Public Health Implications of Pathogenic Fungi Isolated from Open Drains in Port Harcourt

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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 faeces 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 to1.7x10^6 cfu/ml for waste water and 5.7x10^4 cfu/g to 2.9x10^6 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 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 faeces 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, Olu-Obasanjo 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).

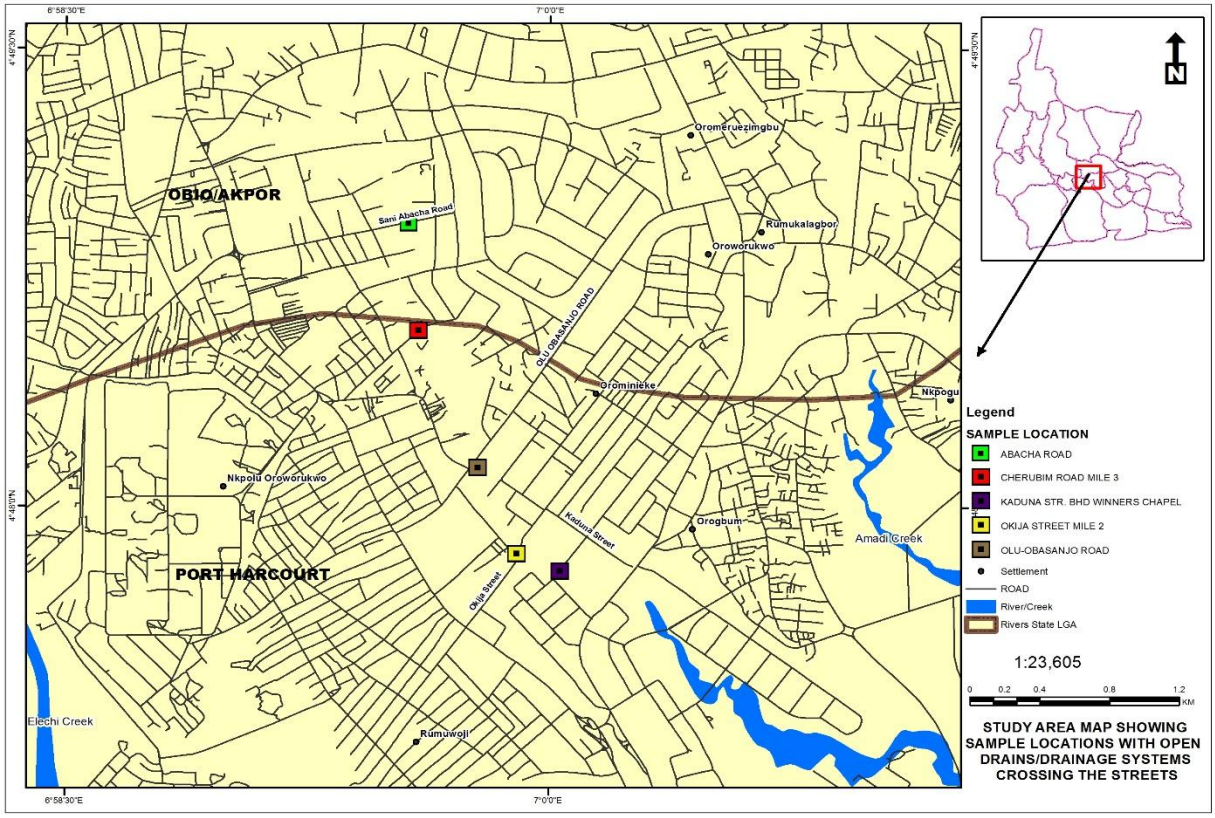


Fig 1. Map of Port Harcourt showing sampling locations along the Ntanwogba creek

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).

