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Filamentous Fungal Quality of a Bovine Abattoir and Associated Water Sources in Akure, Southwestern, Nigeria

O. O. Olusola-Makinde[1*], D. J. Arotupin[1] and F. C. Adetuyi[1]

[1]Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in cooperation between all authors. Authors OOO, DJA and FCA designed the study. Author OOO performed the practical work and the statistical analysis. Author OOO wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study examined the prevalence of filamentous fungi in Onyearugbulem abattoir wastewater samples in Akure, Nigeria.
Methodology: The abattoir's water source, 5 m away from animal washings, the incinerator, 10 m upstream, 10 m downstream and 100 m downstream were sampled between November 2014 and October 2015 for aerobic and anaerobic fungal counts using standard recommended procedures. Fungal isolates were identified macroscopically and microscopically.
Results: The results showed that the water source had the lowest fungal count ($1.4x10^3$ sfu/ml in November and $2.0x10^2$ sfu/ml in February for aerobic and anaerobic counts respectively) throughout the sampling period unlike the incinerator which had the highest fungal count ($5.2x10^3$ sfu/ml in August and $5.5x10^3$ sfu/ml in July for aerobic and anaerobic counts respectively). The aerobic fungal count was lower than the anaerobic fungal count in all the six (6) sampling points except the abattoir water source. The trendline of the data collected also showed a significant increase ($p \leq 0.05$) of the fungal counts in the wet season as compared to the dry season. The fungi isolated are *Rhizopus* spp., *Aspergillus fumigatus, Penicillium chrysogenum, Fusarium oxysporium* and *Saccharomyces cerevisiae*.

**Corresponding author: Email: makanjuolabuks@gmail.com, makanjuolabuks@yahoo.com;*

Conclusion: This work indicated a high dominance of fungi in water bodies associated with the slaughterhouse and therefore warns against environmental and health hazards associated with these microorganisms.

Keywords: Abattoir; fungi; water; aerobic; anaerobic.

1. INTRODUCTION

The grave consequences of untreated abattoir wastewaters in the environment are a major concern. Surveys and technical papers have reported that polluted water bodies from abattoir wastes could constitute significant environmental and public health hazards [1-3]. Several groups of microorganisms have been isolated and characterized in abattoir wastewater [4], they include bacteria, fungi, algae and protozoa, viruses. These microorganisms are usually of medical importance and thus of major public health worries. Fungi are a different assembly of organisms that fits to the kingdom *Eumycota* [5]. This kingdom encompasses five phyla namely *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota* and *Zygomycota* [6]. Fungi can be classified as filamentous fungi (moulds), yeasts, and the mushrooms. Certain fungi are mainly found in water, hence, they are adapted to be aquatic. Fungi can also be found in soil, organic material, and air [6]. These fungi can enter water bodies from various locations [7], especially through the adjoining soils. The filamentous fungi are group of organisms that can be found everywhere, they are present in virtually all ecological niches on earth. They are appraised to be accountable for the spoilage of up to 25% of all plant-derived foods produced annually [8].

Filamentous fungi or moulds are important for the preservation of ecosystems. Nutrient cycling on earth can only be possible through the activities of these fungi on dead organic materials. They can also act plant pathogens which lead to serious crop losses and post-harvest food deterioration [7]. Filamentous fungi are vital in the pharmaceutical and medicine industries, they are sources of commercial enzymes, organic acids, and drugs, such as antibiotics (e.g. penicillin, cefalosporin) [7]. *Penicillium* species have been frequently recovered from water in the various studies performed. Several of the species in genus *Penicillium* and *Aspergillus* are known to produce mycotoxins in other substrates, such as food and beverages [9,10]. Interestingly, detection of aflatoxins produced by *A. flavus* in water from a cold water storage tank was demonstrated by Paterson et al. [11]. *Aspergillus* species is one of the more commonly isolated genus in water. *A. niger* and *A. flavus* are common allergens and may cause opportunistic invasive infections [12].

