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PATHOGENIC FUNGI IN PORT HARCOURT'S OPEN DRAINS: IMPLICATIONS FOR PUBLIC HEALTH

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Abstract: This study describes the occurrence of several fungal species in a wastewater from an open drainage system in Port Harcourt. Open drains create a lot of environmental and health problems and the frequency are increasing since these drains are usually clogged. People dump their garbage into gutters thus making drainage systems the resting place for cans, bottles, plastics, and other household products and due to poor sanitation practices such water runs over the ground during rain storms and picks up feces and contaminates water resources. Wastewater and sediment samples were collected from five (5) sampling sites along the Ntanwogba Creek drainage channel from which fungi were recovered by simple analytical techniques. The mean total fungal count ranged from 4.3x105 cfu/ml to1.7x106 cfu/ml for waste water and 5.7x104 cfu/g to 2.9x106 cfu/g for sediment samples. In total 77 isolates were identified across the open drains sampled and they include Cryptococcus neoformans and Rhizopus the most representative genera (36.4%) and (29.9% respectively) followed by *Torulopsis glabrata* (10.4%), *Penicillium chrysogenum* (7.8%), *Aspergillus versicolor* (5.2%), *Apergillus niger* (3.9%), while *Mucor*, *Aspergillus temairic*, *Scopulariopsis* and *Phoma* (1.3 % each) had the least and finally the species of *Torulopsis* and *Cryptococcus* were isolated as yeasts. The wastewater from the open drainage systems may play a role in fungal dissemination, including opportunistic pathogens causing infectious diseases. Besides, their significant ecological role in nutrient turn over in such environments cannot be overemphasized

Keywords: Pathogenic fungi, open drains, wastewater, pollution, diseases, public health

Introduction

Pathogenic fungi in wastewater distribution systems can cause biofilms which impact foul odour and obstruction of water piping and pigments in water as well as corrosion (Paterson and Lima, 2005; Hussain *et al*., 2010) . They can also cause organoleptic biodeterioraion and act as pathogens or allergens and cause mycotoxin contamination (Oliveira *et al*., 2013). Fungi from soil, air, crops, plant debris, organic matter, etc., may enter such water systems in various ways, through

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increasing human population activities such as expansion of urban centers and industrial setups or through runoffs during heavy rains when top soils and other debris are washed off and these have resulted in the generation of different waste types that discharge into surface water bodies. Much of these wastes are in solid and liquid forms consisting of domestic organic and inorganic wastes, spent oil or lubricants (crank case oil), agricultural pesticides and fertilizers, water from floods (storm water), runoffs (rain water, running through cracks in the ground and into gutters), water from swimming pools, water from car garages and cleaning centers. Many people also dump their garbage into gutters, streams, lakes, rivers, and seas, thus making water bodies the final resting place of cans, bottles, plastics, and other household products. In areas where drainages and sanitation are poor, such waters run over the ground during rain storms and pick up feces and contaminates water resources. These waste materials have in recent times caused blockage of drainages. The widespread use of such wastewater containing toxic substances contaminated by pathogenic fungi may likely cause increase in the incidence of wastewater borne disease which is the most common health hazards associated with untreated drinking and recreational waters. Also, their presence in wastewater can result to breakdown of organic solids which may consume much of the dissolved oxygen in the receiving water bodies (Ogbonna and Ideriah, 2014). This contributes significantly to the spread of diseases like dysentery, diarrhea, cholera, typhoid, malaria and gastroenteric disorders (Van and Pur, 1990; Bicki, 2001; Burabai *et al*., 2007; Ochuko Thaddeus, 2013; Shafi *et al*., 2013; Ogbonna, 2014). Also, improper waste disposal practices on such drainage channels could lead to outbreak of diseases, pollution and nasty odor (Ekugo, 1998; (Aibor and Olorunda, 2006; Ifeoma and Nkiru, 2009; Ogbonna *et al*., 2008 a,b; Owaduge, 2010) which contribute much to deteriorating health of a population (Ezzati *et al*., 2005).

The Ntanwogba stream receives several point and non-point sources of untreated industrial and municipal wastes. The stream finally empties into the brackish water bodies where its impacts on water quality and biological resources which results in loss of water integrity, aesthetics and biodiversity. This paper focuses on the health implications of such open drainage channel which is a source of contamination and pollution of rivers and streams that cause deterioration, impairment of the aesthetic quality and pigmentation of water resources.