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 ± 2^0C 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+2^0C) 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 121^0C 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 37^0C 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	Septate hyphae, conidiophores	*Scopulariopsis*

	with white background	with annelids hyaline, branched.	
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*
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 pienting	+	_	_	_	_	_	_	*Cryptococcus sp.*

Table 3: Mean Counts for Total Heterotrophic Fungi

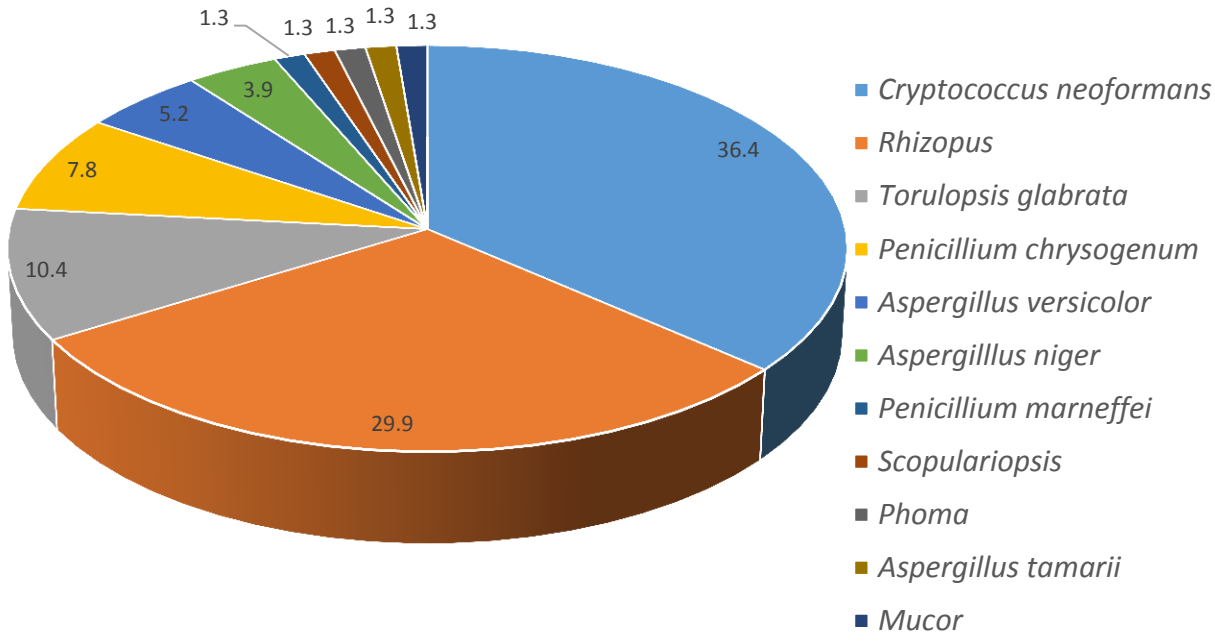
Station	Water sample(cfu/ml)	Sediment sample(cfu/g)
1. Afam street	$4.3x10^5$	$4.6x10^5$
2. Okija road	$4.2x10^5$	$3.5x10^7$
3. Olu-obasanjo road	$1.7x10^6$	$2.9x10^6$
4. Cherubim road	$4.2x10^6$	$5.3x10^6$
5. Abacha road	$3.8x10^4$	$5.7x10^4$

Table 4: Percentage (%) occurrence of fungal isolates

S/N	Organism	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

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 $1.7x10^6$ and $4.2x10^6$ cfu/ml respectively for water samples while for sediment samples, fungal counts had $2.9x10^6$ and $3.5x10^7$ cfu/g for Olu-Obasanjo and Okija Roads respectively. Cherubin Road had $5.3x10^6$ cfu/g while Afam recorded $4.6x10^5$ 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 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).

References

Aibor, M S and Olorunda, J O (2006). A Technical Handbook of Environmental Health in the 21st Century For professionals and students. 1st edition

Anaissie, E J; Stratton, S L; Dignani, M C; Lee, C; Summerbell, R C; Rex, J H; Monson, T P and Walsh, T J (2003). Pathogenic molds (including *Aspergillus* species) in hospital water distribution systems: a 3-year prospective study and clinical implications for patients with hematologic malignancies. Blood 101: 2542–2546.