Predominant fungal genera and species in treated and untreated water are *Aspergillus, Cladosporium, Epicoccum, Penicillium, Trichoderma, Arthrinium phaeospermum, A. flavus, C. cladosporioides, Fusarium culmorum, Mucor hiemalis* and *Trichoderma harzianum* [13]. Fungal genera isolated from Dal lake in Kashmir include *Penicillium caseicolum, P. commune, P. chrysogenum, P. funiculosum, P. lilacinum, P. olivicolor, P. dimorphosporum, Penicillium* sp. *I, Penicillium* sp. *II, Penicillium* sp*. III, Penicillium* sp. *IV, A. flavus, A. fumigatus, A. japonicus, A. niger, A. terreus, A.versicolor, A. wentii, Aspergillus* sp. *Fusarium* sp. *Rhizopus* sp. *Acremonium* sp. *and Mucor* sp. [7]. The biology of aquatic fungi affects their distribution both locally and globally, and the factors influencing the fungi depend on the aquatic environment [14,15].

In this paper, we assessed the occurrence of filamentous fungi associated with Onyearugbulem abattoir wastewater samples, its upstream and receiving streams in Akure, Nigeria.

2. MATERIALS AND METHODS

2.1 Location and Site Description

Akure is the capital of Ondo State in the Southwestern Nigeria. It is located between Latitude 7°12'N - 7°58'N and between Longitude 5°15'E-5°17'E. The climate of Akure is subtropical with two main distinct seasons: rainy and dry season. The humidity of the air masses over the city varies from 60 % in January to 80 % in July [16]. Akure city has a population of approximately 420,000 inhabitants. Onyearugbulem abattoir is located along Owo-Ilesa expressway in Akure. The upstream is located eastward to the abattoir and flows to join the discharged effluent from the abattoir. The receiving stream flows westwards and then curves to the south through the community.

2.2 Collection of Water Samples

The water samples were collected on monthly basis for a period of 12 months between November 2014 to October 2015 from Onyearugbulem abattoir water supply, 5 m from animal killings, incinerator, 10 m upstream, 10 m downstream, and 100 m downstream from the abattoir discharging outlet. Water samples were collected in sterile 500 ml sample bottles according to standard methods of American Public Health Association, [17] and Cheesbrough, [18] for microbiological analysis. The water samples were collected with the bottles facing upstream toward the flow of water. The collection was usually made in early hours of the morning (7:00 am). All samples were collected in triplicate to improve reliability of data. Samples were then transported to the Department of Microbiology laboratory, the Federal University of Technology, Akure for analysis within 6 hour of collection.

2.3 Isolation and Characterization of Fungi

Fungi were isolated using the direct plating method [19]. Isolation of fungi from samples collected from Onyearugbulem abattoir and environs was done using the spread plate method. Successive decimal dilutions up to four folds were obtained with 1 ml of the sample been added to 9 ml of sterile normal saline producing a dilution of 10^{-1}, 0.1 ml from the serial diluted solutions was spread on Petri dishes containing sterilized potato dextrose agar (MERCK, Germany). A set of the inoculated plates were incubated aerobically, while the other set of inoculated plates were incubated anaerobically with the aid of anaerobic jar at 25°C for 72 hours. After incubation, discrete microbial colonies were counted using the colony counter (put maker), sub-cultured and purified colonies were subjected to morphological test. The sub-culture was carried out to purify the fungi isolates. During the sub-culture an inoculating loop flamed in a bursen-burner was used to pick the colony and smeared on the agar plate. This was further incubated at room temperature for 7 days. Fungal colonies were isolated upon formation, stained with lactophenol and observed under the microscope. Fungi so observed were identified using appropriate taxonomic guides [20-22].

2.4 Statistical Analysis

Analysis of variance (ANOVA) test was used to analyze the data for fungal count. Means were separated using Duncan's New Multiple Range Test at 95% confidence level with the aid of SPSS version 18.

3. RESULTS

Figs. 1–6 show the distribution of filamentous fungi throughout a 12 month period in water supply at the Onyearugbulem abattoir, 5 m away from the abattoir killings and washings, the incinerator, 10 m downstream, 100 m downstream and 10 m upstream. The figures also cover both the aerobic and anaerobic counts from the sample sources. Generally, as shown by the trendline, there was significant fungal count increase from the dry season to the wet season. The aerobic fungal count from the abattoir incinerator showed the highest count of $5.2x10^3$ sfu/ml in August and $5.5x10^3$ sfu/ml in July for aerobic and anaerobic fungal counts respectively while the sample from the abattoir water source showed the lowest fungal count of $1.4x10^3$ sfu/ml in November and $2.0x10^2$ sfu/ml in February and March for aerobic and anaerobic fungal counts respectively. The anaerobic fungal count in the water source was lower than the aerobic counts all through the 12 months, unlike the other five (5) sample sources which showed higher anaerobic fungal counts; the aerobic fungal count of wastewater sample from the 5 m away from abattoir washings after killings in October was $1.1x10^3$ sfu/ml while the anaerobic count was $2.7x10^3$ sfu/ml in the same month of October. Analysis of variance on the data obtained showed that there was significant difference ($p \leq 0.05$) in total fungal count between the various samples sources. The isolates that were identified showed presumptive identity to be *Rhizopus* spp., *Aspergillus fumigatus, Penicillium chrysogenum, Fusarium oxysporium* and *Saccharomyces cerevisiae* (Table 1).

