

## REGULAR ARTICLE

## MICROBIOLOGICAL SURVEY AND DISSEMINATION OF FILAMENTOUS FUNGI OF PUBLIC HEALTH SIGNIFICANCE FROM WATER RESERVOIR (STORAGE TANK) IN CROWN ESTATE, IGBINEDION UNIVERSITY, OKADA, NIGERIA

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## ABSTRACT

Results of this investigation revealed that several species of filamentous fungi are present in the reservoir waters. Samples were collected from the surface and walls of the inner storage tank. The isolation process was done by a range of techniques and media, monitored by trained taxonomist to achieve broad arrays of water-borne fungi classified to species level. The direct plate enrichment counts and filtration technique recorded the highest number of counts (59 % and 32 %) respectively. The sabouraud dextrose Agar (SDA) was observed as the medium that recorded the highest colonies (60 cfu/100 ml) for incubation period of 5 d at 30 °C. Six different fungal taxa were recovered from the three examined sites (ST1, ST2 and ST3) and the ST3 was reported to have the highest number of isolated species. However, certain fungi were observed to be more distributed than others, specifically in species of *Aspergillus* and *Penicillium* which tended to be the most common. Although, species of other genera such as *Acremonium* sp., *Rhizopus stolonifer*, *Mucor racemosus* and *Trichophyton* sp. were also present but in low counts. The significance of fungi in water systems is lowly expressed as many of the species isolated from water sources are confirmed to possess the potentiality of secreting toxic secondary metabolites like patulin, produced by *P. expansum* causing immune-suppression in hosts, *A.flavus* secretes aflatoxins that can be carcinogenic, *A. versicolour* releases the musty odours in homes, while some moulds are concerned in food deterioration.

**Keywords:** Filamentous fungi, isolation techniques, mycotoxins, reservoir and health relevance

## INTRODUCTION

The filamentous fungi consists of long structure known as filamentous hyphae which are extended to derive nutrients from substrates and produced for vegetative non-sexual reproduction with spores or conidia and a vast are human pathogens. Fungi are constantly present in different water sources and their impacts lead to many biological processes such as biodegradation, biodeterioration, biofilm and its aesthetic health issues that are of enormous challenges to water-related regulatory agencies.

Fungi secrete digestive enzymes to breakdown biomolecules into simpler elements, prior to utilization as nutrients and in production of mycotoxins such as aflatoxins, fumisinins and zearalenons that are inimical to humans' health. The adverse effects ranged from being used as biological and chemical weapons introduced into water distribution systems, production of cadaverine and putrescine with pungent irritating odour. Though, they are useful in synthesis of antibiotics and other  $\beta$ -lactams and as biological pesticides to control weeds and pests (Espinale & Montero, 2004).

An alarming increase in the number of pathogenic diseases due to filamentous fungi is attributed to the increase in patients undergoing immunosuppressive therapies, organ transplants and acquired immune deficiency syndrome (AIDS) patients. The most common organisms responsible for this trend are the *Aspergillus* and *Penicillium*. The systematic investigation of fungi in drinking water system is on the increase, because it is usually performed when cases of contamination are reported. A limited number of tests revealed that, fungi are present in a significant amount in stored water samples, but the range of species is extremely variable (Ferrti & Demoraes, 2001). The objective of this study was to detect whether the storage tank in Crown Estate of Igbinedion University, Okada, Nigeria is a source of filamentous fungi of public health importance.

## MATERIALS AND METHODS

## Sample Collection

Water samples were collected from three water reservoirs (storage tanks) with each above 100,000 litres in the month of March, 2019 on weekly basis in the Crown Estate, Igbinedion University, Okada, Nigeria. Sterile bottles were sufficiently quantified with sodium thiosulphate to neutralize the chlorine. Five hundred millilitre (500 ml) water samples were obtained and stored at 4 °C before the isolation was performed within 24 hr. A direct collection approach was used

whereby the bottles were inserted onto the storage tank from the top and allowed to fill gently.

## Isolation

This involved the concentration of fungi propagules and filtration processes.

## Concentration

This is the trapping of propagules found in the sample by centrifugation at 2000 resolution per minute (rpm). The fungi contained in the sample were observed directly or allowed to grow and seen as colonies.

## Filtration

One hundred milliliter of reservoir water samples was filtered through a pre-sterilized 0.45  $\mu$ m Millipore filter paper and aseptically transferred onto an agar medium in 10 cm petri dishes for 5 d at 30 °C. Four media that are cheap, simple and with good results were used in the test and these include half corn meal agar (CMA/2), neo-pentose-glucose-rose Bengal agar (NGRBA), sabouraud dextrose agar (SDA) and oomycetes selective medium (OSM). The observation of the filtered sample was carried out by microscopy.

