Journal homepage: http://fens.usv.ro/index.php/FENS
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XXI, Issue 2 - 2022, pag. 152 - 170

MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR IDENTIFICATION OF AUTOCHTHONOUS FUNGAL POPULATION IN SLAUGHTERHOUSE EFFLUENT, SURFACE WATER AND FISH GUT FROM THE OGUN RIVER, NIGERIA

Olanike Maria BURAIMOH[1,5] Temitope Olawunmi SOGBANMU[2], Olusola Abayomi OJO-OMONIYI[3*], Olumide AFOLABI[4] , Chinwe Mary GANOBI[1]

[1]*Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos- Nigeria.*
[2]*Ecotoxicology and Conservation Unit, Department of Zoology, Faculty of Science, University of Lagos, Akoka, Lagos-Nigeria.*
[3] *Department of Microbiology, Lagos State University, Ojo 102101 Lagos- Nigeria, olusola.ojo-omoniyi@lasu.edu.ng*
[4] *Department of Biological Sciences, Faculty of Basic and Applied Sciences, University of Africa, Toru-Orua, Bayelsa – Nigeria.*
[5]*Microbial Diversity, Bioinformatics and Biotechnology Research Group, TETFund Centre of Excellence on Biodiversity Conservation and Ecosystem Management (TCEBCEM), University of Lagos, Lagos-Nigeria.*

*Corresponding author
Received 15th April 2022, accepted 25th July 2022

Abstract: *This study aimed to evaluate and characterize the pathogenic fungi as well as the influence of effluent from the Kara abattoir on the Ogun River, Nigeria. In this study, the fungal population of the abattoir effluent, surface water and a species of fish (Chrysichthys nigrodigitatus) from the Ogun River were sampled and examined during the 2018 dry and rainy seasons using morphological, biochemical and molecular methods. Several pathogenic fungi were characterized both in the effluent and fish gut during both seasons. Penicillium sp., Fusarium oxysporum, and some other unknown species were found in the fish gut during the rainy season. Rhodotorula mucilaginosa was found both in the fish gut and effluent during the rainy season. Talaromyces sp., was found in the fish gut and surface water during the rainy season. Aspergillus flavus, Aspergillus sp., Talaromyces sp. were found in the fish gut and effluent during the dry season. Penicillium citrinum was found in the fish gut and surface water during both seasons. Saccharomyces sp., Candida albicans, Rhizopus stolonifer, Mucor sp. and an unknown fungus were found in all three samples during both seasons. Only Aspergillus niger was present in the surface water during both seasons. The results of this study showed that anthropogenic activities at Kara Abattoir adversely impacts the Ogun River, hence constituting potential environmental and public health risks. Strategic advocacy campaigns, strong evidence - informed policies or regulations, as well as provision of adequate facilities for effluent treatment are recommended to mitigate the current non-sustainable trends at the Kara Abattoir in Ogun state, Nigeria. This will support the efforts towards achieving the United Nations Sustainable Development Goal 3 (good health and wellbeing) and 14 (sustain life below water).*

Key words*: Abattoir, Contamination, Effluent, Fungi, River, sustainable development*

1. Introduction

The impact of wastewater on receiving aquatic ecosystems in developing countries have been variously studied and adjudged to contribute immensely to poor water quality [1]. In developing countries, such effluents which arise from industries, domestic sources, aquaculture and slaughterhouses, among others are often discharged untreated into the receiving surface waters [2]. Although, there are regulations and guidelines which set limits for effluent physico-chemical properties before discharge, attendant adverse impact still persists [2]. The direct nature of effluent inputs into recipient aquatic ecosystems can help in determining the source, nature of effluent and, potential adverse biological effects in receiving aquatic environments. The current study brings to fore the impact of activities at the

DOI: https://doi.org/10.4316/fens.2022.015

Kara Abattoir (a popular slaughterhouse in Lagos, Southwest, Nigeria), on the receiving aquatic ecosystem (Ogun River in particular). The Ogun River which is located in Southwest Nigeria, rises from Oyo State near Shaki at coordinates 8°41'0'N 3°28'0'E, flows through Ogun state and discharges into the Ikorodu axis of the Lagos Lagoon at coordinates 6.745589°N 3.34259°E (Fig. 1). It is a source of water for drinking, cooking and bathing for the Oyo as well as Ogun state communities living close to the River.

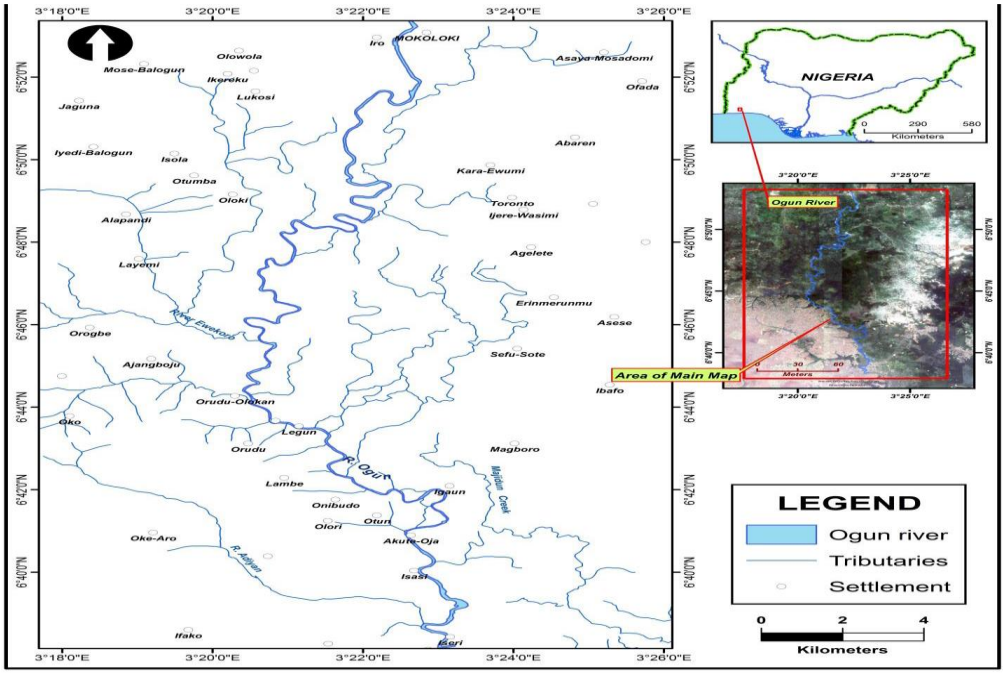


Fig. 1: Map of the Ogun River showing settlement Southwest Nigeria (Source: [3])

The Kara Abattoir is one of the approved centres for slaughtering and trading of cows in Nigeria. Alani *et al*. [1] estimated that more than half of the meat consumed daily in Lagos comes from Kara Abattoir. The Abattoir industry is an important segment of the livestock industry which provides meat (an important protein - rich food item) for many Nigerians and also provides jobs and income for many. Lagos State has a population of over 20 million people. Hence, it is expected that there would be a high demand for meat from such a large population. One of the major facilities meeting that demand is the Kara Abattoir. On a daily basis, animals are slaughtered and packaged at the Abattoir; a process which involves butchering, removal of animal hides, intestine management, rendering, trimming and cleaning [4]. This process generates large volumes of wastewater. Abattoir wastewater usually comprise oil, blood, organic and mineral solids, salts and chemicals added in the handling process [5, 6]. Abattoir wastewater has also been shown to contain microorganisms such as bacteria and fungi capable of causing diseases in living organisms [7, 8, and 9].

