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Research Paper

Isolation and identification of fungi associated with drinking water from various sources in Sokoto metropolis

[1]Umar, S., [2]Baki, A. S. and *[3]Bello, A.

[1]College of Life Science and Technology, Beijing University of chemical technology, Beijing,100029,China.
[2]Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University, Sokoto State, Nigeria.
[3]Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto State, Nigeria.
*Corresponding author E-mail: abccrcfge28@gmail.com

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The study was aimed at isolation and identification of fungi present in drinking water collected a Sokoto metropolis. A total of twenty (20) samples were collected from 4 different areas. Fungi, isolation were identifies using colonial and morphological characteristics. The results obtained indicated that fungal count range from $1.1x10^4$ to $8.0x10^3$cfu/ml. Six (6) species of fungi which include *Aspergillus niger, Aspergillus oryzae, Mucor racemosus, Rhizopus stololnifer, Aspergillus flavus and Talaromyce flavus* of fungi, isolated from drinking water indicated that *Aspergillus niger* with (39.28%) had the highest rate of occurrence followed by *Aspergillus aryzae (17.85%), Aspergillus flavus (17.85-%), Mucor racemosus (10.71%), Rhizopus stolonifer (7.14%) and Taloromyce flavus (7.14%)* which had the least rate of occurrence. It was observed from this study that all the isolated fungi were potential pathogens that can be deleterious to human health.

Keywords: Isolation, identification, fungi, drinking water, *Aspergillus niger*

INTRODUCTION

Biofilm is made up of aggregates of micro organism, such as bacteria, fungi, diatoms, protozoa, algae and any exogenous materials, which are embedded in a hydrated extracellular matrix and attached to a solid surface (pipe, ship hall, teeth, lung, etc). Water is one of the most abundant and essential commodities of man occupying about 70% of the earth's surface, yet greater percentage of the world's population, most especially in developing countries live without access to safe water (Adriano and Joana, 2007). Nigeria for example, is located on coastal West Africa where water is abundant; most of the population lacks adequate and safe drinking water. Biofilms playing a major role in drinking and waste water treatment processes due to their enhanced properties of mineralization, bioaccumulation the organism form assemblages which are irreversibly associated with a surface and enclosed in a matrix of extracellular polymeric substance (EPS) of their own origin which form matrix (Donlan, 2002).

Water quality is crucial for both household and food industry. Many problems in drinking water distribution system are microbial including biofilm growth microbial mediated corrosion and persistence of pathogens (Donlan, 2002). Biofilms may be formed on variety of surfaces, in living tissues, indwelling medical devices, industrial or potable water piping system, or normal aquatic ecosystems. They are suspected of being the primary source of microorganisms in water distribution systems. In addition to health related importance, microbial growth in destruction systems can cause taste and odour problems, which can be contributed by the substances produced by some species of fungi (Adriano and Joana, 2007). Understanding the effect of the environmental conditions on these fungi may help to

improve distribution system management strategy. The mature and concentration of biodegradable compounds present in treated drinking water as the water temperature are some of the factors contributing to biofilm formation in potable water system. In this study the effect of phosphorus concentration, temperature, light and darkness, on the biofilm forming potential of moral these seature have recently been reviewed Flemming, (2008) and Flemming and Wingender, (2010). For long time, biofilms were considered literally as a side issue and they experienced little awareness, although they were a common sight all the time. Their relevance for environmental processes as well as in medicine and public hygiene has gained attention only in past few decades, since then, sophisticated methods have been introduced in to biofilm research such as fluorescence microscopy and confocal laser scanning ceroscopy analysis, and most powerful, molecular biology (Flemming, 2008). When cases of illness are registered, epidemiological studies are conducted in order to demonstrate similarities in heretic profiles of strains isolated from clinical and environmental specimens, to track the source of infection. Drinking water and associated biofilms are often among the prime candidates tested when gastrointestinal diseases and different types of infections are recorded. All this has allowed investigating biofilm biology in much greater detail and, thus, taking views of the life of microorganism in the real world Stewart, (2008). Nigeria is focused on the occurrence and significance of bacteria with little attention to other microorganisms such as fungi. Even in drinking water pathogenic contamination and disease outbreaks may occur demonstrating the imperative requirement for comprehensive water safety plans implementation, (Szewzyk *et al*., 2000; Flemming and Wingender, 2010).

