CURRENT STUDIES IN COMPARATIVE EDUCATION, SCIENCE AND TECHNOLOGY, VOLUME 4, NUMBER 2, 2017, PP. 338-348

Microorganisms in a campus water tank and the effect on the quality of water and human health

BARANU, B. S. & OYIM, O. J.
Rivers State University, Port Harcourt, Nigeria

ABSTRACT Water is essential to life, but many people do not have access to clean and safe drinking water and many die of water borne infections. One of the fundamental needs of a community is to have an access to healthy and safe drinking water. This study focuses on isolating and identifying bacteria and fungi contaminants from the water used by students in Rivers State University in Port Harcourt, Nigeria and determining the effects of these microbes on the quality of the water. Six (6) water samples were taken (four (4) from four different hostels and two (2) from the sources supplying the hostels) and analyzed. The microbiological quality of the water samples were analyzed weekly for three (3) weeks. The study utilizes the wet mount technique using commercially prepared Potato Dextrose Agar (PDA) for the isolation of fungi, and the multiple tube fermentation technique with MacConkey broth and nutrient agar used as media for bacteria isolation. This study revealed that of the six (6) samples which were analyzed, the bacterial population isolated was higher than that of fungi. Based on the results obtained, it indicates a high fecal contamination ranging from 5×10^0 to 2×10^1 coliform (MPN) per 100ml. Also, the total heterotrophic bacterial count ranged from 4.05×10^6 to 1.14×10^7 with the fungal count being the lowest by having 7.0×10^4 to 1.8×10^5. *Aspergillus* species, *Penicillium* species, *Rhizopus* species, *Mucor* species, *Escherichia* species, *Enterobacter* species, *Bacillus* species, *Pseudomonas* species and *Klebsiella* species were the predominant isolates. The results of this study revealed that the water samples are not safe for human use. It is, therefore, necessary to treat water properly before human consumption.

Keywords: bacteria and fungi contaminants, water, human health, Microorganisms

Introduction

According to World Health Organization (WHO, 2014), only 36% of Nigerians have access to potable water and 6% have access to improved sanitation. An estimated 748 million people all over the world lack access to potable water and close to 2.5 billion persons are not provided with adequate sanitation (WHO, 2014). Microorganisms mainly found in water are bacteria and fungi (Moody, 2005). According to Sikoki and Veen (2004), any water body is a potential medium for the production of aquatic organisms but it has been noted that in rural areas, the careless disposal of commercial effluents and other wastes may contribute greatly to their poor quality of

their water which makes it not suitable for domestic activities (Chindah *et al,* 2004; Ugochukwu, 2004).

Waterborne pathogens transmit diseases to around 250 million people each year resulting in 10 to 20 million deaths around the globe (Zamxaka *et al*. 2004; Wilkes *et al*, 2009). The assessment of the microbiological quality of drinking-water aspires to protect consumers from illnesses due to the consumption of water that may contain pathogens such as bacteria, viruses and protozoa, thereby thwarting water-related illness outbreaks. Many studies (Liang *et al,* 2006; Hewitt *et al,* 2007; Maunula *et al,* 2009) have associated the outbreaks of waterborne gastroenteritis with a diversity of enteric bacteria and viruses, although recreational exposure to polluted water has often been more linked to viral infections (Vantarakis and Papapetropoulou 1999). The presence or absence of indicator organisms is fundamental to most drinking water quality guidelines, water supply operating licenses and agreements between bulk water suppliers and retail water companies (Colford *et al,* 2006).

Water intended for drinking, whether treated or not, must meet certain microbiological, physical and chemical standards (WHO, 2003). Any analytical result which does not meet the WHO standard warrants immediate treatment or the provision of alternative source of water supply (WHO, 2003). Water borne pathogens and chemicals when found in large amounts in a body of water can directly or indirectly limit man's legitimate use of that body of water, such as domestic, agricultural and recreational uses (Hagedorn *et al,* 1999). It is therefore necessary to supply safe drinking water free of substances that are harmful to health. WHO (2003), reported that 80% of sicknesses and deaths among children in the world are caused by unsafe drinking water.

The aim of the study is to identify the microorganisms (mainly bacteria and fungi) present in some water tanks of hostels in Rivers State University and their impacts on water quality and human health. The objectives of the study were to determine the microorganisms present in some water tanks of hostels in Rivers State University, to determine the microbiological parameters of the water and to evaluate the water quality of the water in the hostels.