The lack of facilities for effective wastewater management coupled with ignorance on the short- and long-term consequences of improper disposal of the wastewater are potential cursors of indiscriminate discharge of untreated wastewater into the Ogun River. The consequences of this environmentally unsustainable practice, though considered

easy and convenient are multifaceted. The wastewater alters the physical, chemical and biological characteristics of the water. These changes disrupt the aquatic ecosystem and can lead to significant environmental and public health hazards [8]. Researchers have reported high presence of microbes and faecal coliforms, as well as unfavourable physical and chemical characteristics such as high turbidity, low dissolved oxygen, high Biological and Chemical Oxygen Demand and high nutrient status in the Ogun River around the Kara Abattoir and elsewhere [1, 2, 10, and 11].

While some researchers have reported bacterial contamination of the Ogun River as a result of activities at the Kara Abattoir [11], this study aimed to isolate and identify indigenous fungi in the abattoir effluent, the Ogun River surface water at the point of receipt of the effluent from the Kara Abattoir and in the gut of dominant fish (*Chrysichthys nigrodigitatus*) sample from the Ogun River near the Abattoir. The results from this study would identify any potential risk posed to fisheries and humans based on the nature of fungi identified as well as contribute to the holistic assessment of the Abattoir and its receiving water body in order to proffer targeted interventions to promote good health and wellbeing (United Nations Sustainable Development Goal (UN SDG) 3) and sustain life below water (UNSDG 14) from any fungal contamination at the Ogun River from the Kara Abattoir effluents.

2. Materials and methods

Sample Collection

The abattoir wastewater samples were collected at the point of its discharge into the Ogun River with a sterile glass container. The surface water samples were collected at some distance away from where the Abattoir wastewater was discharged into the river with a sterile glass container. Live fish samples (*Chrysichthys nigrodigitatus*) were also collected from the same points as the surface water. The samples were then properly labelled and placed in a cooler containing ice cubes which was transported to the laboratory at the Department of Microbiology, University of Lagos, Nigeria. The samples were collected during 2018 dry and rainy seasons. Serial dilutions of each of the effluent and water samples were done aseptically in the laboratory. The fish sample was dissected and the gut was removed aseptically. Approximately, 1 g of the gut was weighed and placed in 9 mL of sterile distilled water and shaken thoroughly, then serial dilution was carried out on the mixture up to 10^{-5} dilution. Thereafter, 10^{-2} and 10^{-3} dilutions were inoculated using spread plate method onto potato dextrose agar (PDA) plates in duplicates. The plates were incubated at 27°C (for both the dry and rainy season samples) for a period of five (5) days. Thereafter, the resulting moulds and yeast were sub-cultured on Sabouraud dextrose agar and left for another five (5) days at the same temperature [12].

Media preparation

Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were prepared following Manufacturer's instruction. All microbiological media were sterilized at 121ºC for 15 minutes [12].

Morphology and Biochemical Characterization of Isolates

Macroscopic and microscopic examination of isolates were determined following standard methods [12, 13].

DNA extraction and amplification of the ITS gene

DNA extraction

A measurement of 80 mg (wet weight) of fungal cell that had been re-suspended in

up to 200 µL of deionized water to a ZR BashingBead™ Lysis Tube was obtained. It was secured in a bead beater fitted with a 2.0 mL tube holder assembly (Scientific Industries' Disruptor Genie™, Cat. No. S6001-2 from Zymo Research Corp.) and processed at maximum speed for 5 minutes. The ZR BashingBead™ Lysis Tube was centrifuged in a micro-centrifuge at ≥10,000 x g for 1 minute. Up to 400 µL supernatant was transferred to a Zymo-Spin™ IV Spin Filter (orange top) in a Collection Tube and centrifuged at 7,000 rpm (7,000 x g) for 1 minute. The base of the Zymo-Spin™ IV Spin Filter was snapped off prior to use. 1,200 µL of Fungal DNA Binding Buffer was added to the filtrate in the collection tube of step four. Thereafter, 800µl of the mixture from step five was transferred to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at 10,000 x g for 1 minute. The flow through was discarded from the Collection Tube and step six was repeated. Then, 200 µL DNA Per-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new Collection Tube and centrifuged at 10,000 x g for 1minute. Thereafter, 500 µL Fungal DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IIC Column was transferred to a clean 1.5 mL micro-centrifuge tube and 100µl DNA Elution Buffer was added directly to the column matrix. It was then centrifuged at 10,000 x g for 30seconds to elute the DNA [14].

PCR Amplification of the ITS Gene and Sequencing

Polymerase chain reaction (PCR) was carried out to amplify the ITS gene of the fungal isolates using the primer pair ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction was carried out using the Solis Biodyne 5× HOT FIREPol Blend Master mix.

PCR was performed in 25 µL of a reaction mixture, and the reaction concentration was brought down from 5× concentration to 1× concentration containing 1× Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphates (dNTP)(Solis Biodyne), 25pMol of each primer (BIOMERS, Germany) and 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne). Additional Taq DNA polymerase was incorporated into the reaction mixture to make a final concentration of 2.5 units of Taq DNA polymerase, Proofreading Enzyme, 2µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in an Eppendorf Vapo protect thermal cycler (Nexus Series U.S.A.) for an initial denaturation of 95°C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95°C; 1 minute at 58°C and 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder was used as DNA molecular weight standard [14, 15].

Biochemical Identification of Yeast Isolates

Biochemical tests which include Gram reaction, Carbohydrate fermentation and Germ Tube Test (GTT) were used to characterize Yeast isolates following the methods [12, 13].

Germ Tube Test (GTT)

This is a test used for differentiating *Candida albicans* from other yeast species, when *Candida albicans* is grown in human

or sheep serum at 37°C for 3 hours, they form a germ tube which can be detected with a wet KOH film as filamentous outgrowth extending from their cells. Approximately, 0.5mls of human serum was placed in test- tubes, a little inoculum was then placed into it using a well flamed inoculating loop. The tubes were then incubated at 37°C for 3 hours after which slides were made then viewed under the OPTIKA microscope Camera (magnification × 100 – × 400) and results were taken [16].

Data Analysis

In order to identify organisms, DNA sequence data generated from this study was blasted against ITS/16S rRNA type strain database on NCBI. Threshold for identification was set with reference to [17], with modification to accommodate species with high congeneric sequence divergence range. An isolate was assigned to species level if the best matching reference species showed ≥98% homology and the next best matching reference species showed at least 0.8% less sequence homology. A strain or isolate was assigned to genus level when there was 95% to 98% homology to the best matching species, or when more than one sequence entry of several species from the same genus showed ≥98% homology. For sequences with <95% homology to the best match, an assessment of the congeneric sequence divergence range was carried out by blasting an arbitrarily chosen reference sequence of a type organism for the genus of interest against the type organisms' ITS/16S rRNA gene sequence database. The sequence divergence observed in this blast hits was used to adjudge the congeneric sequence divergence range and this range was used to determine the plausibility of the identification for sequences with <95% homology to blast's best match. Where homology fell below 95% and without the support of congeneric sequence divergence value, Identification was considered unsuccessful. Phylogeny was constructed to support the identification by ITS sequence using Fast Minimum Evolution algorithm as deployed on NCBI and visualized on MEGA11 [18]. The counts of species and genus were utilized as an estimate of species and genus diversity respectively. In the estimation of the species diversity, unidentified isolates were considered as different from the identified species and therefore counted as different species. In the estimation of the genus diversity, as opposed to the treatment in the estimation of species diversity, unidentified isolates were removed from the analysis. Other descriptive and inferential statistics were carried out on R using the follow R packages: base [19], reshape2 [20], dplyr [21], ggplot2 [22], ggpubr [23], qpcR [24], knitr [25, 26 and 27]. The data wrangling was achieved using Reshape2 and dplyr; inferential statistics were carried out on R base; and data was visualized using ggplot2, ggpubr, qpcR and knitr.