4. DISCUSSION

The continuous discharge of untreated abattoir wastewater into receiving streams especially in developing countries like Nigeria has gained a degree of attention in academia. The waste produced at these slaughterhouses poses a severe danger to the environment with adverse consequence on land, air and water [23]. This adverse consequence embrace increased level of microorganisms including fungi in the environment especially the receiving water bodies.

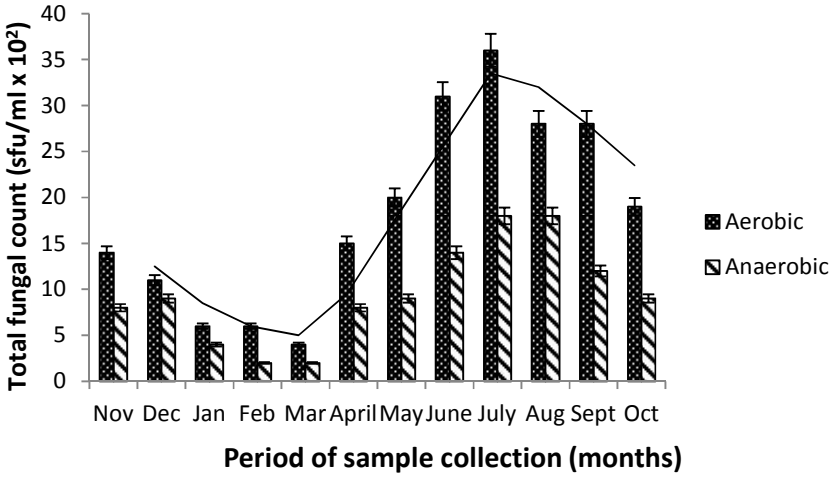

Fig. 1. Aerobic and anaerobic fungal count of Onyearugbulem abattoir water source

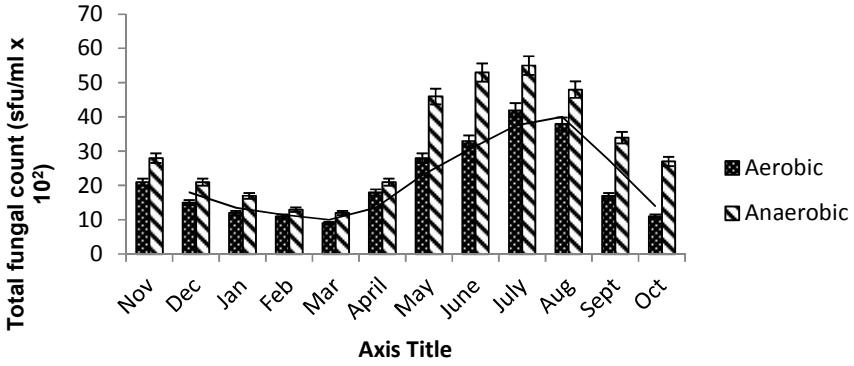

Fig. 2. Aerobic and anaerobic fungal count of wastewater sample from 5 m away from Onyearugbulem abattoir washings

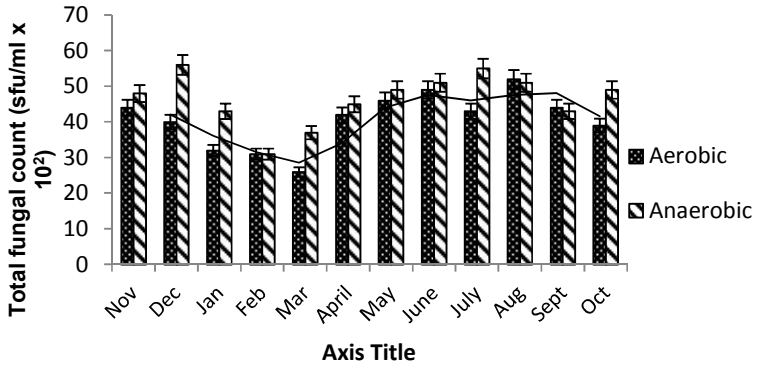

Fig. 3. Aerobic and anaerobic fungal count of wastewater sample from Onyearugbulem abattoir incinerator