Materials and Methods

Study Area and Sample Collection

The study area was Rivers State University main campus at Nkpolu-Oroworukwu in Port Harcourt. Water samples were collected from four hostels that were randomly selected and from the sources supplying water to the hostels. The hostels are Hostel C, Hostel E, Hostel F and Hostel H. The water sources are the water supplying tank in Estate and Works and water supplying tank on Road B. The water from these hostel tanks and water sources were labeled A, B, C, D, E, and F respectively. The water samples were collected using sterile containers. The tip of the tap was flamed so as to sterilize the external surface before collection. During collection, care was taken so as to avoid splashing the water on the body of the container. After collection, the containers were labeled and dated appropriately. The samples were collected three times a week from each hostel water tank and from the water sources for a period of three weeks.

Enumeration of Total Heterotrophic Bacteria and Fungi

One milliliter (1ml) of each of the water sample was pipetted into nine milliliter (9ml) of the normal saline and diluted serially up to 10^{-5} for all samples. Aliquots (0.1ml) of 10^{-1} to 10^{-5} of each sample were inoculated into freshly prepared nutrient agar (NA) and potato dextrose agar (PDA) plates in triplicate; and the plates were labeled accurately. The spread plate method was used to ascertain the total heterotrophic bacteria and Fungi. A sterile glass rod was used to spread the sample evenly on both the nutrient agar and potato dextrose agar plates. The nutrient agar plates were incubated at 25^0C for 24 hours while the potato dextrose agar plates were incubated at 25^0C for 48 hours to obtain discrete colonies. The colonies that grew on both the nutrient agar and potato dextrose agar plates were counted and the average was taken and recorded as total heterotrophic counts of aerobic bacteria and fungi.

With regards to color, texture, size, elevation, transparency and colonial characteristics of the colonies, pure cultures were obtained by sub-culturing each discrete colony onto freshly prepared NA and PDA plates for both the bacteria and fungi respectively. The plates were incubated at 25^0C for 24 and 48 hours for bacteria and fungi respectively. The pure cultures when grown were stored in bijou bottles containing 2ml glycerol at 4^0C for further biochemical tests.

Bacteriological analysis of water samples

The bacteriological analysis involves the use of multiple tube method for the water analysis. This technique is called the most probable Number (MPN) technique. It comprises of three (3) standard steps: Presumptive, confirmed and completed tests.

Characterization and Identification of Bacteria isolates

The colonies that grew in the nutrient agar plates were characterized based on the following standard bacteriological tests; Gram Staining, Motility Test, Biochemical Tests, Catalase Test, Oxidase Test, Coagulase Test, Citrate Test, Starch Hydrolysis, Urease Production, Methyl Red-Vogues Proskauer (MRVP), and Sugar Fermentation Test. The isolates were presumptively identified based on the results of their cultural, microscopic and biochemical characteristics, and with comparison with those of known taxa of Bergey's manual of determinative bacteriology (1999).

Isolation of pure fungal cultures

Discrete fungal colonies were sub cultured in order to obtain pure colonies. A subculture was done using an inoculating needle which was used to cut part of the fungal colony and transferring aseptically onto fresh already prepared SDA plates. These were incubated at room temperature for 5days. The pure cultures obtained were then stored in the refrigerator for further studies.

Characterization of pure fungal isolates

For qualitative detection of isolated fungal species, routine macroscopic and microscopic procedures were performed. In the macroscopic examination, the colony structure was observed, nature of growth, aerial & substratum region were also observed for colour. In the microscopic examination, two drops of lactophenol cotton blue was used to stain the center of a sterilized clean glass slide, and then a small portion of the fungal isolate from the sub-cultured plate was transferred unto the

slide, using an inoculating needle and covered with a coverslip. This was examined under the microscope first at low power (×10) and then at high power (×40). The fungal features noted were the;

- Somatic structure.
- Reproductive structure
- Vegetative structure
- Structure of hyphae
- Colony colour
- Structure of Colony margin
- Surface appearance

Results

The result of the mean total aerobic heterotrophic bacterial count in all the tank water samples is shown in Table 1 below. The count ranged from 4.20×10^6 CFU/ml to 1.83×10^7 CFU/ml for all the samples. Sample B recorded the highest bacterial count while sample F recorded the lowest count.

