ismael Z. And Ahmed, A. A.(2013). Bacteriological and Mycological Assessment for Water Quality of Duhok Reservoir, Iraq. *Jordan Journal of Biological Sciences.* 6(4): 308 – 315.