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Incidence of Moulds in Treated and Untreated Drinking Water of Selected Local Governments in Ibadan, South-Western Nigeria

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Abstract

Water is an essential component of biological make up and it is one of the most abundant life resources. However, scarcity of potable water is an issue of concern worldwide. In this study, incidence of moulds in treated (bottled and sachet) and untreated (bore-hole) drinking water in Ibadan south-western Nigeria was investigated. Samples of sachet and bottled water, and bore-hole water were gotten from selected locations in Ibadan north, Ibadan north-east, Ibadan north-west, Ibadan south-west, Idoo and Akinyele local governments, they were analyzed for the presence of moulds by pour-plating technique on Sabouraud Dextrose Agar plates. Characterization of mould species was done morphologically based on macroscopic and microscopic characters. Seventy one colonies of moulds were isolated but seven species with highest percentage incidence belonging to the genera *Penicillium, Aspergillus, Fusarium, Rhizopus, and Neurospora* were identified. The genera *Aspergillus* (53.52%) and *Penicillium* (29.58%) occurred most frequently. Mould contamination in sachet water (42.25%) and bottled water (29.58%) was higher than that in bore-hole water (28.17%). The mean total viable counts for moulds were 6.29 CFU/ml in sachet water, 6.05 CFU/ml in bottled water and 5.67 CFU/ml in bore-hole water. The presence of moulds in drinking water is non-negligible, therefore moulds should be considered in assessing microbiological quality of drinking water and setting standards in drinking water regulations.

***Keywords:** Bore-hole water; Bottled water; Moulds; Characterization; Nigeria; Sachet water*

Introduction

Water is one of the most important resources useful in biological metabolisms without which life would not be possible [1], humans can stay alive for many days without food but not without water. However, water becomes unsafe for human consumption when it contains pathogenic or disease-causing microorganisms [2]. Drinking water may be contaminated during packaging, transferring, Storing if the not handle hygienically. Water is usually treated to purify it, but some treatment techniques may not properly reduce or eradicate this contamination [2]. Moreover, many microorganisms inhabit water and some have being attributed to water contamination some of which are fungi. Therefore, good knowledge of indigenous

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moulds contaminating our drinking water may form a basis for their control and also may be employed in quality control of our drinking water.

Fungi are diverse group of eukaryotic heterotrophic organisms belonging to the kingdom Eumycota. They comprise of five phyla; Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota and Glomeromycota [3]. Some groups of fungi have been classified into groups of multicellular filamentous fungi called moulds, yeasts, and mushrooms [4]. Moulds are fungi that have branch and threadlike filaments while yeasts are single-celled organisms that reproduce by budding [5]. Fungi are widely found in nature, many fungal species can survive in oligotrophic environments, scavenging nutrients from the substrate, air and water [6,7]. Several studies reported that filamentous fungi are common on water surface pipes, even when they have been treated with chlorine [8].

Furthermore, over 7000 species of fungi and fewer than 300 of them have been implicated in human diseases, and fewer than a dozen cause about 90% of all fungi infections [9]. They are involved in different forms of diseases including allergies to fungal antigens, or direct invasion of hosts [10]. Fungi also produce secondary metabolites, of which toxins are included [11]. The moulds implicated in human mycoses include species of the genera *Aspergillus*, *Fusarium* and *Rhizopus* [12,13]. Allergenic responses are produced by *Candida* and *Aspergillus* spp. [14], while toxic secondary metabolites (mycotoxins) are produced mainly by *Aspergillus*, *Fusarium* and *Penicillium* species [4,15-17]. Taste and odour problems in water are caused by *Penicillium*, *Phialophora* and *Acremonium* spp. [18]. Waterborne illnesses due to microbial contamination are major threats to human health globally of which fungi are included and there is limited information on the occurrence and characterization of moulds from drinking water in Nigeria. This study therefore investigated the occurrence of moulds in treated bottled and sachet and untreated bore-hole water drinking water from selected locations in Ibadan.