Table 1: Average total aerobic heterotrophic bacterial counts of the tank water samples

Water Sample	Week 1	Week 2	Week 3
A	1.04×10^7	7.30×10^6	4.20×10^6
B	1.83×10^7	1.08×10^7	5.09×10^6
C	1.38×10^7	5.08×10^6	3.99×10^6
D	4.33×10^6	4.36×10^6	3.64×10^6
E	5.35×10^6	5.66×10^6	4.21×10^6
F	6.27×10^6	3.46×10^6	2.41×10^6

The result of the mean total fungal count in all the tank water samples is shown in Table 2 below. The count ranged from 7.0×10^4 CFU/ml to 4.0×10^5 CFU/ml for all the samples. Sample F recorded the highest fungal count while sample C recorded the lowest count.

Table 2: Average total fungal counts of the tank water samples

Water Sample	Week 1	Week 2	Week 3
A	1.3×10^5	1.9×10^5	8.0×10^4
B	1.5×10^5	3.0×10^5	3.0×10^5
C	7.5×10^4	8.0×10^4	8.5×10^4
D	1.4×10^5	8.5×10^4	1.9×10^5
E	7.0×10^4	1.2×10^5	2.0×10^5
F	1.8×10^5	4.0×10^5	3.4×10^5

The average estimated total coliform bacterial count from the MPN index per 100ml of the water samples that indicated the presence of acid and gas in the tubes is shown in Table 3 below.

Table 3: Average Estimated Total coliform Bacterial count in the tank water samples

Water Sam-ple	Total Coliform Bacteria (MPN /100ml)		
	Week 1	Week 2	Week 3
A	9	9	9
B	26	14	11
C	14	9	11
D	22	17	14
E	9	11	7
F	11	9	5

The bacterial genera isolated in this study were identified as *Escherichia*, *Enterobacter*, *Klebsiella*, *Bacillus*, *Salmonella* and *Pseudomonas*. While the fungal genera and species were identified as *Aspergillus niger, Penicillium chrysogenium, Penicillium sp, Rhizopus sp, Mucor sp,* and *Aspergillus sp.*

The frequency of isolation (%) of bacterial isolatesfrom the tank water samples is shown in Figure 1. The highest frequency in Sample A is *Escherichia* sp and the lowest is *Bacillus.* The lowest for Sample B is *Bacillus* spp and the highest is *Escherichia* spp. while the lowest in Sample C is *Pseudomonas s*pp and *Bacillus* spp and the highest is *Escherichia* spp. From the table below, it is seen that *Escherichia* spp has the highest occurrence in all samples.

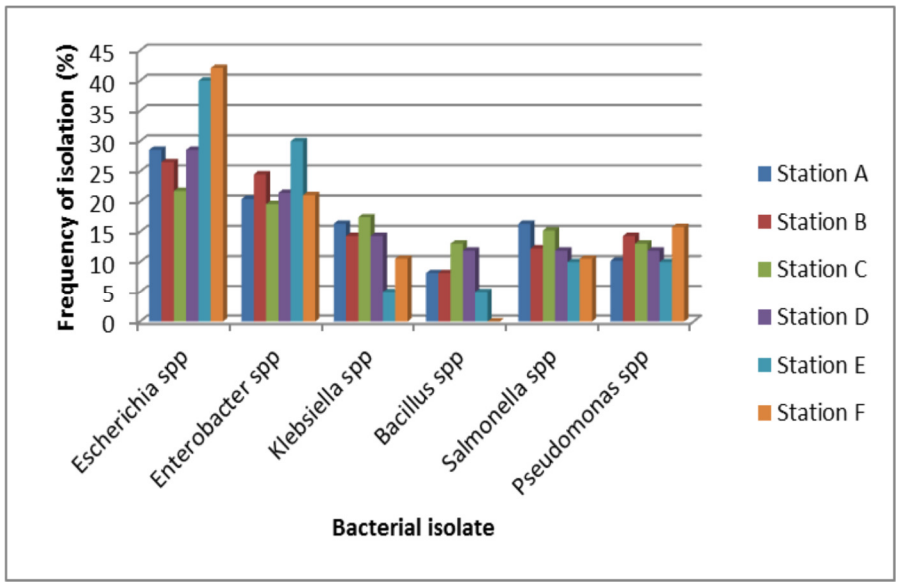

Fig 1: 4: Frequency of isolation of bacterial isolates in the tank water samples

TABLE 4.7: Frequency of occurrence of fungi isolated from the tank water samples

ISOLATE	FREQUENCY	PERCENTAGE (%)
Aspergillus niger	3	12
Penicillium chrysogenium	5	20
Rhizopus sp	4	16
Mucor sp	3	12
Aspergillus sp	4	16
Penicillium sp	6	24
TOTAL	25	100