3. Results

After five (5) days of incubation of the PDA plates which was inoculated with diluted effluent, surface water and fish gut samples (obtained both in the wet and dry seasons), the isolates described in Table 1 were observed. The moulds isolated were identified based on colonial morphology and microscopy at the end of the experiment, they were; *Aspergillus niger, Aspergillus flavus,* and other *Aspergillus* sp., *Fusarium oxysporum, Penicillium* sp., *Rhizopus stolonifer* and *Mucor* sp. (Table 2). The yeast isolates were identified based on biochemical test and colony morphology to be *Rhodotorula* sp., *Saccharomyces* sp. and *Candida albicans* (Table 3). PCR was done on six (6) isolates (3 from each season) in order to amplify their ITS gene (Figure 2). The fungal

species identified in the study are listed in Table 4.

In the abattoir effluents, *Saccharomyces* sp., *Candida albicans, Rhizopus stolonifera* and *Mucor* sp. were observed in both dry and wet seasons. *Aspergillus flavus* was observed in the effluents only in the dry season while *Rhodotorula mucilaginosa* was observed only in the wet season. In the surface water, *Saccharomyces* sp., *Candida albicans, Rhizopus stolonifera, Mucor* sp. *Penicillium citrinum* and *Aspergillus niger* were found present both in the dry and wet seasons. *Talaromyces* sp. was found in the surface water only during the wet season.

In the gut of fish samples collected from the Ogun River surface water, *Saccharomyces* sp., *Candida albicans, Rhizopus stolonifera, Mucor* sp., *Penicillium citrinum* and *Talaromyces* sp. were identified both in the dry and wet seasons. Fungal species found only in the dry season were *Aspergillus flavus* and *Aspergillus* sp. while *Penicillium* sp., *Rhodotorula mucilaginosa, Fusarium oxysporum* and *Penicillium* sp. were identified from the fish gut only in the wet season.

During the dry season it was observed that two (2) isolates that were present in the fish gut were absent in the effluent. Also, four (4) isolates that were in the fish gut and effluent, were absent in the surface water. More isolates were obtained in the fish than the effluent and surface water for both seasons. During the rainy season about seven (7) isolates were present in the fish gut which were not found in the effluent and surface water. There was only one (1) isolate that was present in the surface water and absent in both the fish gut and abattoir effluent for both the rainy and dry season. Only one (1) isolate was peculiar to the fish gut and effluent during the rainy season. For the molecular identification of selected isolates, quality genomic DNA was extracted from pure cultures and successful PCR amplification of the ITS region was achieved.

Sequences of the identified isolates have been submitted to the GenBank and the accession numbers are provided in Table 4. Identification by blast (homology search) was supported by *Fast Minimum Evolution phylogenetic analysis* (Figure 3A and 3B) and congeneric sequence divergence range (Table 5 and 6). Isolates R9 and R62 possessed sequence homologies of 99.13% and 98.73% to *Rhodotorula mucilaginosa* and *Aspergillus welwitschiae*, respectively (Table 4). However, these isolates did not fulfil the second condition for allocation to species: difference of at least 0.8% between the query sequence's homology to the best match and its (query sequence) homology to the second-best match. The second-best match to R9 is *Rhodotorula alborubescens* JCM 5352 (NR_153197.1) with a homology of 99.07%, and homology difference of 0.06% between the best and the second-best match. The uncertainty in identification created by this circumstance can also be seen in the phylogeny (Figure 3B (D)), where R9 clusters with both *R. mucilaginosa* and *R. alborubescens*. Similarly, the difference between the homology of R62 to *A. welwitschiae* CBS 139.54 (NR_137513.1) and its homology to *Aspergillus tubingensis* NRRL 4875 (NR_131293.1) is 0.7% and it is also observed that R62 clusters with *A. welwitschiae, A. foetidus* and *A. niger* on the phylogeny (Figure 3B [D]) This suggests that the ITS region alone cannot sufficiently identify these isolates to the species level.

Isolates C56 and R4 presented lower sequence homologies than the set threshold of 95% for genus level identification (Table 4). C56 presented a homology of 93.03% to *Penicillium citrinum*; the best match from the blast search. To justify the allocation of this isolate to the genus *Penicillium*, the congeneric ITS sequence

divergence range for the genus *Penicillium* was assessed through blast search: an independent blast search of GenBank's type organisms ITS database was carried out, using the standard organism *P. citrinum* NRRL 1841 (Accession Number: NR_121224.1) as the query ITS sequence. The result (Table 5) showed that *P. nothofagi* CBS 130383 (NR_121518.1), the tenth blast hit, has a sequence similarity of 87.89% to *P. citrinum* NRRL 1841. This suggests a congeneric sequence divergence range of ~12% and therefore justifies the allocation of C56, which has a sequence divergence of ~7% to *P. citrinum* NRRL 1841, to the genus *Penicillium*. Similar analysis was carried out with respect to R4 which has a similarity of ~93% with *Talaromyces annesophieae* and *Talaromyces domesticus*, using *Talaromyces domesticus* NRRL 58121 (Accession Number: NR_171608.1) as the query ITS sequence. The result (Table 6) reveals that *Talaromyces unicus* CBS 100535 (NR_157429.1), the 100th blast hit, has a sequence similarity of 84.97% with *T. domesticus* NRRL 58121. This suggests a congeneric sequence divergence range of ~15%, and thus justifies the allocation of R4, which has a sequence divergence of ~7% form *T. domesticus* NRRL 58121, to the genus *Talaromyces*.

A comparison of the identification by the different methods, showed slight disparity in the identification by morphology and identification by molecular method. Species diversity is higher in the wet than in the dry season (Figure 3A & amp; B). The Fish gut (FG) presented the highest species diversity (Figure 4A & amp; C. Similar patterns were observed in the Genus diversity, with Fish gut possessing the highest genus diversity among studied locations and wet season presenting a higher diversity than dry season (Figure 4 D, E & F). However, none of the observed values were statistically significant at p = 0.05. *Aspergillus flavus* and two other unidentified species of *Aspergillus* (C55 and C58), were unique to the dry season, while *Fusarium oxysporum*, *Rhodotorula* sp., two unidentified species of *Talaromyces* (R4 and R57) and three unidentified isolates (R6, R7 and R32) were unique to the wet season. No genus is unique to the dry season, meanwhile *Fusarium, Rhodotorula* and *Talaromyces* were isolate from only the wet season (Table 10). No unique species was isolated from the Abattoir Effluent (EF), meanwhile an unidentified species of *Aspergillus* (R62) was found unique to the Surface Water (SW), while the fish gut present 5 unique species which include *Fusarium oxysporum*, one unidentified species of *Talaromyces* (R4) and three unidentified isolates (R6, R7 and R3). No unique genus was found in the Abattoir Effluent and Surface water, while *Fusarium* is the only identified unique genus isolated from the Fish Gut (Table 10). Comparatively, the fish gut showed marked difference from the Abattoir Effluent and Surface Water, in its fungal flora. Common genera isolated from the wet and dry seasons were: *Aspergillus, Candida, Mucor, Penicillium, Rhizopus and Saccharomyces* which include *Candida albicans* and *Rhizopus stolonifera.* C53 is an unidentified isolate that is common to all locations and seasons. Common genera isolated from all sampled locations (Abattoir Effluent, Shallow Water and Fish Gut) are: *Aspergillus, Candida, Mucor, Rhizopus* and *Saccharomyces*. These also include *Candida albicans* and *Rhizopus stolonifera* (Tables 1, 3 & 4).