Material and Methods

Sample collection

Three sources of water used in this study were; bore-hole water, sachet water and bottled water. Sixty-three samples were collected (twenty one samples in triplicates) comprising seven brands each of sachet and bottled water, and bore-hole water from selected locations in Ibadan north (7.443902°N, 3.887 906°E; 7.452002°N, 3.902258°E; 7.434310°N, 3.896365°E; 7.436896°N, 3.918640°E; 7.421527°N, 3.919557°E; 7.421484°N, 3.925284°E), Ibadan north-east (7.443139°N, 3.910756°E; 7.445433°N, 3.916538°E; 7.440775°N, 3.916457°E), Ibadan north-west (7.392989°N, 3.885781°E; 7.396680°N, 3.892670°E; 7.3905053°N, 3.882804°E), Ibadan south-west (7.367024°N, 3.862136°E; 7.367184°N, 3.866363°E; 7.365503°N, 3.865848°E), Idoo (7.411511°N, 3.85892°E; 7.411060°N, 3.873409°E; 7.437260°N, 3.822182°E) and Akinyele (7.469946°N, 3.911272°E; 7.467117°N, 3.913313°E; 7.466823°N, 3.917041°E) local governments in Ibadan, Oyo state. Bore-hole water was collected by allowing the water from the tap to run to waste for two or three minutes and then the water was aseptically collected in sterile containers and sealed with screw cap, making sure that the containers did not touch the tap before, during and after collection. After collection, all samples were sealed firmly, labelled with sample number, date of collection and sample source and transported to the laboratory in ice coolers. Analyses commenced within 24 hours after sampling.

Isolation of moulds

Moulds were isolated by pour-plating technique according to the procedure of [19] Sabouraud Dextrose Agar was prepared at the rate of 65 g per liter of distilled water. 1 ml of each water sample was placed in Petri dish and then still molten medium was incorporated into the plates containing the sample and rotated carefully. This method gave for better dispersal for counting and enumeration. The isolated colonies that showed different morphological characters were sub-cultured onto fresh Sabouraud Agar medium as soon as they were seen to get pure single colonies for identification tests according to the method described by Mesquita-Rocha et al. [20].

Characterization of moulds

Moulds were identified morphologically using cultural and morphological traits with the aid of suitable references [21,22]. Microscopic analysis was done according to the method of [23] in which a drop of 95% ethanol was placed on microscope slide, a small portion of fungal growth was gently removed, placed on the microscope slide on the 95% ethanol and then gently spread out with two dissecting needles so that it could be easily identified for viewing. When most of the ethanol had evaporated, a drop of Lacto-phenol Cotton Blue was added to the slide and covered with a cover glass. Identification of moulds was done by comparing the macro and microscopic characters [8,21,24,25].

Macroscopic Examination the colonies of the organism were observed for peculiar characteristic colonial morphology and this was done using the under listed feature.

1. Rate of growth followed at regular intervals.
2. Colour of colonies.
3. Texture of colonies.
4. Colonial appearance.
5. Reverse side or colour of underside.
6. Vegetative pattern.

These were compared with microscopic characteristics such as vesicle shape, conidial head and arrangement of conidia, type of conidiophore and type of hyphae.

Statistical data analysis

All the experiments were carried out in triplicates. The data generated were subjected to analysis of variance (ANOVA) at the probability level of $P < 0.01$ and $P < 0.05$. The tests of significant were carried out using Duncan's multiple range test (DMRT) at probability level of $P < 0.05$ using the SAS 9.0 statistical package

Results and Discussion

A total of 71 isolates of moulds from 5 genera (*Penicillium, Aspergillus, Fusarium, Rhizopus, and Neurospora*) were obtained. The most frequently isolated genera were *Aspergillus* (53.52%), *Penicillium* (29.58%) and *Fusarium* (12.68%), while *Neurospora* was the least with 1.41% occurrence (TABLE 1).

TABLE 1. **Mould genera in water samples.**

Mould genera	**Frequency (X = 71)**	**Percentage (%)**
Aspergillus	38	53.52
Penicillium	21	29.58
Fusarium	9	12.68
Rhizopus	2	2.82
Neurospora	1	1.41
Values are means of three replicate determination.		

The identified mould species (FIG. 1 and FIG. 2) and their frequency of occurrence are represented in TABLE 2. Three species of *Aspergillus* - *A. flavus* (8.45%), *A. niger* (16.90%) and *A. fumigatus* (28.17%), and one species each of *Penicillium* (*P*. *notatum*), *Fusarium* (*F*. *oxysporum*), *Rhizopus* (*R. nigricans*) and *Neurospora* (*Neuerospora crassa*) were identified. *Penicillium notatum* was the highest occurred (29.58%) species.

TABLE 2. **Mould species in water samples.**

Mould species	**Frequency (X = 71)**	**Percentage (%)**
Aspergillus fumigates	20	28.17
Aspergillus niger	12	16.90
Aspergillus flavus	6	8.45
Penicillium notatum	21	29.58
Fusarium oxysporum	9	12.68
Rhizopus nigricans	2	2.82
Neurospora crassa	1	1.41
Values are means of three replicate determination.		