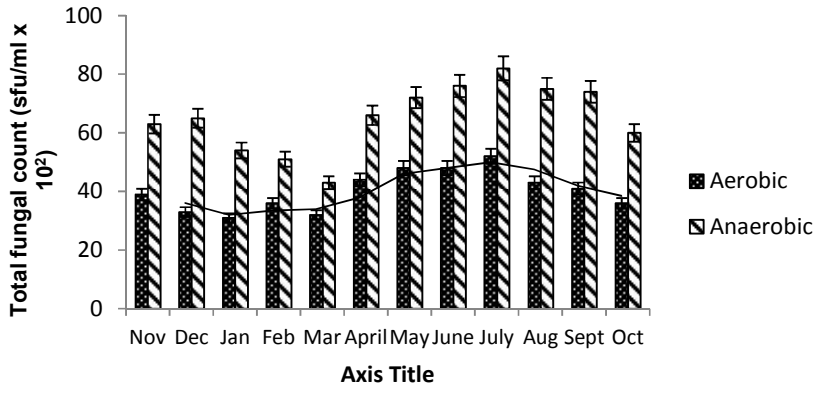

Fig. 4. Aerobic and anaerobic fungal count of water sample from Onyearugbulem abattoir 10 m downstream

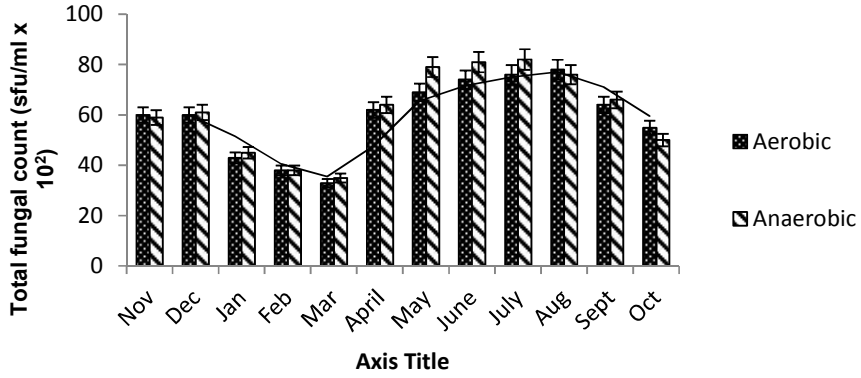

Fig. 5. Aerobic and anaerobic fungal count of water sample from Onyearugbulem abattoir 100 m downstream

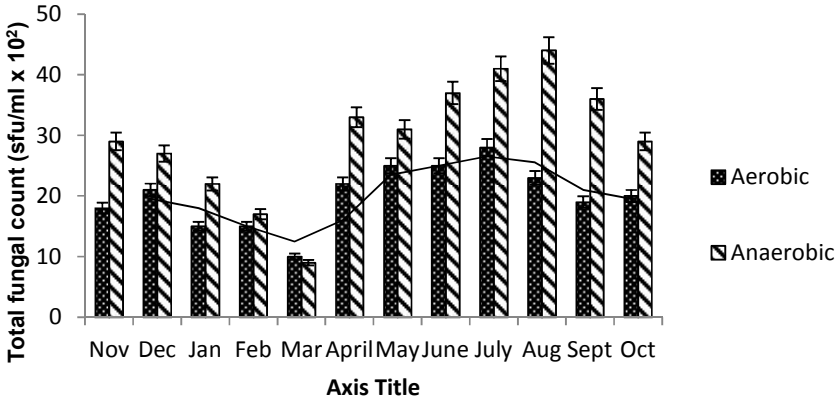

Fig. 6. Aerobic and anaerobic fungal count of water sample from Onyearugbulem abattoir 10 m upstream

Table 1. Characteristics of fungal isolates from Onyearugbulem abattoir wastewater samples