Discussion

From Table 4.1, it is seen that the amount of total coliform bacteria is high ranging from 5 x 10^0 to 26 x 10^1 coliform (MPN) per 100ml. this indicates high fecal contamination and does not meet the required standard. However, the World Health Organization (WHO) stated that water intended for human consumption should contain no indicator organism (WHO, 2006). The contamination of the water samples by coliform could be through broken underground pipes when the pressure within the pipes becomes lower than that outside; thus, making the bacteriological quality of the wa-

ter samples to be unsatisfactory. The total heterotrophic bacteria (THB) counts ranged from 4.05 x 10^6 to 1.14 x 10^7 cfu/ml with sample B (Hostel E) having the highest. The bacterial genera isolated in this study were *Escherichia, Enterobacter, Klebsiella, Bacillus, Salmonella* and *Pseudomonas*. The potential health effects that may be caused by these bacterial genera include abscesses, ulcers, food poisoning, inflammation of breast and conjunctivitis in new born, nausea, vomiting, diarrhea, urinary tract infections, appendicitis, meningitis, abdominal pain, pneumonia and bacteriaemia (Cheesbrough, 2000;WHO, 2011).

The fungi isolated from the water samples were *Aspergillus niger, Penicillium chrysogenium, Penicillium sp, Rhizopus sp, Mucor sp,* and *Aspergillus sp.* This is an indication that the water samples are not well treated. It could also be that the use of chlorination as a chief purification procedure, which has remained dogmatic in the treatment of water is probably not suitable to eliminate fungi. De Maria (1996) and Oni (2001) independently reported that purification procedures such as chlorination do not eliminate fungal spores, which implies that perhaps the treatment given to our sachet water is usually not effective enough to eliminate these microorganisms. Also, Gunhild et al. (2006) suggested that several mold species survive disinfection and water treatment and could thus contaminate the water after sometime.

Among the water samples, sample F has the highest fungi counts (3.1 x 10^5) indicating that it is the most polluted. Furthermore, all the water samples examined showed evidences of contamination with 2, 3 or more species of fungi (Table 4.7). Hence, the presence of fungi in the water samples probably indicates poor treatment techniques. Also, poorly designed septic tanks, poor drainage, human waste water disposal and poor sanitation (WHO, 2006; Adriano and Joana, 2007) can add endangering fungi to the water.

The genus Aspergillus which was isolated is known to produce aflatoxins (B1, B2, G1 and G2), the most toxic and potent hepatocarcinogenic natural compounds ever characterized (Bennett and Klich, 2003). This fungi genus cause a wide range of diseases in humans, ranging from hypersensitivity reactions to invasive infections associated with angio invasions (Bennett and Klich, 2003). Penicillium species were mostly abundant and they are known to cause allergy, asthma and some respiratory problems (Cooley et al., 1998; Frisvad et al., 1998 and Gunhild et al., 2006). The genus Mucor is known to be a major cause of thrombosis, infarction, nasal or paranasal sinus infection and GI disorders.

Although it is unlikely that concentrations as low as those reported in this study can cause fungal infection in healthy people, immunosuppressed persons are however at risk of infection. Kanzler *et al*., (2007) suggested that routine microbiological investigations should be made in hospitals or institutions where immunosuppressed individuals are treated.

The areas sampled in this study are the most populated hostels in the University, which means the number of visitors will also be higher than other hostels. This implies that whatever infection these hostels under study are exposed to is significant relative to the total population of majority of the student on campus.

Conclusion

Six (6) fungal species and six (6) bacterial genera were isolated from the water samples. The predominant fungal genera identified were *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor*. Whereas the predominant bacterial genera associated with the water samples were Escherichia, *Enterobacter*, *Klebsiella*, *Bacillus*, *Salmonella*, and *Pseudomonas*. The presence of these fungal and bacterial genera poses a significant threat to water quality and may be hazardous to human health. Thus, the results of the water quality analyses reveals that the microbiological parameters analyzed in the water samples were not within the acceptable water quality standards and therefore indicate the existence of pollution in the water samples studied. This implies that consumption of water from these tanks by students and staff of the University may lead to water borne infections which if not detected in time or poorly managed may cause death.

Recommendations

The presence of indicator bacteria and some fungal genera shows contamination of the water samples. It is therefore recommended that; Treatment of the water and periodic monitoring should be enforced, waste materials should not be dumped near the reservoir and water tanks, and the underground pipes should be monitored regularly to detect leakage.