Drinking water associated biofilm induce aesthetic problems consisting in colour, odour and taste degradation due to chemical compound release and more important, they pose a threat to human and animal health by hosting pathogenic or toxins producing bacteria, viruses, protozoa, algae, fungi and invertebrates. All of them share common feature and take substantial ecological benefits from these structures, the great majority of water related health problem are the result of microbial contamination (Riley *et al*., 2011). Naturally occurring biofilms contact with drinking water were identified and described as microbial reservoirs for further contamination (Szewzyk *et al*., 2000; Flemming and Wingender, 2010). Fungal infections are becoming more and more important because of increasing numbers of immunesuppresed patient. None less, water borne fungi is associated with taste and odour problems, contamination of food and beverage preparation and in a variety of health related effects. Opportunistic pathogens, potentially causing superficial or systemic infections, allergenic or toxigenic species of fungi (Yeasts and moulds) have been Isolated from drinking water worldwide (Nagy and Olson, 2011). This study was aimed at determining the microbiological quality of drinking water in Sokoto Metropolis and was achieved with the following objectives:

(a) To determine the occurrence, distribution and significance of mould species in selected public drinking water in Sokoto metropolis.
(b) To determine and analyzed the biofilms potential of mould and filamentous fungi in drinking water.
(c) To determine the microbiological quality of drinking water.

MATERIALS AND METHODS

Sample collection

Twenty (20) samples of water were collected from four different areas within Sokoto metropolis viz: Bado, Arkilla, Gidan Dare and Kwakwalawa. Standard method described by American public Health Association (APHA, 1999), was adopted. 300 ml of water was poured aseptically in to 300 ml sterilized bottle. For tap water and borehole water, the samples were collected by allowing the water to run to waste for 2 or 3 min and then the water were aseptically collected in sterile bottles. Water from wells was collected in a sterilized bottle fitted with a weight at the base. Water from river was collected by dipping the sterilized bottle into the water. The entire collected samples were then labelled accordingly and were taken to the laboratory for analysis.

Sample processing and analysis

Nine milliliters of distilled water was poured with the aid of sterilize syringe into the three test tubes each per sample and autoclaved at 121°C for 15 min, 1 ml of each sample was transferred into the already sterilized tubes thereby making it to be homogenized (10^{-1}, 10^{-2}, 10^{-3}) respectively for the whole number of samples. The last (1 ml) of the final tube was discarded. The essence is to reduce the microbial load present in the sample, (Adrano and Joana, 2007):

Isolation and identification of fungi

Colonies growing on the SDA plate, after were sub cultured on to another freshly prepared sterile SDA plates for identification test.

During identification, a drop of lacto-phenol blue was placed on a microscope slide. Using sterile inoculating needle, a small portion of fungal growth was gently removed and placed of lacto-phenol blue and then gently spread it out with two dissecting needles so that it can easily be identified when viewing and covered with a cover glass and then examined microscopically. The slide was placed on a stage using x10 objective lens, the fungal structure was observed and fungi were identified (Chei *and* Kalhatkar*,* 2000).

Anova

A complete randomized design (CRD) f – test was used to compare between the mean colony counts of different source of drinking water. Null hypothesis says there is significant difference between the mean colony counts of different sources of drinking water while alternative hypothesis says there is no significant difference between the mean colony counts of different source of drinking water. If the calculated f – value is greater than tabular value then there is significant difference between the various sources of drinking water. But if the tabular value is greater than calculated f – value then there is no statistically significant difference between the various sources of drinking water. Therefore, the result is not significant different.