Cultural characteristics	Microscopic observation	Presumptive identity
Cotton-like mycelia at 24 hours turning dirty with development of black spores on mycelium	Non-septate hyphae thin sporangiophore with a sporangium in umbrella-like form	*Rhizopus* spp.
Blue-green with a narrow white border. Powdery surface.	Conidiophores are short, smooth walled and have conical shaped terminal vesicles. Septate hyphae	*Aspergillus fumigatus*
Blue-green growth	Septate mycelium bearing single conidiophores which are branched near the apex ending in phialides that carry conidia	*Penicillium chrysogenum*
White cotton-like mycelia spreads round whole plate	Mycelium extensive in a cottonwool-like form. Having phialides that is bearing a beanpod-like microconidia borne singly or in chain	*Fusarium oxysporium*
Flat, smooth, moist, glistering cream	Blastoconidia are unicellular, globuse and ellipsoid to elongate in shape	*Saccharomyces cerevisiae*

This study examined the level of fungi present in Onyearugbulem abattoir in Akure, Nigeria, this took into consideration the abattoir's water source, the sample from drainage at 5 m away from animal killings, the incinerator, 10 m downstream, 100 m downstream and 10 m upstream. Among the six (6) sample points, the abattoir water source recorded the lowest fungal count throughout the 12 month sampling duration, this may be due to lower exposure of the borehole (water source) to anthropogenic activities that could lead to contamination [24]. In contrast, the samples from the abattoir incinerator showed the highest fungal count. The abattoir incinerator is poorly managed, overflowing and presently damaged hence non-functional. It only receives the wastewater, which then flows directly into the receiving bodies, this allows microbial growth while the wastewater stays in the incinerator before discharge. The fungal count from the 10 m upstream water sample may be due to bird droppings, animal feeding and defeacation, and other anthropogenic activities. Farell and Nieuwenhuijsen [25], reported that anthropogenic activities contaminate surface waters. The higher occurrence of anaerobic fungi compared to the aerobic fungi may be due to the nature of wastewater entering the sampled water bodies. The abattoir wastewater comprises washings from the animal intestines. Also, these animals are majorly ruminants such as cows. Valente et al., [26], reported that the anaerobic fungi are part of the natural microorganisms of the rumen.

There was significant difference between the fungal count in the dry season and the wet season, this may be due to increased water level and washings of adjoin soils into surface waters. The findings are in line with those of Ana et al. [27] and Omole and Ogbiye [23]. During the wet season, there is increase in water table because of infiltration, thereby, microorganisms from wastes may be added to water [23]. The fungal species isolated from the sample points were *Rhizopus* sp., *Aspergillus fumigatus, Penicillium chrysogenum, Fusarium oxysporium* and *Saccharomyces cerevisiae,* this is an indication of contamination. Arvanitidou et al. [28] and Gunhild et al. [29] reported that *Aspergillus* is the most common isolated genera in water. *Aspergillus* sp. are known to produce aflatoxins (B1, B2, G1 and G2), the most toxic and potent hepatocarcinogenic natural compounds ever characterized [30]. These fungi cause a wide range of diseases in humans, ranging from hypersensitivity reactions to invasive infections associated with angioinvasions. *Fusarium* sp. was reported in this study. *Fusarium* sp. *has* been recognized as an agent of superficial infections (keratitis and cutaneous infections, onychomycosis and infections of wounds and burns) [31]. In recent years, deep-seated and disseminated infections have been increasingly described in immunocompromised patients, especially in neutronpenic patients [31]. The prognosis is very poor and death occurs in up to 70% of cases despite antifungal therapy [32]. *Penicillium* sp. were also identified in this study. *Penicillium* is known to cause allergy, asthma

and some respiratory problems [33,34,29]. Therefore, the species isolated in this study may have allergic potentials if susceptible individuals are exposed. *Rhizopus* was reported in this study. *Zygomycetes* are known to cause diseases in immunocompromised patients [35,27]. The genus *Mucor* is known to be a major cause of thrombosis, infarction, nasal or paranasal sinus infection and GI disorders.

5. CONCLUSION

This work has established that there is a high level of fungal presence in water bodies which is not environmentally acceptable by standard authorities such as WHO and FEPA. The presumptive identification also indicates fungi that are of public health interest. Therefore, further studies on the molecular identification of these isolates are suggested so as to confirm their identity and treatment methods. The discharge of untreated abattoir wastewater into the environment should also be discouraged by all stakeholders including the government and industrialists.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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