## Baiting

This test was mainly to determine the zoospore fungi (Oomycetes) and conducted as described (Virginin *et al.*, 2011) with slight modifications. Many pieces of 1.0 cm sterilized snake skin and hallucinogenic seeds of tobacco plants were added to 100 ml fraction of samples in shake-flask incubated for 5 d at 30 °C, during which period, any baits accompanied with presumed evidence of fungi colonies were aseptically accepted and transferred onto oomycetes selective medium (OSM) and samples plated out in duplicates.

## Swapping

The swab was collected by using a scalpel-fixed with cotton wool at the tip to scrap the wall of the inner surface of the water reservoir. The obtained swab was then rinsed in saline solution (NaCl, 1.0 % w/v). A sample (0.1 ml) spread evenly over the medium surface, then dispensed into petri-dishes and incubated for 5 d at 30 °C. The media employed were CMA/2; NGRBA and SDA.

## Enrichment

This was done by inoculating 10 ml of diluted sample suspension onto 90 ml of SDA in shake-flask. Sub-samples (1.0 ml) were then inoculated onto the medium in plates, incubated for 48 hr at 25 °C and observed for the growth of fungal colonies. This test was conducted to cater for samples with low fungi colonies.

## Enumeration and Identification

All colonies on agar plates were counted and recorded as colony forming unit (cfu/100 ml) of water. The fungi were identified on the platform of macroscopic and microscopic characteristics. However, most *Aspergillus* was identified to species level according to procedures outlined (Kimsey et al., 2010).

## RESULTS

**Table 1** Number and percentage of different fungal colonies recorded by various methods and media

Media	Filtration	baiting	Plating	swabbing
CMA/2	18	-	20	1
NGRBA	10	2	34	3
OSM	2	3	14	3
SDA	25	1	32	2
All media	55 (32.0 %)	6 (3.5 %)	100 (59.0 %)	9 (5.3 %)

**Key:** NGRBA – Neo-pentose- glucose Bengal agar; OSM – Oomycetes selective medium; SDA – Sabouraud dextrose agar; DP – direct plating NGRBA – Neo-pentose- glucose Bengal agar; OSM – Oomycetes selective medium; SDA – Sabouraud dextrose agar; DP – direct plating

The baited and swabbed methods employed in this study recorded low number of fungal isolates. There was an estimated reduction of counts compared to the overall fungal counts (Table 1). The surfaces of the baits tended to be completely covered with bacteria and yeast cells which reduced the number of counts and the dryness of the inner wall of the water reservoir also contributed to the low counts recorded. Nevertheless, 6 fungal (CFU/ml) was obtained from baiting and 9 fungal (CFU/ml) was recorded from swabbing methods respectively. The direct plating and filter techniques recorded highest values of fungal colonies (100 and 55 CFU/ml). The NGRBA provided the highest recovering of filamentous fungi (34 out of 100) were detected using enrichment plate method (Table 1).

The fungal propagules microscopically examined showed that, the cornidia were more produced in all the media used with average score (5.3) and the sclerotia recorded the least mean (0.3) (Table 2)

**Table 2** Mean of propagules on 1.0 cm pieces of filter employed from stored water sample sites

Isolation	hyphae	Spores (cornidia)	sclerotia
ST1	-	4	1
ST2	3	10	-
ST3	2	2	-
Mean	1.7	5.3	0.3

The table below showed the dissemination of organisms based on the isolation methods and media used; the species obtained were of 5 genera, the most abundant were *Aspergillus* and *Penicillium* which accounted for 63 %. The other organisms were *Acremonium simplex*, *Rhizopus stolonifer*, *Mucor racemosus* and *Trychophyton* sp. estimated at 37 %. The *Aspergillus* identified to species level included; *A. flavus*, *A. fumigatus* and *A. versicolor*, while the *Penicillium* species were *P. brevicompactum* and *P. expansum*. Overall, all fungi were accordingly isolated from the three surveyed sites (ST1, ST2 and ST3) though the highest number of fungi was recorded in ST3, *A. fumigatus* and *P. brevicompactum* were present in all the sites investigated.