Table 1

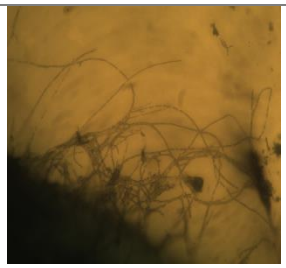
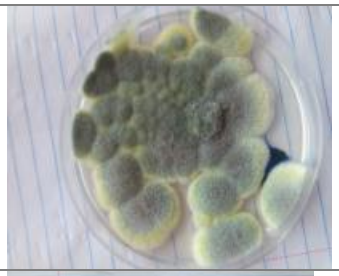
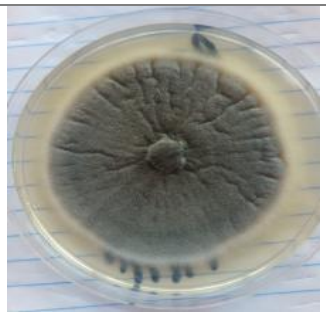
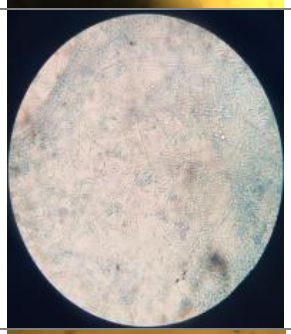
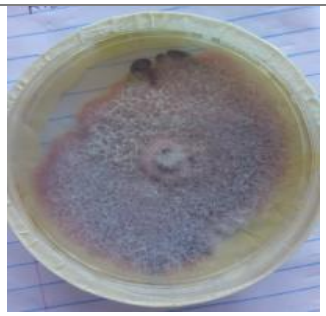
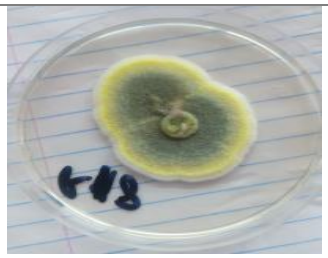
Colonial Morphology of Isolates and their Location

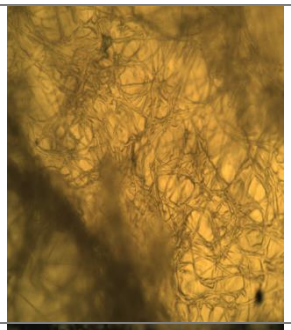
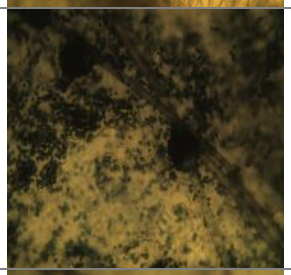
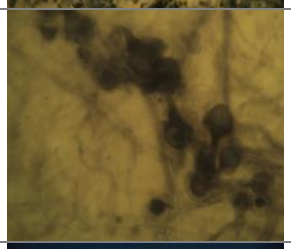
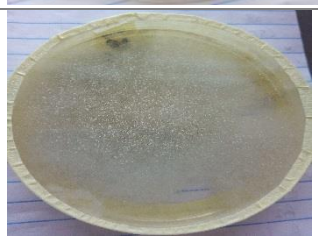
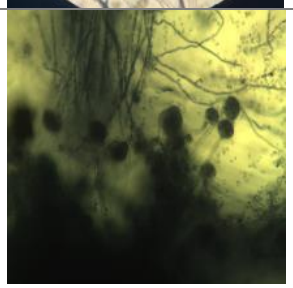
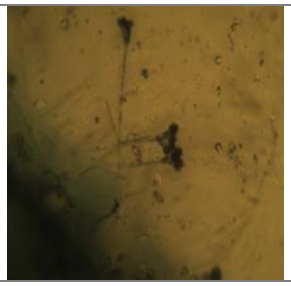
ISOLATES	COLONIAL MORPHOLOGY						DRY SEASON			RAINY SEASON		
	FORM	SIZE	ELEVATION	MARGIN	SURFACE	OPACITY	COLOUR	ET	FG	SW	ET	FG	SW
R2	Circular	Small	Raised	Entire	Wrinkled	Opaque	Cream	+	+	+	+	+	+
R4	Filamentous	Small	Raised	Curled	Rough	Opaque	Yellow ring with a blue-green centre	-	-	-	-	+	-
R6	Circular	Small	Flat	Filiform	Rough	Opaque	Light grey	-	-	-	-	+	-
R7	Circular	Big	Flat	Filiform	Wrinkled	Opaque	Grey	-	-	-	-	+	-
R9	Circular	Small	Raised	Entire	Smooth	Opaque	Pink	-	-	-	+	+	-
R11	Circular	Small	Raised	Entire	Smooth	Opaque	Cream	+	+	+	+	+	+
R12	Filamentous	Small	Flat	Filiform	Rough	Opaque	Whitish surface with pink background	-	-	-	-	+	-
R18	Filamentous	Small	Raised	Curled	Rough	Opaque	Yellow ring with a blue-green centre	-	-	-	-	+	-
R32	Filamentous	Small	Flat	Filiform	Rough	Opaque	Grey with white surrounding	-	-	-	-	+	-
C34	Circular	Small	Raised	Entire	Smooth	Opaque	Cream	+	+	+	+	+	+
C45	Filamentous	Big	Flat	Filiform	Rough	Opaque	White surrounding with dark green centre.	+	+	-	-	-	-
R47 And C47	Filamentous	Big	Convex	Filiform	Rough	Opaque	White with black patches.	+	+	+	+	+	+
R48	Filamentous	Big	Convex	Filiform	Rough	Opaque	White	+	+	+	+	+	+
C53	Circular	Small	Raised	Entire	Smooth	Opaque	Cream	+	+	+	+	+	+
C55	Irregular	Small	Flat	Lobate	Rough	Opaque	Pink with white surrounding	+	+	-	-	-	-
C56 and R56	Circular	Moderate	Raised	Filiform	Rough	Opaque	Greyish blue	-	+	+	-	+	+
C58	Circular	Moderate	Raised	Filiform	Rough	Opaque	Dirty green	+	+	-	-	-	-
R57	Circular	Moderate	Raised	Filiform	Rough	Opaque	Dirty green	-	-	-	-	+	+
C61	Filamentous	Big	Flat	Filiform	Rough	Opaque	Black	-	-	+	-	-	+
R61	Circular	Small	Raised	Entire	Smooth	Opaque	Cream	+	+	+	+	+	+
C62	Filamentous	Moderate	Flat	Filiform	Rough	Opaque	Light green	+	+	-	-	-	-
R62	Filamentous	Big	Flat	Filiform	Rough	Opaque	Black	-	-	+	-	-	+

Legend:
ET- Effluent
\+ Present
FG- Fish Gut
\- Absent
SW - Surface Water

Table 2

Identification of Fungal Isolates based on Microscopy and Colonial Morphology

Isolate	Microscopy image	Colony images	Identification of isolates based on microscopy and colonial morphology
1-R4			*Penicillium* sp.
2-R6			Unknown species
3-R7			Unknown species
4-R12			*Fusarium oxysporum*
5-R18			*Penicillium* sp.