MATERIALS AND METHODS

DESCRIPTION OF AREA OF STUDY

The Ntanwogba Creek is located on the western flank of Port Harcourt city of Rivers State, Nigeria. The stream lies between latitude 40 50'' and 50 00''N and longitude 60 55''N and 70 00''E. The climate of the area is that of tropical equatorial latitude with rainfall occurring almost all year round (Gobo *et al*., 2008). The Ntanwogba is a black water stream with its water source running through Orazi forest of Rumueme town across Abacha Road, Cherubim Road, OluObasanjo Road, Okija Road and Afam Street (D/line), and meanders through the densely populated city of Port Harcourt into the Upper Bonny Estuary. Five sampling sites in Port Harcourt metroplois, were studied. Sampling was done 500m apart along the stream (Fig 1).

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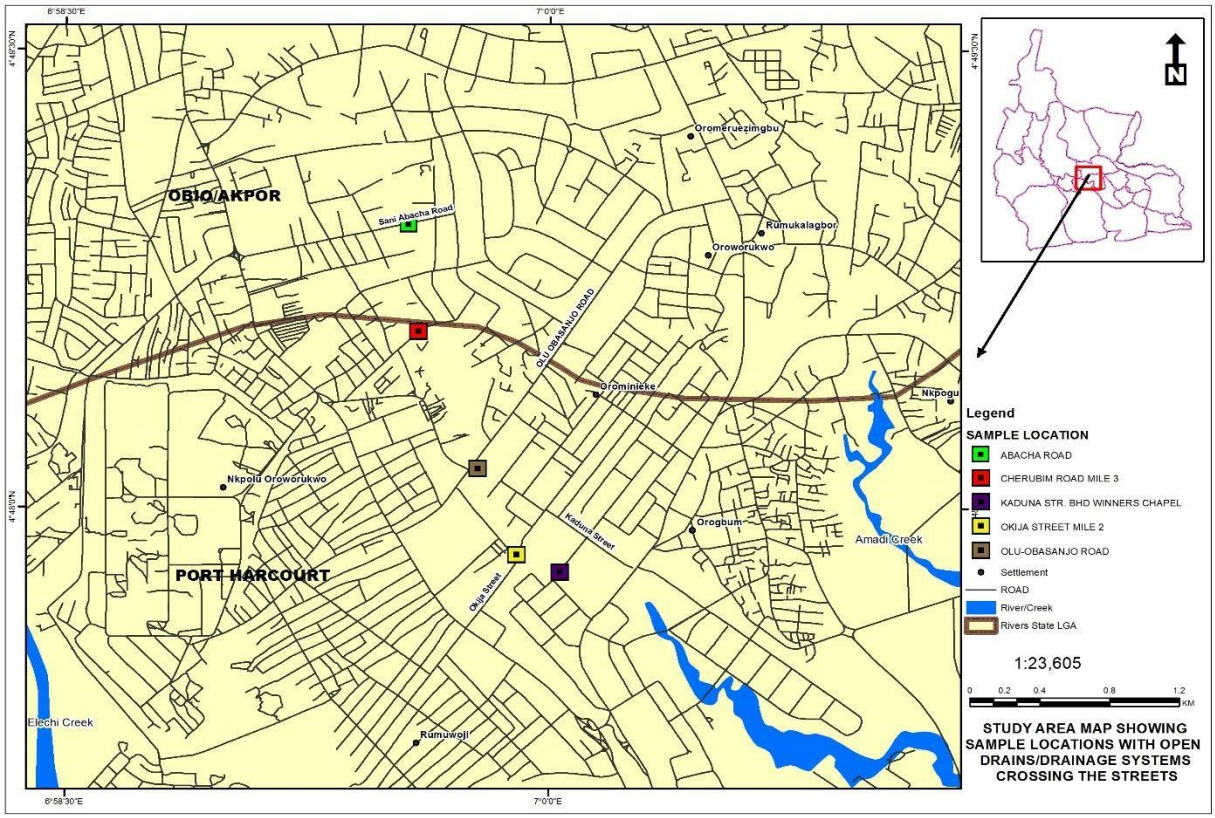

Fig 1. Map of Port Harcourt showing sampling locations along the Ntanwogba creek

COLLECTION OF SAMPLES

Wastewater samples were collected with sterile containers (already sterilized in the laboratory). Each sample bottle was rinsed with the appropriate sample before the final collection. To collect the water sample, base of the sterilized sample bottle was held with one hand, plunged about 30cm below the water surface with the mouth of the sample container positioned in an opposite direction to water flow (APHA, 2012). The container was filled with wastewater samples and this repeated at all the sampling stations starting from the upstream (Afam /Kaduna Street behind the Winners Chapel) to the downstream (at Abacha Road) leaving a gap of about 2cm and then covered.