RESULTS AND DISCUSSION

The results obtained from this study showed that all the fungi isolated were present in drinking water sample collected from Sokoto metropolis. Pure cultures obtained from the growth of the samples were subjected to microscope for identification and fungi identified from 20 samples of drinking water were *Aspergillus niger, Aspergillus oryza, Aspergillus flavus, Mucor recemosus, Talaromyce flavus and Rhizopus stolonifer*. The mean fungal count of the fungi isolate is presented in (Table 1). The sample D had the highest fungal count of 8.0 x 10^3cfu/ml and sample C had the least count of 1.1 x 10^4. The fungal isolated and identified in this research are

Table 1. Fungal colony count cfu/ml in the different samples of drinking water.

Sample	Fungal colony counts	Fungal counts (cfu/ml)
A	18	$1.8x10^4$
B	15	$1.5x10^4$
C	11	$1.1x10^4$
D	8	$8x10^3$

Key A = Bado; B = Gidan dare; C = Arkilla; D = Kwakwalawa

Table 2. Percentage frequency of occurrence of fungi isolated from drinking water sample collected at Bado.

Isolated organisms	Frequency of occurrence	Percentage(%) of occurrence of the organisms
Apergillus niger	3	50
Aspergillus oryzae	2	33.33
Mucor racemosus	1	16.67
Total	6	100

presented in (Table 2) showing *Aspergillus niger, Aspergillus oryzea a*nd *Mucor racemosis* respectively. The percentage frequency of the fungal isolates in Arkilla area are shown in (Table 3) and *Mucor racemosis* had the highest percentage frequency of occurrence of (40%) and *Aspergillus niger*, *Aspergillus oryzea* and *Stolonifer rhizypus* had the least percentage frequency occurrence of (20%). Table 4 represented the percentage frequency of occurrence of fungi from drinking water analysis which includes three (3) species of fungi. *Aspergillus flavus* (33.33%), *Aspergillus niger (*50%) and *Talaromyces flavus* (16.67%). Table 5 indicated the percentage frequency of occurrence of fungi isolated from drinking water collected at kwakwalawa which include five (5) species of fungi. *Aspergillus oryzae* (18.18%), *Aspergillus flavus* (27.27%), *Aspergillus niger* (36.40%), *Talaromyces flavus* (9.10%) and *Rhizopus stolonifer* (9.10%). The percentage frequency of occurrence of fungi isolate in all area are show in (Table 6) and *Aspergillus niger* had the highest percentage frequency of occurrence of (39.28%) followed by *Aspergillus oryzae* (17.85%), *Aspergillus flavus* (17.85%), *Mucor racemosus* (10.71%), *Rhizopus stolonifer* (7.14%) and *Talaromyce flavus* (7.14%) had the least rate of occurrence.

In this study the most frequently isolated fungi was genus *Aspergillus*. These findings are consistent with works conducted by Arvanitidou *et al*., (1990, 2000) and Gunhild *et al*. (2006), that *Aspergillus* was the most commonly isolated generally in water. *Aspergillus* are known to produce aflatoxins (B1, B2, G1 and G2), the most toxic and potent hepatocarcinogenic natural compounds ever characterized (Bennett and Klich, 2003). These fungi cause a wide range of diseases on humans, ranging from hypersensitivity reactions to invasive infections associated with angio-invasions, *A. niger, A. oryzae* and *A. Flavus* where found on several occasions during this study. The finding of *A. niger* in agreement with the work conducted by (Hageskal *et al.,* 2006), in which he also reported the frequency occurrence of *A. niger* in drinking water. However, the presence of *A. Oryzae* and *A. fumigatu* was not consistent with the work of (Okpako *et al.,* 2009), in that he reported the frequency

Table 3. Percentage frequency of occurrence of fungi isolated from water sample collected at Arkilla.