6-R32			unknown species
7-C45			*Aspergillus flavus*
8-R47			*Rhizopus stolonifera*
9-R48		Not available	
10-C55			*Aspergillus* sp.
11-C56 and R56			*Penicillium citrinum*

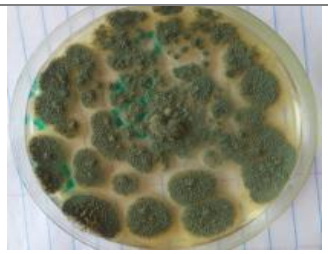
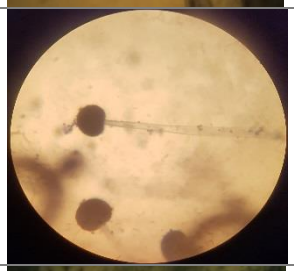
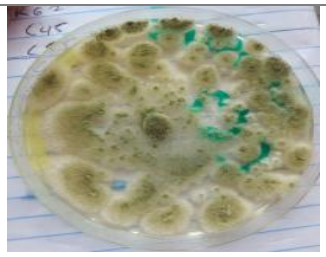
12-C58 and R57			*Talaromyces* sp.
13-C61			*Aspergillus niger*
14-C62			*Aspergillus flavus*
15-R62			*Aspergillus niger*

Table 3

Biochemical Characterization of Yeast Isolates

SAMPLES	GRAM STAIN	GF	SF	LF	GTT	PROBABLE IDENTITY
1-R2	+ve	+ve	+ve	+ve	-ve	*Saccharomyces* sp.
2-R9	+ve	-ve	-ve	-ve	-ve	*Rhodotorula mucilaginosa*
3-R11	+ve	+ve	+ve	+ve	+ve	*Candida albicans*
4-C34	+ve	+ve	+ve	+ve	-ve	*Saccharomyces* sp.
5-C53	+ve	+ve	+ve	+ve	-ve	Unknown species.
6-R61	+ve	+ve	+ve	+ve	-ve	*Saccharomyces* sp.

Legend:
GF- Glucose Fermentation; SF- Sucrose Fermentation LF- Lactose Fermentation
GTT- Germ Tube Test +ve = Positive -ve = Negative

Table 4

Molecular Identification of Selected Isolates.

Isolate	Identification		Sequence Accession Numbers	
	Description	**Percentage Identity**	**Reference Strain**	**Isolate**
C53	No Identification			
C56	*Penicillium* sp. *(*Closest species: *Penicillium citrinum*)	**93.03%	NR_121224.1	MK542006
C58	*Aspergillus* sp. (Closest species: 1. *Aspergillus austwickii* or 2. *Aspergillus aflatoxiformans*)	97%	1. NR_171607.1 2. NR_171606.1	MK542007
R4	*Talaromyces* sp. (Closest species: 1. *Talaromyces annesophieae* or 2. *Talaromyces domesticus*)	**92.88%	1. NR_170732.1 2. NR_171608.1	MK542008
R9	*Rhodotorula* sp. (Closest species: *Rhodotorula mucilaginosa*)	*99.13%	NR_073296.1	MK542009
R62	*Aspergillus* sp. (Closest species: *Aspergillus welwitschiae*)	*98.73%	NR_137513.1	MK542010

***Identification requires congeneric divergence range support.*
**The difference in percentage similarity between best and second-best matches is lower than 8%*

Table 5

Assessment of the congeneric sequence divergence range in *Penicillium sp.*

Blast hit position	Description (ITS Region from Type Material)	Accession	Percentage Identity
1	*Penicillium citrinum* NRRL 1841	NR_121224.1	100.00%
2	*Penicillium hetheringtonii* CBS 122392	NR_111482.1	99.40%
3	*Penicillium malacaense* NRRL 35754	NR_121344.1	93.43%
4	*Penicillium terrigenum* CBS 127354	NR_121515.1	88.40%
5	*Penicillium copticola* CBS 127355	NR_121516.1	88.25%
6	*Penicillium gallaicum* CBS 167.81	NR_103657.2	88.03%
7	*Penicillium raphiae* CBS 126234	NR_121511.1	88.24%
8	*Penicillium anatolicum* NRRL 5820	NR_121225.1	88.13%
9	*Penicillium argentinense* CBS 130371	NR_121523.1	87.99%
10	*Penicillium nothofagi* CBS 130383	NR_121518.1	87.89%

Table 6

Assessment of the congeneric sequence divergence range in *Talaromyces sp.*

Blast hit position	Description (ITS Region from Type material)	Accession	Percentage Identity
1	*Talaromyces domesticus* NRRL 58121	NR_171608.1	100.00%
2	*Talaromyces annesophieae* CBS 142939	NR_170732.1	99.45%
3	*Talaromyces pratensis* NRRL 62170	NR_165529.1	99.26%
4	*Talaromyces adpressus* CBS 140620	NR_171595.1	98.90%
5	*Talaromyces malicola* NRRL 3724	NR_165531.1	98.89%
96	*Talaromyces tiftonensis* NRRL 62264	NR_155920.1	84.41%
97	*Talaromyces endophyticus* ACCC 39141	NR_171599.1	87.71%
98	*Talaromyces yelensis* CBS 138209	NR_145183.1	84.99%
99	*Talaromyces emodensis* CBS 100536	NR_137077.1	84.88%
100	*Talaromyces unicus* CBS 100535	NR_157429.1	84.97%

Table 7

Fungal isolates from Kara Abattoir effluent, Fish gut and Surface water of Ogun River

Fungi Isolate	**Dry Season**			**Wet Season**		
	ET	FG	SW	ET	FG	SW
Saccharomyces sp.	+	+	+	+	+	+
Talaromyces sp. (R4)	-	-	-	-	+	-
Rhodotorula sp.	-	-	-	+	+	-
Candida albicans	+	+	+	+	+	+
Fusarium oxysporum	-	-	-	-	+	-
Aspergillus flavus	+	+	-	-	-	-
Rhizopus stolonifera	+	+	+	+	+	+
Mucor sp.	+	+	+	+	+	+
Aspergillus sp. (C55)	+	+	-	-	-	-
Penicillium sp (C56)	-	+	+	-	+	+
Aspergillus sp. (C58)	+	+	-	-	-	-
Talaromyces sp. (R57)	-	-	-	-	+	+
Aspergillus sp. (R62)	-	-	+	-	-	+
R6 (Unknown)	-	-	-	-	+	-
R7 (Unknown)	-	-	-	-	+	-
R32 (Unknown)	-	-	-	-	+	-
C53 (Unknown)	+	+	+	+	+	+