APHA (American Public Health Association; American Water Works Association (AWWA); Water Environmental Federation (WEF)) 2012. *Standard Methods for the Examination of Water and Wastewater, 2*2nd ed.; American Public Health Association; American Water Works Association; Water Environmental Federation:

Besner, M C; Prévost, M and Regli, S (2011). Assessing the public health risk of microbial intrusion events in distribution systems: Conceptual model, available data, and challenges. *Water Resources.* 45: 961–979.

Bicki, T (2001). Onsite sewage disposal: the influence of system density on water quality**.** *Journal of Environmental Health.* 53(5):39-42

Burubai, W; Akor, A J and Lilly, M T (2007). Performance evaluation of a septic system for high water-table areas. *American Eurasian Journal of Scientific Research* 2(2): 112-116

Collins, C H and Lyne, M P (1976). *Microbiological Methods*. Butterworth and Co publishers Ltd., London, Boston. 524.

documents/2007_05_18_disinfection_tcr_whitepaper_tcr_biofilms.pdf

Demain, A.L and Davies, J.E (1999). *Manual of Industrial Microbiology and Biotechnology*, 2nd Ed, American Society for Microbiology Press, Washington D.C

Douterelo, I; Boxall, J B; Deines, P; Sekar, R; Fish, K E and Biggs C A (2014). Methodological approaches for studying the microbial ecology of drinking water distribution systems. *Water Resources.* 65, 134–156.

Ekugo, E I (1998). Public Health and Urban Sanitation. *Environmental News* 5, 7

EPA (Environmental Protection Agency) (2016). "Health Risk from Microbial Growth and Biofilms in Drinking Water Distribution Systems". Available online: *http://www.epa.gov/sites/production/files/2015-09/*

Ezzati, M; Utzinger, J; Cairncross, S; Cohen, A J and Singer, B H. (2005). "Environmental risks in the developing world: exposure indicators for evaluating interventions, programmes, and policies". *Journal of Epidemiology and Community* Health 59:15-22.

Environmental Protection Agency () (2000). Nutrient criteria technical guidance manual-rivers and Streams. *EPA-822-B-00-002. Washington DC.*

EPA (Environmental Protection Agency) (2002). "Ghana Landfills Guidelines", Accra, 2002.

Eynard, F; Mez, K and Walther, J L (2000). "Risk of cyanobacterial toxins in Riga waters (LATVIA)". *Journal of Water Research.* 30(11): 2979-2988.

Gobo, A E; Ubong, I U and Ede, P N (2008). "Relationship between rainfall trends and flooding in the Niger-Benue river basins". *Journal of Metrology.* 13:13

Hageskal, G; Lima, N and Skaar, I (2009**)**. "The study of fungi in drinking water". *Mycological Research.* 113**:** 165–172.

Harding WM, Marques LLR, Howard RJ, Olson ME. (2009). "Can filamentous fungi form biofilms? *Trends in Microbiology* **17 (11):** 475-480.

Harrigan, W. F and McCance, M. E (1976). *Laboratory Methods in Food and Dairy Microbiology*. Academic Press, London

Hussain, T; Ishtiaq, C M; Hussain, A; Mahmood, T; Sultana, K and Ashraf, M (2010). "Incidence of fungi in water springs of Samahni Valley, District Bhiimber, Azad Kashmir, Parkistan". *International Journal of Biology* 2**:** 94–101

Hussein, M M; Hatai, K and Nomura, T (2001). "*Saprolegniasis* in salmonids and their eggs in Japan". *Journal of Wildlife Diseases* 37, 204-207

Ifeoma, M and Nkiru, E (2009). Public Health Implication of Household Solid Waste Management in Awka South East Nigeria. *The Internet Journal of Public Health,* 1: (1)

Liney, K E; Hagger, J A; Tyler, C R; Depledge, M H; Galloway; T S and Jobling, S (2006). "Health Effects in fish of long-term exposure to effluents from wastewater treatment works". *Environmental Health Perspective* 114(S-1): 81-89.