**Table 3** Methods and media used in isolation for various fungal group

Organisms	Sites of isolation	Methods	Media
<i>Acremonium simplex</i>	2, 3	Filtration, direct Plating(DP), swab	CMA/2, SDA
<i>Aspergillus flavus</i>	1, 3	Baiting filtration	NGRBA, CMA/2, OSM
<i>Aspergillus fumigatus</i>	1, 2, 3	Baiting, DP, Filtration	CMA/2, SDA, NGRBA
<i>Aspergillus versicolor</i>	1, 2		
<i>Penicillium brevicompactum</i>	1, 2, 3		NGRBA, OSM
<i>Penicillium expansum</i>	1, 3	DP, filtration, baiting	NGRBA, OSM, SDA
<i>Mucor racemosus</i>	1, 3	DP, filtration, swab	CMA/2, SDA
<i>Rhizopus stolonifer</i>			
<i>Trychophyton</i> sp.	3	DP, filtration, swab	All media

**Key:** NGRBA – Neo-pentose- glucose Bengal agar; OSM – Oomycetes selective medium; SDA – Sabouraud dextrose agar; DP – direct plating

## DISCUSSION

The identification of vast number fungi is primarily based on morphological and microscopic convectional techniques of spore-bearing structures. Six genera were recovered from the three sites analyzed to include *Aspergillus*, *Penicillium*, *Acremonium*, *Rhizopus*, *Mucor* and *Trychophyton*. However, they contain variable number of species and therefore needed much attention to identify them to species level. The investigative methods and resources available as enunciated by Mirian et al. (2007) have enabled broad spectrum of isolation and identification realistic and a lot of fungi observed in the reservoir which were not water-related microorganisms.

The method employed that gave the highest count was the serial dilution enrichment plate method involving different media at incubation time of 5 d for 30 °C. This study disclosed that, reservoir of Crown Estate, Igbinedion University, Okada is a source of filamentous fungi (ff) ultimately due to prevailing environmental conditions, isolation and growth media used. The growth of ff could be inhibited by the presence of bacteria and yeast cells competing for nutrients and toxins often released by ff can clear the proliferation of other microbes in the system. In this analysis, few organisms were recorded by the swabbing approach, thereby indicating that biofilm development had not occurred on the inner wall of reservoir.

The media devoid of antibiotics with raised ff level may indicate that chemotherapeutic substances have been produced by the fungi. The *Aspergillus* and *Penicillium* recorded highest number of occurrence (Table 3). Briza et al. (1989); Jose (1967) declared that *P. expansum*, secretes chemicals that damage post-harvest diseases of apples and patulin (immune-suppressive toxin), *P. bravicompactum* produces mycophenolic acid that causes animal and human diseases such as oesophageal cancer, hormonal disorder and benign endemic nephropathy. *Aspergillus flavus* produces gliotoxin that suppresses the function of immune system and aflatoxin responsible for chronic granulomatous sinusitis, keratitis and wound infection. *A. versicolor* causes musty odours in homes and produces hepatotoxic and carcinogenic mycotoxins, while *A. fumigatus* produces gliotoxin that is immuno suppressive, and fumagillin that is cyclohexane derivative, known as inhibitor of endothelial cell proliferation. The *Acremonium* releases octenol, linked to odours, *Trychophyton* sp. is concerned with skin, toe, hair and nail infections and the *Rhizopus* and *Mucor* are involved in decomposition and spoilage of food substances.

In the study of Virginin et al. (2011) *Alternaria alternata*, *Aspergillus niger*, *P. expansum*, *P. bravicompactum*, few molds and *Trychophyton mentagrophyte* were detected in water systems in low counts. Differences in genera observed is added to conditions such as raw water sources, system hydraulics, water temperature patterns, maintenance and infrastructural systems from which the samples were collected. Despite the fact that all the fungi were obtained from the suspension, deriving reliable data for this analysis was difficult. This is thought to be due to the mycelial type of filamentous fungi which is capable of disintegrating into irregular structures to initiate their own growth and also as a result of the difference in fungi cornidia hydrophobicity which are uniformly distributed or aggregated, as some agglutinate due to thick gelatinous coatings (Cast, 2013). Realizing this fact, it is not possible to suggest a specific medium for growth, though numbers were recovered in all media. Although, all the fungi were recovered from the suspensions, obtaining ideal data for these organisms proved tedious.

## CONCLUSION

Fungal proliferation in drinking water may be influenced by raw water source, system hydraulics, nutrient concentration and biotic interactions causing severe myco-infections in immunosuppressed hosts resulting from drinking, contact and inhalation of aerosol spores. If further scientific studies suggest an increase in disease risk, pilot epidemiological analysis and surveillance could be performed to checkmate the deleterious menace of fungi dissemination in drinking water systems.

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