Legend:
ET*- Effluent*
FG*- Fish Gut*
SW*- Surface Water*
+ Present
- Absent

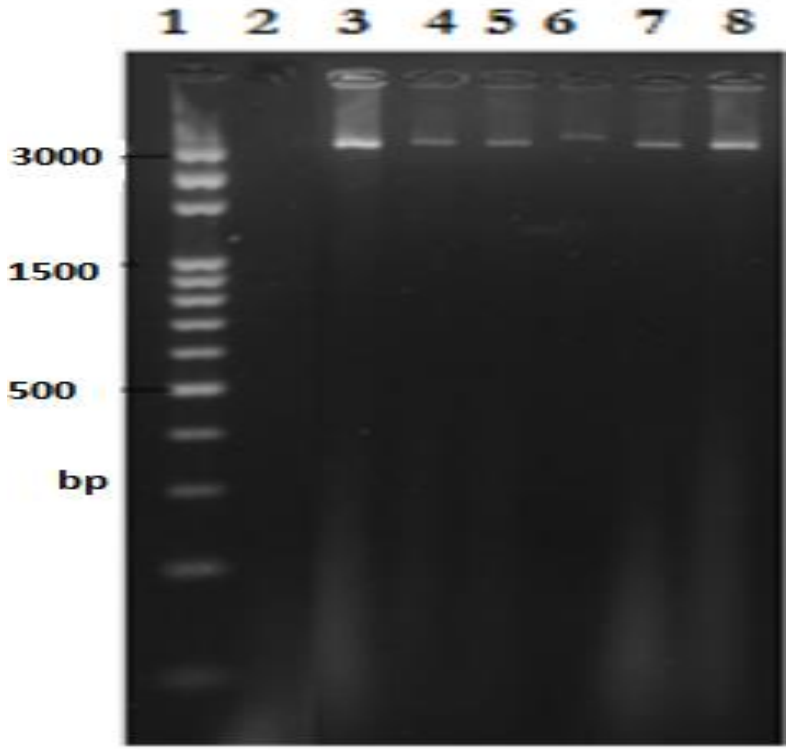


Fig. 2. DNA Extraction and Amplification

Lane 1= DNA Marker Lane 2 = Negative Control Lane 3= C53 Lane 4= C56
Lane 5 = C58 Lane 6 = R4 Lane 7= R9 Lane 8= R62

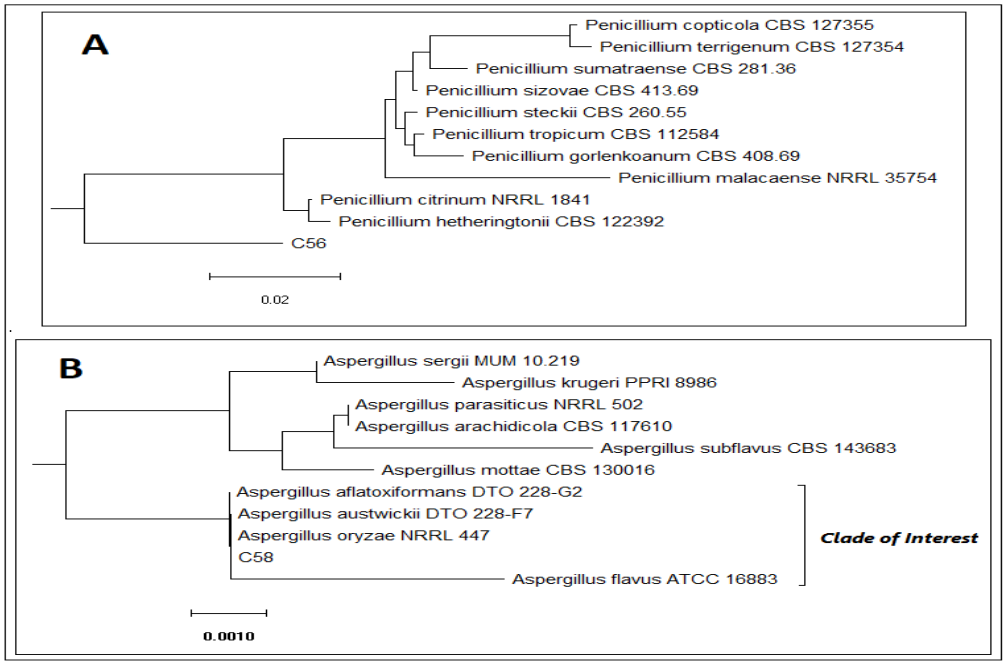


Fig. 3A. Molecular Identification of Selected Fungal Isolates: A. Isolate C56, B. Isolate C58

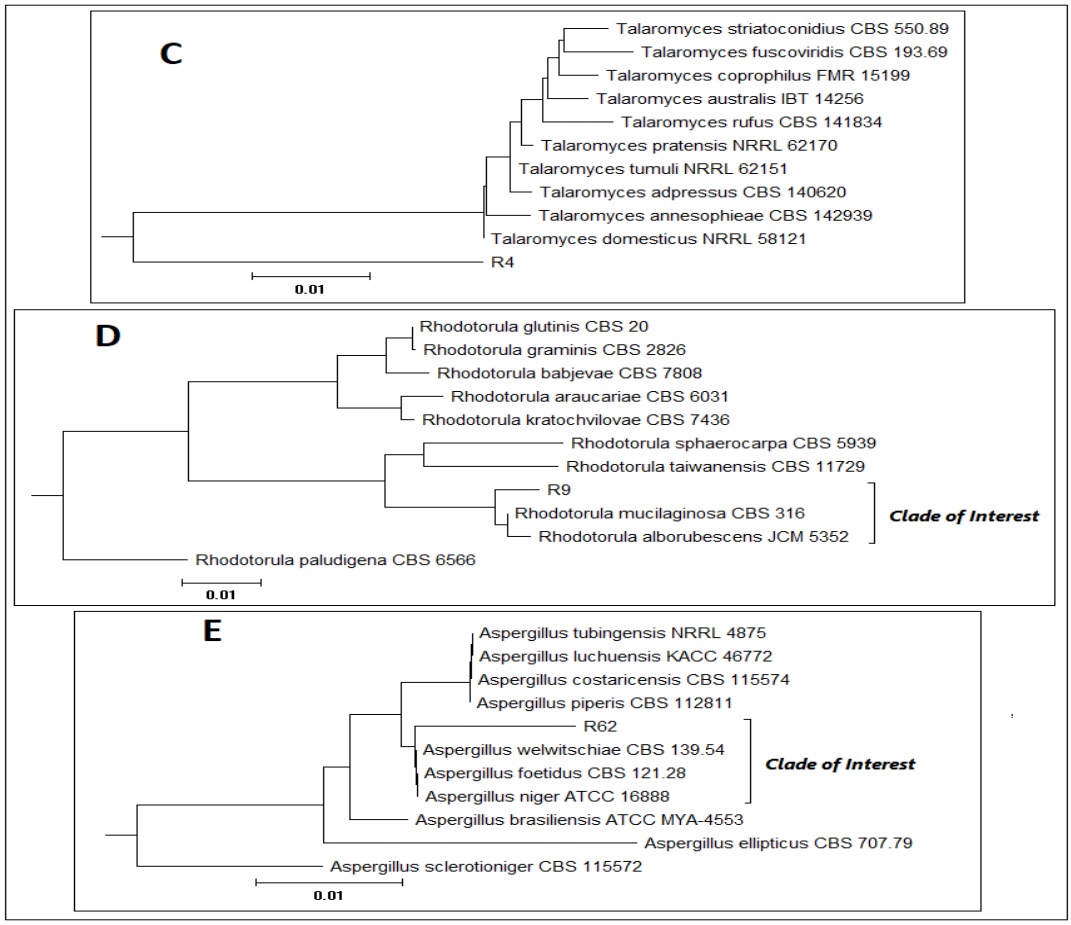
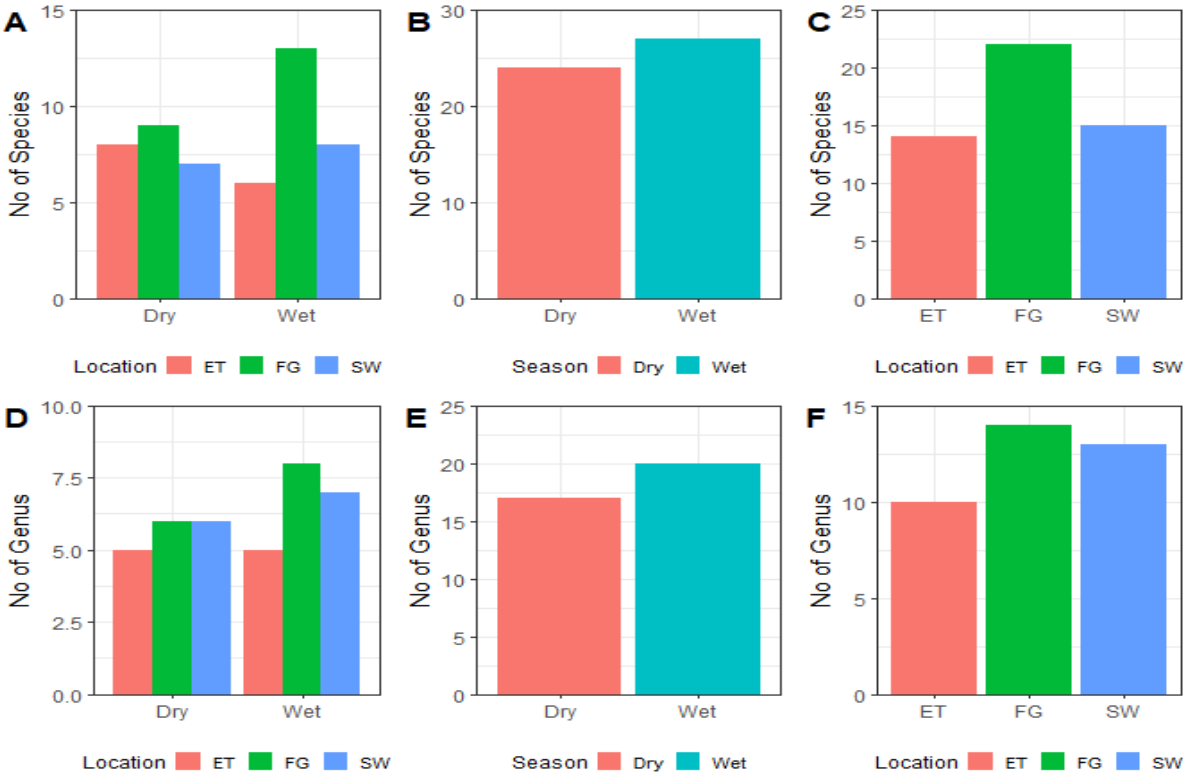