Correspondence
Baranu, B. S.
Department of Microbiology
Rivers State University, P.M.B 5080
Port Harcourt, Nigeria
E-mail: barisiale.baranu@gmail.com; out.oyim@ust.edu.ng

References

Adetunde, L.A and Glover, RLK. (2010). Bacteriological quality of borehole water used by students of University of Development Studies, Navrongo campus in Upper east region of Ghana. Current Research *journal of Biological Sciences,* **2**(64) 361-364.

Adriano, A.B. and Joana, S.B. (2007). The quest for safe drinking water: An example from Guinea-Bissau (West Africa). *Water Resources* **41**: 2978-2986.

Akpoveta, O.V., Okoh, B.E. and Osakwe, S.A. (2011). Quality Assessment of Borehole Water used in the Vicinities of Benin, Edo State and Agbor, Delta State of Nigeria. *Current Research in Chemistry,* **3**: 62-69.

Anaissie, E.J., Stratton, S.L., Dignani, M.C., Summerbell, R.C., Rex, J.H., Monson, T.P., Spencer, T., Kasai, M., Francesconi, A. and Walsh, T.J. (2002). Pathogenic *Aspergillus* species recovered from a hospital water system: a 3-year prospective study. *Clinical Infectious Diseases*, **34**: 780-789.

Ashbolt, N.J., Grabow, W.O. and Snozzi, M. (2001). Indicators of microbial water quality. World Health Organization. *Assessing Microbial Safety of Drinking Water*. 293–295.

Baroni, L., Cenci, L., Tettamanti, M. and Berati, M. (2007). Evaluating the environmental impact of various dietary patterns combined with different food production systems. *European Journal of Clinical Nutrition*. **61** (2): 279–286.

Bennett, J.W. and Klich, M. (2003). Mycotoxins. *Clinical Microbiology Review*. **16**: 497-516.

Bonde, G.J. (1977). Bacterial indicators of water pollution, in Advances in Advanced Aquatic Microbiology, (eds M.R. Droop and H.W. Jamasdi). Academic Press Inc., London.

Bonde, G.J. (1977). Bacterial Indicators of Water Pollution. In Advances of aquatic Microbiology Vol 1. H.W. Academic press Inc. (London) ltd. 273-356.

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries, Pt- 2. Cambridge University Press, 64-65.

Chindah, A.C. (2003) .Effects of tankfarm wastewater on water Quality in the Lower Bonny River, Niger Delta. *Journal of Nigerian Environmental Society, **2** (2), 210*-214

Cooley, J.D., Wong, W.C., Jumper, C.A. and Straus, D.C. (1998). Correlation between the prevalence of certain fungi and sick building syndrome. *Occupational Environmental Medicine*. **55**: 579-584.

De Hoog, G.S., Mayser, P., Haase, G., Horré, R. and Horrevorts, A.M. (2000). A new species, *Phialophora europaea*, causing superficial infections in humans. *Mycoses*, **43** (11-12): 409-416.

DHSS, Department of health and Social Security (1977). The Biological Examination of Water Supplies. *Department of Health and Social security*. Report No. 71 London.

Eguabor, V (1998). Strategies for Teaching water pollution in secondary schools. STAN Mycological Research, 113: 165-172.

Environmental Services. (2003). Environmental Fact Sheet. Available: http:// des.nh.gov/organization/commissioner/pip/factsheets/wwt/documents/web-18.pdf.

Fenwick, A. (2006). Waterborne Diseases—Could they be Consigned to History? Science.; 313:1077–1081.

Frisvad, J.C., Bridge, P.D. and Arora, D.K. (1998). Chemical fungi taxonomy. Marcel Dekker, Inc., New York, N.Y.

George, M., Garrity, J., Bell, A. and Timothy, G. (2004). Bergey's manual of systematic bacteriology. *Taxonomic outline of the prokaryotes bergey's manual of systematic bacteriology*. **1** (1) 45-212.

Gerardi, M.H. and Mel, C.Z. (2005). Wastewater Pathogens. *Wastewater Microbiology Series*. Hoboken, NJ: John Wiley & Sons, Inc. 147

Graun, G.F. and Maccabe, L.J. (1983). Review of the cause of waterborne disease outbreaks. *Journal of American Water Works association.* **65:** 71-81.

Gunhild, H., Ann, K.K., Peter, G., De Hoog G. and Ida, S. (2006). Diversity and significance of Mould species in Norwegian drinking water. *Applied and Environmental Microbiology* **72**(12): 7586-7593.