Aspergillus was the most frequently isolated mould genus revealed by this study. *Aspergillus* has been reported in drinking water by some researchers. [26] reported 21% *Aspergillus* spp. contamination of water samples studied while [27] recorded more than 70% *Aspergillus* spp. contamination of drinking water samples examined. *Aspergillus* species are opportunistic and can cause health problems in immune-compromised patients. Of the *Aspergillus* species recovered from the water samples, *Aspergillus* fumigatus was the most frequently isolated (28.17%). *A. fumigatus* is a cosmopolitan mould found almost everywhere and on every conceivable type of substrate. It is an important human pathogen and is the most common cause of all forms of invasive and non-invasive aspergillosis [13]. *A. flavus* produces mycotoxins called aflatoxins which are toxic to humans and animals [21]. Aflatoxin contamination occurs when *A. flavus* successfully colonizes a substrate, grows in

it, and subsequently produces aflatoxins as secondary metabolites [28]. Aflatoxins are potent carcinogens which are responsible for many thousands of human deaths mostly in non-industrialized tropical countries [29].

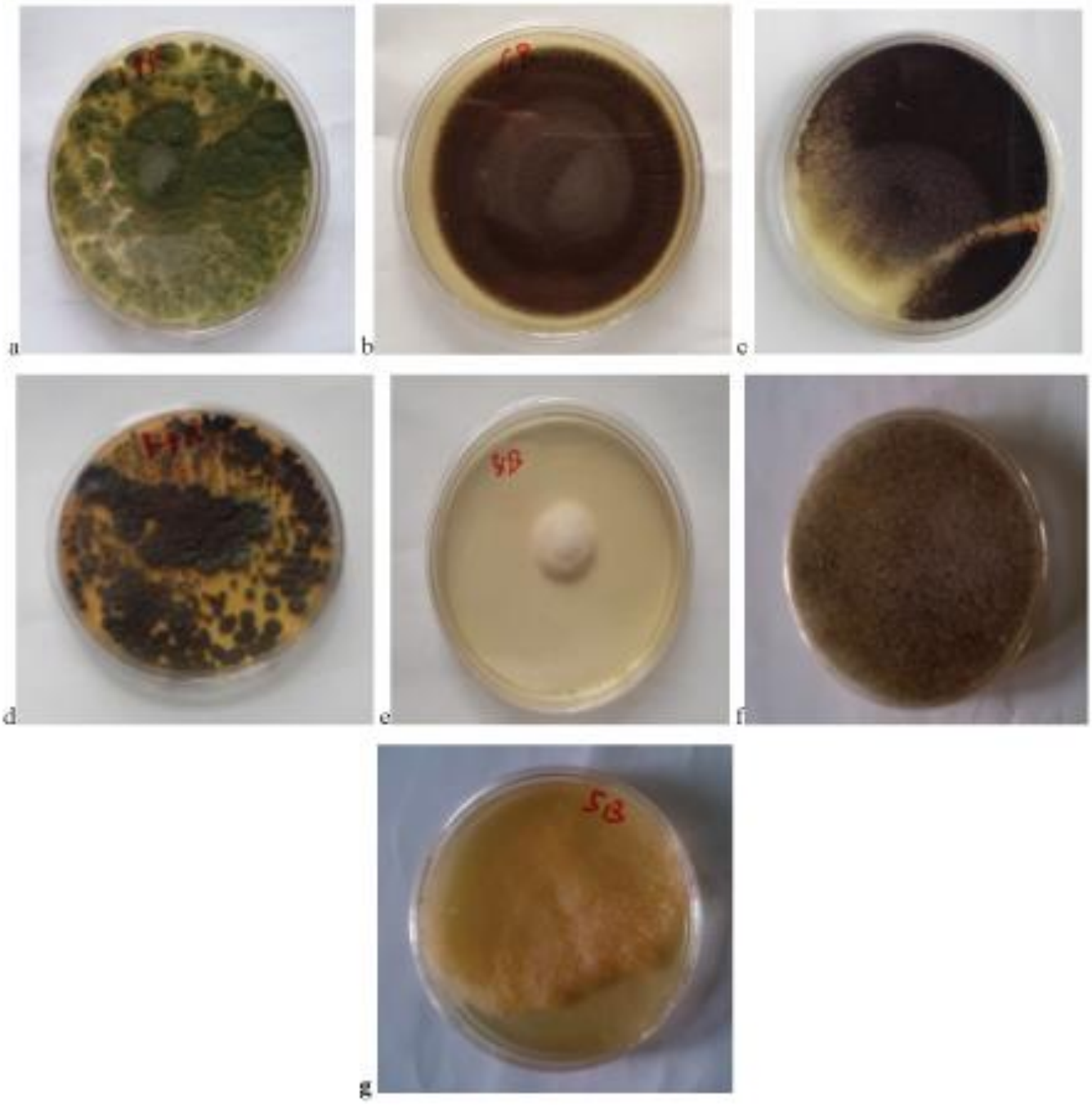

FIG. 1. **a.** ***Aspergillus flavus*** **b.** ***Aspergillus fumigatus*** **c.** ***Aspergillus niger*** **d.** ***Penicillium notatum*** **e.** ***Fusarium oxysporum*** **f.** ***Rhizopus nigricans*** **g.** ***Neurospora crassa on*** **Sarbouraud dextrose agar.**

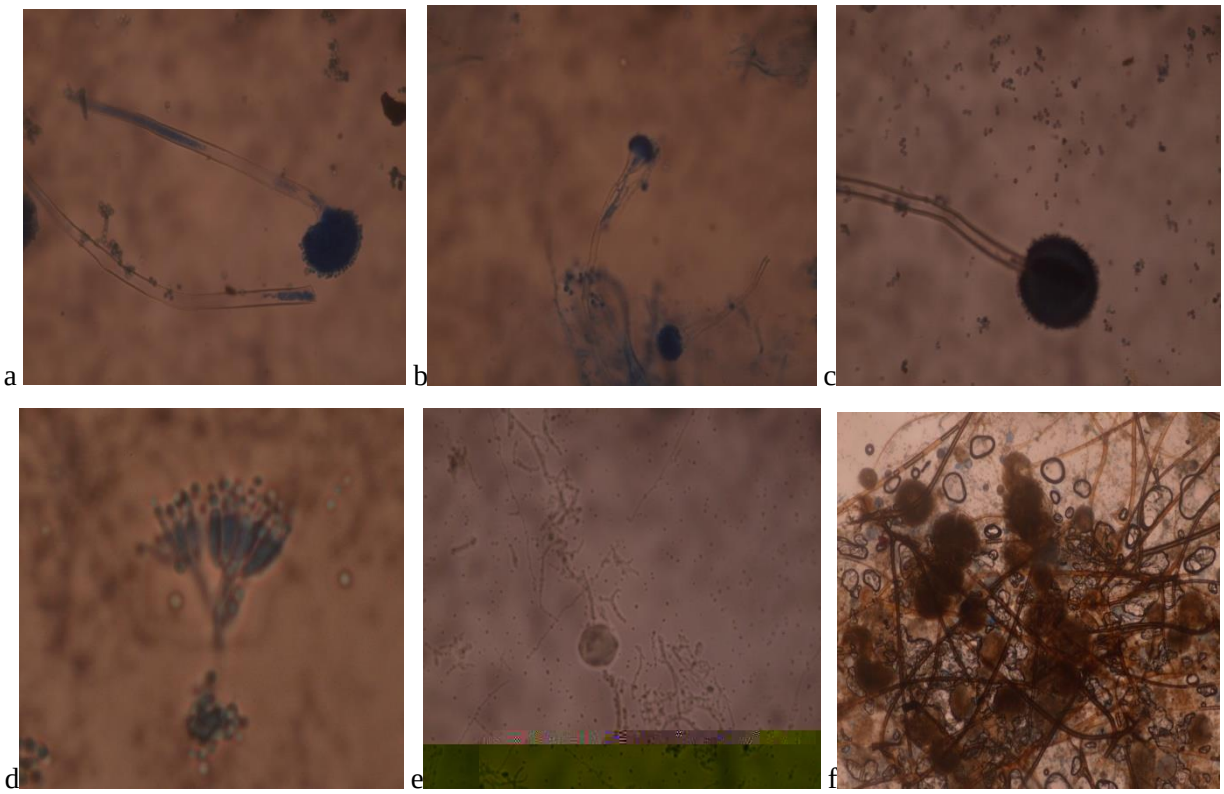

FIG. 2. **Micrographs of a. *Aspergillus flavus* (x40) b. *Aspergillus fumigatus* (x40) c. *Aspergillus niger* (x40) d. *Penicillium notatam* (x100) e. *Fusarium oxysporum* (x40) f. *Rhizopus nigricans* (x40).**

High contamination (29.58%) of *Penicillium* was observed in all samples analyzed. Similar results have been reported by other researchers [30,31]. *Penicillium* species are opportunistic and capable of causing infections such as allergies, asthma and other respiratory problems in immune-compromised patients [32,33]. The specie isolated in this study may have these potentials if susceptible individuals are exposed to it.