Sediment samples for analysis were also collected along the same water course. To collect the sediment sample, the bottles were opened and held with the left hand while using the right hand with a plastic scooper to scoop the sediment sample. The sample bottles were filled with sediment sample and covered immediately. After collection, the samples were immediately labelled and transported in a cooler packed with ice blocks to the laboratory for analysis. Sample collection was carried out twice monthly from February to June.

Microbiological analyses

Samples were analyzed for total fungal counts by spread plate method using Sabouraud Dextrose agar (SDA).

Serial dilution

Ten-fold Serial dilutions of the samples were made according to the methods of Collins and Lyne (1976) and Harrigan and McCance (1976).

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Inoculation and incubation

One milliliter of appropriate ten- fold serial dilution of the sample were inoculated onto appropriate sabouraud dextrose agar in triplicates using pour plate methods of Collins and Lyne(1976) and Harrigan and McCance (1976) and spread plate methods of Demain and Davies(1999). Inoculated plates were incubated at 28 ± 20C for 48-72 hours. Visible discrete colonies on incubated plates were counted and expressed as colony forming units per gram (cfu/g) of sediment samples and colony forming units per milliliter (cfu/ml) of waste water samples.

Maintenance of pure culture

Fungi grown onto Sabauraud dextrose agar (SDA) were purified by repeated sub-culture unto gari slide culture media. Pure cultures were preserved on gari slide culture media at ambient temperature (28+20C) for further tests.

Garri Slide Culture Method

According to the method of Sokari *et al* (1996), individual, fairly large grains of garri were placed in a glass petri dish and autoclaved at 1210C for 15minutes. Using a flamed forcep, one granule of the garri was transferred from the glass petri dish unto a glass slide. After which fungal growth was touched with a wire inoculating needle and then placed on garri granule on glass slide. Inoculated granule was transferred into a sterile petri dish layered with moistened cotton wool, and incubated at 370C for 3-5days. After incubation, structures of fungal growth were enhanced by touching edge of coverslip with lactophenol cotton blue. Identification of species was done phenotypically based on macroscopic and microscopic morphological features of cultivation in gari slide culture medium.

Characterization and Identification of Fungal Isolates.

Pure cultures of fungal isolates were identified based on cultural parameters, microscopic technique and biochemical tests including carbohydrate utilization as described by Cruickshank *et al* (1975). Characterization and identification of fungal isolates was done according to Domsch *et al* (1980) and Barnett and Hunter (1987).

RESULTS AND DISCUSSION

Fungi are supposed to be common constituents of water distribution systems. Fungi are ubiquitous in soil and wastewater preferring cool and moderate climate, commonly present whenever organic material is available (Nicoletti *et al*., 2009). The fungal species were identified as *Aspergillus niger*, *Penicillium chrysogenum Aspergillus tamarii*, *Cryptococcus neoformans*, *Aspergillus versicolor*, *Torulopsis glabrata*, *Rhizopus, Mucor sp*., *Scopulariopsis, Penicillium marneffei*, and *Phoma* (Tables 1, 2). These species except *Scopulariopsis, Phoma, Aspergillus tamarii, Penicillium marneffei* and *Mucor*, were isolated from both water and sediment samples. *Mucor* occurred only in water sample from station 4 (Cherubim Road), Scopulariopsis, Phoma and *Penicillium marneffei* occurred only in sediment samples from station 5 (Abacha Road), *Aspergillus temairic* occurred only in sediment samples from station 2 (Okija Road) while *Cryptococcus neoformans* and *Rhizopus* species were isolated more frequently from both water and sediment samples in the open drains. However, the patterns and rate of growth of the fungal counts/ species were more in sediments than in water samples. Similar species of Fungi have been

isolated from municipal water distribution networks and from hospital plumbing systems (Anaissie *et al*., 2003; Warris *et al*., 2003; Hageskal *et al*., 2009; Harding *et al*., 2009) with pathogenic properties causing formation of biofilms. Fungi are more often diseases of water used for recreation, bathing, hot tubs, swimming, washing and water uses other than drinking, in contrast to many viral and bacterial diseases which occur from ingestion of the water. Nutrient compounds in the wastewater becomes valuable substances for enhanced growth of the microbes. Discharge of these nutrients into rivers and lakes without pretreatment can cause adverse influences in our environment and life, thus resulting in the ecological imbalance of such aquatic environments which may cause eutrophication. This may subsequently cause nuisance conditions and predispose the public to poor health conditions