Fig. 3B. Molecular Identification of Selected Fungal Isolates: C. Isolate R4, D. Isolate R9, E. Isolate R62.



Legend: ***ET****- Effluen,* ***FG****- Fish Gut,* ***SW****- Surface Water*

Fig. 4. Genus and Species Diversities: A. Species Diversities in different seasons disaggregated by location, B. Species diversities in different seasons, C. Species Diversities in Different Locations, D. Genus diversities in different seasons disaggregated by location, E. Genus diversities in different seasons, F. Genus diversities in different locations.

4. Discussion

The results from this study showed that pathogenic fungi of different species were present in the Kara Abattoir effluent as well as in the surface water and fish gut from the Ogun River. The presence of fungi in Kara cow market effluent was also reported [9], who reported the presence of *Aspergillus niger, Fusarium sp.* and *Trichoderma* sp. in the Kara cow market effluent. [5] reported the presence of different species of fungi, namely *Trichoderma* sp. *Trichophyton* sp. *Aspergillus* sp. and *Scedosporium* sp. in wastewater from Lafenwa Abattoir in Abeokuta, Ogun state Nigeria. *Aspergillus* sp., Coccidioides sp., *Trichophyton* sp. and *Scedosporium* sp. were also reported to be present in surface water receiving Lafenwa Abattoir wastewater [5]. This corroborated the findings of this study.

In this study, there were similarities and variations in the fungal species found in the effluent, in the surface water and those found in the gut of the fish samples. Also, there were variations in the fungi species identified during rainy and dry seasons. The results showed that greater species variation was observed during the rainy season than during the dry season. This suggested climatic influence on fungal population dynamics. Increase in fungi variety during rainy season can be explained by the fact that the lower temperatures and higher humidity during rainy seasons favour propagation and growth of fungi in the respective media.

Abattoir effluents are rich in nutrients and organic matter, hence the presence of microbes such as bacteria and fungi which facilitate biodegradation of organic molecules. Some of these fungi are known to be pathogenic and can cause various types of diseases. The release of untreated effluent into the Ogun River exposes the water and the living organisms in it to various chemical and biological agents of pollution already present in the wastewater. This practice constitutes several environmental and public health hazards. *Rhodotorula mucilaginosa*, for example was observed in this study to be present in the effluent and fish gut during the wet season. *Rhodotorula* sp. are considered an emerging pathogen and were reported as the main cause of invasive fungal infections by non-Candida yeasts in China [28, 29]. *Rhodotorula* sp. were also reported as the most common microorganism isolated from the hands of hospital employees and patients and the third most commonly isolated yeast from blood cultures [29, 30]. *Rhodotorula* sp. have also been reported to cause meningeal, skin, ocular, peritoneal and prosthetic joint infections in humans whether immune-compromised or not [31]. *Aspergillus flavus* were also identified in the effluents and in the fish gut during the dry season while *Aspergillus niger* was observed during the wet season. *Asperillus flavus* is well known to produce aflatoxins (a toxic carcinogen) in the seeds of crops before and after harvest. Consequently, when spores of *A. flavus* are inhaled by humans, this leads to Aspergillosis (a health condition often characterised by lung infections and allergic reactions) especially in people with weak/suppressed immunity [32]. *Aspergillus niger* also produces mycotoxins (fumonisins and ochratoxins) which have been reported to be carcinogenic. When surface water becomes contaminated with such pathogenic fungi, they constitute risks to the living organisms in the water, to man and animals dependent on the water body, and even to crops (if such water is used in irrigation). For example, if such contaminated water is used for irrigation, crops become exposed to fungal contamination with consequences on food security and safety. The problem of plant pathogen in irrigation water has been noted as a growing challenge. Pathogens in irrigation water are often the primary/sole source of inoculum for diseases in crops [33]. The channels through which diseases can be spread from a fungi polluted water body to other living organisms are numerous (it could be through direct consumption by man/animals, consumption of contaminated fish /other aquatic organisms, use in irrigation of crops, domestic uses, and so on). It was also observed from the study that the batch slaughter system (in which animals are killed and processed sometimes on the bare floor or on corrugated roofing sheets) employed in Kara Abattoir is highly unhygienic and not environment friendly. A more eco-friendly slaughter systems should be adopted in the abattoir. In a study carried out previously to compare contamination levels in three different abattoir systems (namely: line, slaughter slab and batch), their findings proved that the batch slaughter system was least healthy/hygienic as it brought greater levels of contamination to the floor, butchering knives, workers' hands and effluent water. High level of contamination in the batch slaughter system is due to the fact that the system compromises standard operating procedures for running Abattoirs recommended by the World Health Organisation [34].