Fusarium spp.. isolated in several samples in this study agrees with the results from similar investigations [34] reported that the water distribution system of a hospital located in Houston, Texas, USA, is a potential indoor reservoir of *Fusarium* spp.. [35] also reported contamination by *Fusarium* spp.. in surface water samples of Norwegian drinking water. *Fusarium oxysporum* is associated with broad spectrum of superficial keratitis and cutaneous infections, infections of wounds [12,36] and fusariosis, onchomycosis [37] and locally invasive and disseminated diseases in immune-compromised individuals [38].

Rhizopus spp.. present in this study supports the findings of [30] who reported 10% occurrence of *Rhizopus* in treated tap waters in Portugal. *Rhizopus nigricans* is implicated in immune reactions, contributing to increased severity of asthma and respiratory allergies, skin allergies, opportunistic infections, acute pneumonitis and hypersensitivity reactions [39,40].

Neurospora crassa recorded in this study has not been reported in drinking water. The genus *Neurospora* has not been implicated in any human illness [41].

Mould contamination was found in all the water samples regardless of the source. Sachet water (42.25%) and bottled water (29.58%) had higher mould contamination than bore-hole water with an occurrence of 28.17%. There were no significant differences ($p \geq 0.05$) in the occurrence of moulds in sachet, bottled and bore-hole water (TABLE 3).

TABLE 3: **Mould contamination in bottled, sachet and bore-hole water.**

Water source	Mean of occurrence	Total number of species	Frequency of occurrence	Percentage (%)
Bottled	6.05[a]	7	21	29.58
Sachet	6.29[a]	5	30	42.25
Bore-hole	5.67[a]	4	20	28.17
Means with same letters are not significantly different at $p \geq 0.05$.				

Mould contamination in bore-holes could be from poorly designed septic tanks, poor drainage, waste water disposal and poor sanitation since they are sited within residential areas [42,43]. Fungal contamination in bottled water [44] and sachet water [45] has been known to occur. The high demand for packaged water by consumers led to springing up of many water producing industries where water is packaged without proper regard for hygiene. This results in a lack of guarantee that the products will meet set standards for drinking water quality [46]. Mould contamination in sachet and bottled water suggests that treatment procedures used are not enough to suppress growth of these moulds. It could be possible that the moulds were only partially inactivated by the treatment and recovered, and remained present after treatment and multiplied within the system. Although there were no significant differences ($p \geq 0.05$) in the occurrence of mould species in the different drinking water sources, the frequency of occurrence and the average mean counts (CFU/ml) in bore-hole water were lower than that in bottled and sachet water. Bottled and sachet water since they are treated, should naturally contain less mould contamination than untreated bore-hole water, but the reverse was obtained in the study. This is in accordance with the findings of [35] who demonstrated the increase in diversity of mould species from raw water to treated water. The higher mould contamination in bottled and sachet water also suggests entry of moulds via pathways of secondary contamination (post-treatment handling). Most vendors display these bottled and sachet water on bare ground, under the sun and even on dirty slabs. These could also be routes of contamination by these moulds.

Conclusion

Most of the moulds isolated from drinking water in this study could be pathogenic to humans, and are likely to be responsible for infections in immune-compromised individuals. Therefore there is need for certification and monitoring agencies to conduct regular inspections and audits on water packaging industries. This is to ensure that treatment methods are sufficient to handle the mould contamination in water, and also that processes used in collection, packaging and storage of bottled and sachet water do not threaten the safety of water users and the general public. In households using bore-hole water for drinking, citing of bore-holes close to polluted areas should be avoided and appropriate efforts should be made to ensure safe

collection, storage and perhaps treatment of bore-hole water to eliminate mould contamination. Public health, surveillance and/or other local authorities should also provide guidance to support households and individual consumers in ensuring the safety of their drinking-water.

Since there are no guidelines regarding moulds in drinking water, conclusions cannot be made about their occurrence in the water being within permissible levels. Hence suggestions for guidelines of mould contamination in drinking water should be reviewed and mould contamination addressed.

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