Table 1. Morphological Characterization and identification of fungal Isolates

Isolates code	Morphological Characteristics	Microscopic Characteristics	Probable organism
1	White cottony mycelium	Non-septate hyphae, large globose many spored sporangia on single sporangiophore.	*Rhizopus*
2	Green dense velvet mycelium	Hyaline conidiophores, phialides borne on versicles. Green chain of conidia with septate hyphae.	*Aspergillus versicolor*
3	Dark green granular dense mycelium	Conidiophores with inflated branches.	*Pencillium chrysogenum*
4	Compact white basal dark colony	Hyaline conidiophore phylides borne on vesicles. Green chain of conidia, with septate hyphae.	*Aspergillus niger*
5	Loose cotton wool-like aerial mycelium	Non septate mycelia, that bear sporangiophores scattered all over the mycelium.	*Mucor*
6	Oval, creamy with slimy surface	Oval yeast cells with single terminal budding.	*Torulopsis glabrata*
7	White with blackish brown pyonidia	Conidia are unicellular, hyaline are ellipsoidal to cylindrical with septate hyphae.	*Phoma*
8	Hairy light brown surface with white background	Septate hyphae, conidiophores with annelids hyaline, branched.	*Scopulariopsis*
9	Small yeast-like colony milkish with a slimy surface	Single yeast cell undergoing budding.	*Cryptococcus neoformans*
10	Brown hairy elevated surface	Hyphae are septate, hyaline and conidia heads are radiates.	*Aspergillus tamarii*

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11	White coily elevated surface	Conidiophores with inflated branches at the top, conidia in chains.	*Penicilium marneffei*

Table 2. Morphological Characterization and identification of yeasts Isolates

Isolates Code	Cell morphology	Gram reaction	Oxidation and fermentation	Glucose	Fructose	Maltose	Lactose	Sucrose	Probable organism
1.	Oval and creamy	+	F	_	+	+	_	_	Torulopsis sp.
2.	Spherical and piented	+	_	_	_	_	_	_	Cryptococcus sp.

Table 3: Mean Counts for Total Heterotrophic Fungi

Station	Water sample (cfu/ml)	Sediment sample (cfu/g)
1. Afam street	$4.3x10^5$	$4.6x10^5$
2. Okija road	$4.2x10^5$	$3.5x10^7$
3. Olu-obasanjo road	$1.7x10^6$	$2.9x10^6$
4. Cherubim road	$4.2x10^6$	$5.3x10^6$
5. Abacha road	$3.8x10^4$	$5.7x10^4$

Table 4: Percentage (%) occurrence of fungal isolates

S/N	Organisms	No. of fungi Isolate	Percentage(%) of occurrence
1	*Cryptococcus neoformans*	28	36.4
2	*Rhizopus*	23	29.9
3	*Torulopsis glabrata*	8	10.4
4	*Penicillium chrysogenum*	6	7.8
5	*Aspergillus versicolor*	4	5.2
6	*Aspergilllus niger*	3	3.9
7	*Penicillium marneffei*	1	1.3
8	*Scopulariopsis*	1	1.3
9	*Phoma*	1	1.3
10	*Aspergillus tamarii*	1	1.3
11	*Mucor*	1	1.3