Isolated organisms	Frequency of occurrence	Percentage(%) of occurrence of the organisms
Aspergillus niger	1	20
Aspergullus oryzae	1	20
Mucor racemosus	2	40
Rhizoipus stolonifer	1	20
Total	5	100

Table 4. Percentage frequency of occurrence of fungi isolated from Gidan Dare drinking water.

Isolated organisms	Frequency of occurrence	Percentage(%) of occurrence of the organisms
Apergillus flavus	2	33.33
Aspergillus nigers	3	50
Talaromyces flavus	1	16.671
Total	6	100

Table 5. Percentage frequency of occurrence of Fungi isolated from sample collected at Kwakwalawa river.

Isolated organisms	Frequency of occurrence	Percentage(%) of occurrence of the organisms
Aspergillus oryzae	2	18.18
Aspergillus flavus	3	27.27
Aspergillus niger	4	36.40
Talaromyces flavus	1	9.10
Rhizopus stolonifer	1	9.10
Total	11	100

Table 6. Percentage frequency of occurrence of fungi isolated from drinking water sample representing all collection areas.

Isolated organism	Frequency of occurrence	Percentage (%) of occurrence of the organisms
Aspergillus niger	11	39.28
Aspergillus oryzae	5	17.85
Mucor recemosus	3	10.71
Rhizopus stolonifer	2	7.14
Aspergillus flavus	5	17.85
Talaromyce flavus	2	7.14
Total	28	100

occurrence of *A. flavus* in borehole water sample. *A. niger* is a common allergen and may cause opportunistic invasive infections in hospitalized immunized patients (De Hoog et al., 2000). Very small percentage of *Rhizopus* was found in the study. Okpako et al. (2009), recovered a significant percentage of *Rhizopus* in river and borehole water. *Zygomycetes* are known to causes diseases in immune compromise patients (Ana et al., 2006). The genus *Mucor* is known to be a major cause of thrombosis infarction, nasal or paranasal sinus infection and GL disorders. The presence of these filamentous fungi may be mainly associated with post treatment contamination from outside sources, or post collection contamination, or from populations growing within biofilm or other materials (such as pipe joints and seals) in the distribution system, or they were able to escape the treatment procedures or the contamination is the source (Bay *et al.,* 1970; Grabinska *et al.,* 2007). The detection of pathogenic microorganisms in different sources of drinking water also reveals the alarming situation for the well water. The high prevalence of filamentous fungi in well drinking water is a matter of serious concern.

On comparism, there was no significant difference in the occurrence of fungi between water collected from boreholes, wells, taps and rivers. Also a significant difference of fungi in water collected from Bado, Arkilla, Gidan Dare and Kwakwalawa. The lack of a difference between these sites may be associated to the fact that most of them have the same sources of water. It is unlikely that the occurrence of fungi in water at the concentrations observed in this study would cause disease in healthy individuals. However, if the right conditions are present and regrowth of fungi occurs in water systems, exposure of humans to large amounts of potentially harmful fungi species could become a problem. Several the fungi are potential toxin producers

and exposure to small amount of toxins for several years may have negative effects on the immune system (Letscher-Bru *et al*., 2002).

Conclusion

The species of fungi isolated from drinking water sample are *Aspergillus niger, Aspergillus oryzae, Aspergillus flavus, Mucor racemosus, Talanomyce flavus* and *Rhizopus stolonifer.* Where the colony count of fungi ranges from $1.1x10^4$ to $8.0x10^3$ cfu/ml. Aspergillus which has the highest occurrence of 39.28% causes a wide range of diseases on humans, ranging from hypersensitivity reaction to invasive infections associated with angio-invasions.

Recommendation

The following recommendations are necessary:

(i) Prevention of storm flooding in to spring and wells.
(ii) If source of water is microbiologically clean, then use of containers with a narrow mouth and lid, would render boiling unnecessary.
(iii) Governments should improve dissemination of information on private water testing personal hygiene and sanitation.

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