Ochuko, U and Thaddeus, O (2013). Effect of underground on-site sewage disposal system on the quality of water from hand dug wells in the urban centre of Ughelli, Delta state Nigeria. *Standard Journal of Education and Essay* **1(6):** *8*1-90

Ogbonna, D N and Ideria, T J K (2014). "Effect of abattoir on the physico-chemical characteristics of soil and sediment in Southern Nigeria". *Journal of Scientific Research and Reports* 3 (12):1612-1632

Ogbonna, D N; Chilaka, S N; Gobo, A E and Amangabara, G T (2008a). "Effect of waste disposal practices and perennial flooding in Port Harcourt Metropolis, Nigeria". *Journal of Research in Bioscience* 4(3): 103-110

Ogbonna, D N Chilaka, S N; Gobo, A E and Amangabara, G T (2008b). "Health Implications of poor waste disposal practices and perennial flooding in Port Harcourt Metropolis, Nigeria Nigeria". *Journal of* Medical *and Pharmaceutical Science* 4(2): 87-94

Ogbonna, D N (2014). Distribution of Microorganisms in Water, Soils and Sediment from Abattoir Wastes in Southern Nigeria. *International Journal of Current Microbiology Applied Science* 3(9): 1183-1200

Oliveira, B R; Barreto-Crespo, M T; San-Romão, M V; Benoliel, M J; Samson, R A and Pereira, V J**.** (2013) **"**New insights concerning the occurrence of fungi in water sources and their potential pathogenicity". *Water Resources* 47: 6338–6347.

Oribhabor, B J (2016). "Impact of Human Activities on Biodiversity in Nigerian Aquatic Ecosystems". *Science International***.** 4: 12-20

Owaduge, S (2010). Solid waste management in Lokoja metropolis. Accessed online july *http:///www.greatestcities.com/users/owagde*

Owuli, M A (2003). "Assessment of impact of sewage effluents on coastal water quality in Hafnarfjordur, Iceland". *The United Nations Fishery Training Program,* Final Report.

Pailwal, P and Sati, S C (2009). "Distribution of Aquatic fungi in relation to physiochemical factors of Kosi River in Kumaun Himalaya". *Natural Science* 7(3), 70- 74.

Paterson, R R M and Lima, N (2005). "Fungal contamination of drinking water". In *Water Encyclopedia;* Lehr, J., Keeley, J.,Lehr, J., Kingery, T.B., III, Eds.; JohnWiley & Sons: New York, NY, USA, pp. 1–7.

Prescott, L M; Harley, J P and Klein, D A (2005). *Microbiology*. 6th ed. McGraw Hill, London.Pp. 135 -140.

Refai, M; Laila, K; Mohamed, A; Kenawy, M and Shimaa, E L SMS (2010). The assessment of Mycotic settlement of freshwater fishes in Egypt. *Journal of American Science* **6**(11): 595- 602 (2010).

Richardson, M D and Richardson, R (2015). *Aspergillus* and aspergillosis. *In Molecular Biology of Food and Water Borne Mycotoxigenic and Mycotic Fungi;* Paterson, R.R.M., Lima, N., Eds.; *Food Microbiology Series*; CRC Press: Boca Rotan, FL, USA, pp. 151–164.

Shafi, S; Kamili, A N; Shah, M A and Bandh, S A (2013). Coliform bacterial estimation: A tool for assessing water quality of Manasbal Lake of Kashmir, Himalaya. *African Journal of Microbiology Research* 7(31): 3996-4000

Sokari, T G; Yubedee, A G and Lugbe, P B (1996). Garri as an effective medium for certain Mycological studies. *Niger Delta Biologia*. 1(1): 86-89.

Van, P and Pur, A (1990). The importance of clean water to Industries in Developed World 4th ed. Hong Kong, Grovener Press

Warris, A; Klaassen, C H W; Meis, J F G; deRuiter, M T; deValk, H A; Abrahamsen T G;Gaustad, P and Verweij, P E (2003). Molecular epidemiology of *Aspergillus fumigatus* isolates recovered from water, air, and patients shows two clusters of genetically distinct strains. *Journal of Clinical Microbiology* 41: 4101–4106.

WHO (World Health Organization) (2006). Guidelines for the Safe Use of Wastewater, Excreta and Greater.Vol.3**.** *World Health Organization Press Geneva, Switzerland*.