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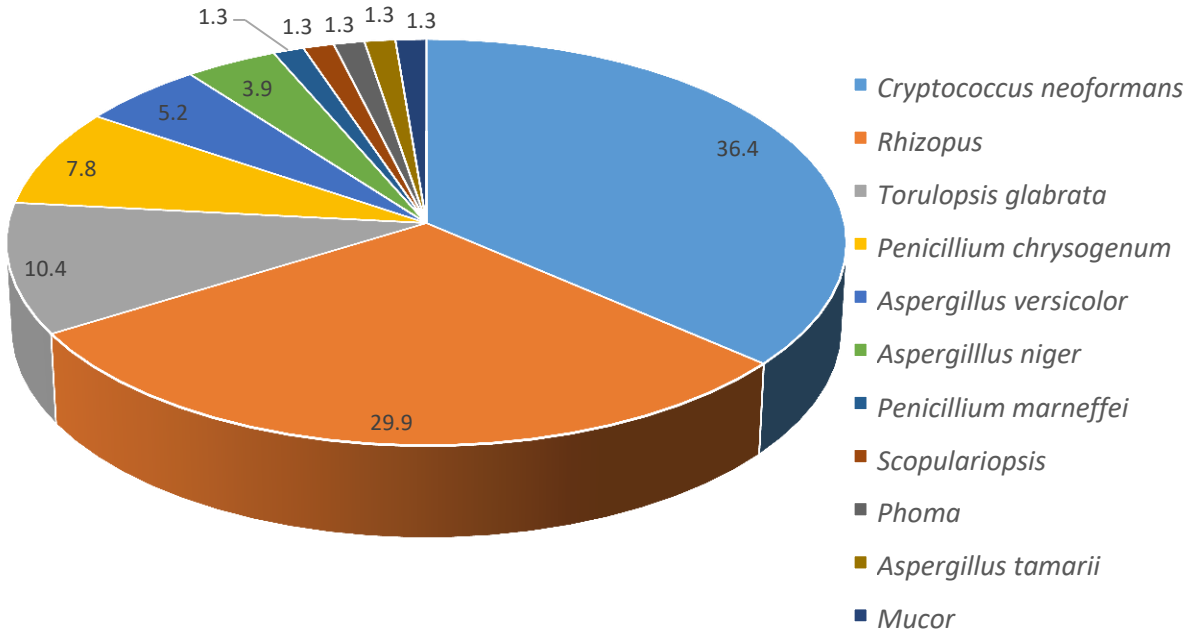
Fig 3. Percentage occurrence of individual isolates



Microbial counts for mean total fungal from five stations, both for water and sediment samples increased at Olu-Obasanjo and Cherubim Road stations with 1.7×10^{6} and 4.2×10^{6} cfu/ml respectively for water samples while for sediment samples , fungal counts had 2.9×10^{6} and 3.5×10^{7} cfu/g for Olu -Obasanjo and Okija Roads respectively. Cherubin Road had 5.3×10^{6} cfu/g while Afam recorded 4.6×10^{5} cfu/g for sediment (Table 3).

This study revealed that the presence of these species of microorganisms in wastewater indicates that there may be possible contamination by fungal pathogens (Prescott *et al.*, 2005) if anthropogenic and other industrial activities around such areas are not controlled. The source of contamination may also come from runoffs of fertilizers used on farms or sewage which contain excess nutrients that plants, algae, fungi can utilize for growth. Worrisome enough is the deleterious effect of polluted water effluent on quality of receiving water bodies which are manifold, as well as the volume of discharge, the chemical and biological concentrations or composition of the effluents. It also depends on the amount of suspended solids or organic matter or organic pollutants like heavy metals and organochlorines, and the characteristics of the receiving waters (Owuli, 2003). Most fungi especially the species isolated from these open drainage systems produce toxins in wastewater bodies and can cause health related problems such

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as gastroenteritis, liver damage, nervous system impairment, skin irritation and liver cancer in animals (EPA, 2000; Eynard *et al*., 2000; WHO, 2006). However, Refai *et al* (2010) reported that species of *Penicillium*, *Aspergillus* and *Rhizopus* are the normal mycoflora found in water bodies. Many of the fungal genera have virulence factor which cause various diseases under favourable predisposing environment. The role of ecology is also an important factor, which influence the diversity of fungus genera in the aquatic environment (Hussein *et al*., 2001]. According to Pailwal and Sati, (2009) diversity of water molds depends upon the interaction of physicochemical factors. It may be stressed that poor management of aquatic environments increases the chances of occurrence of diseases in such systems. Infection can also occur by ingestion of such contaminated water or inhalation of aerosols containing pathogens or contact of skin, mucous membranes, eyes and ears (WHO, 2006). Exposure to waste water treatment effluents containing estrogenic chemical can also disrupt the endocrine functioning of aquatic life, thereby causing permanent alterations in the structure and functioning of the reproductive systems (Liney *et al*., 2006).). Their presence in water bodies releases metabolic products such as hydrogen sulfide and nitrite or endotoxins which causes biofilms affecting the hygienic quality of water and also impairs the aesthetic quality by discoloration, turbidity and presence of odours (Harding *et al*., 2009) causing obstruction of water piping and pigments in water bodies (Paterson and Lima, 2005; Hussain *et al*., 2010).

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