Hagedorn,C. and Robinson, R.B., Jr. (1999). Determining Sources of Faecal Pollution in Rural Virginia. Watershed With Antibiotic Resistance Patterns in Faecal *Streptococci. Applied and Environmental Microbiology. **65**:5522-5531*

Hageskal, G., Lima, N. and Skaar, I. (2009). The study of fungi in drinking water. *Journal of Environmental Education series* (2), 49

Hayette, M.-P., Christiaens, G., Mutsers, J., Barbier, C., Huynen, P., Melin, P. and de Mol, P. (2010). Filamentous fungi recovered from the water distribution system of a Belgian university hospital. *Medical Mycology*, **48**: 969-974.

Kimura, M., Goto, A., Furuta, T., Satou, T., Hashimoto, S. and Nishimura, K. (2003). Multifocal subcutaneous phaeohyphomycosis caused by *Phialophora verrucosa*. *Archives of Pathology and Laboratory Medicine*, **127** (1): 91-93.

Kulshreshtha, S.N (1998). A Global Outlook for Water Resources to the Year 2025. *Water Resources Management.* **12** (3): 167–184.

Larry, (2006). World Water Day: A billion people worldwide lack safe drinking water. http://environment.about. com/od/environmental events/a/ waterdayqa.htm,March 22$_{nd}$, 2006.

Medema, G.J., Payment, P., Dufour, A., Robertson, W., Waite, M., Hunter, P., Kirby, R. and Anderson, Y. (2003). Assessing Microbial Safety of Drinking Water Improving Approaches and Method. WHO & OECD, IWA Publishing; London, UK. Safe drinking water: an ongoing challenge. 11–45.

Olaniran, N.S. (1995). "Environment and Health: An Introduction", in Olaniran, N.S. etal (Ed) Environment and Health. Lagos. Macmillan Nig. Pub. Co for NCF, 34-151.

Prescott M, Harley JP, Klein DA (2005). Microbiology 3rd ed., WMC Brow Publishers, New York, p. 935.

Rivers State University of Science and Technology. (2016). In Wikipedia, The Free Encyclopedia. https://en.wikipedia.org/w/index.php?title=Rivers_State_ University_of_Science_and_ Technology&oldid=728877810

Seas, C., Alarcon, M., Aragon, J.C., Beneit, S., Quiñonez, M., Guerra, H. and Gotuzzo, E. (2000) Surveillance of Bacterial Pathogens Associated with Acute Diarrhea in Lima, Peru. *International Journal Infectious Diseases*; **4**:96–99

Sikoki, F.D. and Veen, F. (2004) Aspects of water quality and the potential for fish production of Shinro Reservoir. *Nigeria Living System Sustainable development*, **2**:1-7.

Tamiskar, J., Naidu, J. and Singh, S.M. (2006). Phaeohyphomycotic sebaceous cyst due to *Cladosporium cladosporioides*: a case report and review of literature. *Journal of Medical Mycology*, **16** (1): 55-57.

United Nations. (2008). *The Millennium Development Goals Report.* Available: http://mdgs.un.org/unsd/mdg/Resources/Static/Products/Progress2008/MDG_Report_2008_En.pdf#page=44. Last accessed 16th August, 2016.

Vanittanakom, N., Cooper, C.R., Fisher, M.C. and Sirisanthana, T. (2006). *Penicillium mareneffei* infection and recent advances in the epidemiology and molecular biology aspects. *Clinical Microbiology Reviews*, **19** (1): 95-110.

Webster.com. (2016). Definition from Webster Dictionary 08-13

WHO (2006). *WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater*. Geneva, Switzerland: World Health Organization (WHO). 23-42.

WHO (World Health Organization) (2008) Guidelines for Drinking-water Quality, Incorporating 1st and 2nd Addenda, Volume 1, Recommendations. 3rd ed. WHO; Geneva, Switzerland.

Wilkes, G., Edge, T., Gannon, V., Jokinen, C., Lyautey, E., Medeiros, D., Neumann, N., Ruecker, N., Topp, E. and Lapen, D.R. (2009). Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Resources*. **43**:2209–2223.

World Health Organization (2003). *Guidelines for Drinking Water Quality* 3rd Edition. Geneva: World Health Organization. 23-54.

World Health Organization (2004). *Guidelines for Drinking-water Quality*. London: World Health Organization. 225-250.

Zamxaka, M., Pironcheva, G. and Muyima, N.Y.O. (2004). Microbiological and physico-chemical assessment of the quality of domestic water sources in selected rural communities of the Eastern Cape Province, South Africa. Water SA. **30**:333–340.