The release of Kara abattoir wastewater into the Ogun River has gone on for years despite several reports emphasising the negative effects of such practice [1, 9 and 11]. A careful analysis of the issue reveals that ignorance on the part of people, weak

policies, and poor infrastructure/facilities have caused this ecologically harmful practise to go on unhindered. To ensure meat is provided for people without threatening public health directly/indirectly through the process, we recommend the strategic advocacy/enlightenment, strong policy/regulation, and state of the art facility/infrastructure for Abattoir management. First, enlightenment campaigns should be designed and executed to reach the people carrying out these activities as knowledge on Abattoir best practises and on the harms caused by bad practises would stimulate a willingness to change and adjust; such willingness is an important precursor for change. Also, since people's practice are usually guided by set policies/standards, government must ensure that policies based on scientific evidence are crafted and implemented to regulate the slaughter houses and ensure adherence to best practises. It is important that government provide strong policies/regulations that compels provision of sufficient facilities/infrastructures that guarantees sustainable waste management development. Facilities such as slaughter floor, lairage, chiller, tripe room, power, water supply, effluent disposal, hide and skin processing should be provided so that the abattoir can function in harmony with the sustainable development goals. Lack of adequate facilities and poor management of abattoirs besides leading to environmental pollution could also lead to meat contamination and occupational hazard to the workers on the site [35]. It must also be stated that though sufficient facility, sound policies and strong advocacy require funds to institute, such cost must be seen as an investment because it yields tremendous gains for man and the environment. Besides preventing negative outcome in the environment and health, proper abattoir management has the potential of also yielding more financial gains and making abattoir management more economically and socially sustainable (thus cutting across the three arms of sustainability: planet, profits and people)[36]. Meanwhile, by properly managing waste and integrating secular economic principles in abattoir management, maximal value will be obtained from every by-product/left-over of the abattoir processes. Hence, there is need to overlook the short-term cost of eco-friendliness and focus on the long-term gain which far outweighs the cost [36, 37].

5. Conclusion

In conclusion different species of pathogenic fungi were isolated from Kara abattoir wastewater, Ogun River surface water and fish from the river. Some of the fungi species identified in this study were *Aspergillus flavus*, *Aspergillus niger*, *Rhodotorula mucilaginosa*, *Saccharomyces* sp., *Candida albicans*, *Rhizopus stolonifera* and *Mucor* sp., *Penicillium* sp., *Fusarium* oxysporum, *Talaromyces* sp. and *Penicillium citrinum*. Many of these species are known to cause various forms of diseases in living organisms, thus making the river unsafe for those dependent on it for various activities. In order to remedy this situation and protect the environment in this and other similar cases we recommend strategic advocacy campaign on eco-friendliness and harmony with nature.

6. Statements and declarations

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript. This is a self-funded research by the listed Authors.

Competing Interests: The authors have no competing interest to disclose because this study was self-sponsored by all the listed authors as a collaborative study. The

authors did not receive support from any organization for the conduct of the study and the submitted manuscript.

Author Contributions: All authors contributed to the study conception and design as well as preparation, data collection and analysis. All authors contributed equally to all the previous versions of the manuscript. All authors read and approved the final manuscript.

Dataset: All Dataset are available in GenBank and NCBI repository, the Accession numbers for isolates are displayed and trackable. (*www.ncbi.nlm.nih.gov/genome*).

Ethical Consent: Ethical approval is not required for this type of work in Nigeria, since we did not test any material on human and animal subjects.

Consent to Participate: The consent to participate in this study was given by each author.

Consent to Publish: The consent of each author was obtained prior to the commencement of the publishing process.

7. References

[1]. ALANI, R., ALO, B., UKOAKONAM, F. Preliminary investigation of the state of pollution of Ogun River at *Kara* Abattoir, Near Berger, Lagos. *International Journal of Environmental Science and Toxicology*, **2**(2): 11-23. (2014).

[2]. OLANIRAN, E. I., SOGBANMU, T. O., SALIU, J. K. Biomonitoring, physico-chemical and biomarker evaluations of abattoir effluent discharges into the Ogun River from Kara Market, Ogun State, Nigeria using *Clarias gariepinus*. *Environmental Monitoring and Assessment* **191**: 44. (2019).

[3]. FASHAE, O. A., FANIRAN, A. Downstream morphologic characteristics of alluvial section of lower river Ogun, Nigeria. *J. Environmental Geography* **8**(1-2): 1-10. (2015).

[4]. ELEMILE, O. O., RAPHAEL, D. O., OMOLE, D. O., OLORUNTOBA, E. O., AJAYI, E. O., OHWAVBORUA, N. A. Assessment of the impact of abattoir effluent on the quality of groundwater in a residential area of Omu-Aran, Nigeria. *Environmental Sciences Europe*, **31**:16. (2019).

[5]. ADEBOWALE, O. O., ADEYEMO, O. K., JAYEOLA, A. O., OJO, O. E., ADEBOWALE, O., KEHINDE O. O., KPEREGBEYI, E. A. Microbial characterization of the waste water from a major abattoir and its receiving surface water in Abeokuta, Nigeria. *Proceedings of the XVth International Congress of the International Society for Animal Hygiene, Vienna, Austria, 3-7 July 2011,* **3***:* 1131-1134. . (2011).

[6]. AKANGE, E. T., CHAHA, J. A., ODO, J. I. Impact of Wurukum abattoir effluent on River Benue Nigeria, using macroinvertebrates as bioindicators. *Int. J. Aquac*, **6**(22):1–11. (2016).

[7]. ADESEMOYE, A., OPERE, B., MAKINDE, S. Microbial content of abattoir wastewater and its contaminated soil in Lagos, Nigeria. *African Journal of Biotechnology* **5:**1963-1968. (2006).

[8]. NAFARNDA, W. D., AJAYI, I. E., SHAWULU, J. C., KAWE M. S., OMEIZA, G. K., SANI, N. A., TENUCHE, O. Z., DANTONG, D. D., TAGS, S. Z. Bacteriological quality of abattoir effluents discharged into water bodies in Abuja, Nigeria. *International Scholarly Research Notices (ISRN) Vet. Sci. Volume 2012 Article ID: 515689.* (2012).

[9]. SOGBANMU, T. O., SOSANWO, A. A., UGWUMBA, A. A. A. Histological, microbiological, physicochemical and heavy metals evaluation of effluent from Kara Cow Market, Ogun State, Nigeria in guppy fish (*Poecilia reticulata*). *The Zoologist* **17**: 54-61. (2019).

[10]. BALOGUN, S., AYANGBENRO, A., OGUNSANYA, G., AZEEZ, A., MUONAKA, C., IHONGBE, M. Bacteriological pollution indicators in Ogun River flowing through Abeokuta metropolis. *Journal of Science and Technology* **36:** 54-63. (2016).

[11]. ADESINA, A. O., OGUNYEBI, A. L., FINGESI, T. S., OLUDOYE, O. O. Assessment of Kara abattoir effluent on the water quality of Ogun River, Nigeria. *Journal of Applied Sciences and Environmenta*l Management **22**(9):1465-1470. (2018).

[12]. GERHARDT, P., MURRAY, R. G. E., COSTILOW, R. N., NESTER, E.W., WOOD, W. A., KRIEG, N. R., PHILLIPS, G. B. *Manual of methods for General Bacteriology*. American
a. Society for Microbiology (1981) Pp. 524.

[13]. SMITH, G. *SMITH'S INTRODUCTION TO INDUSTRIAL MYCOLOGY 7TH EDITION* (EDS.) A. H. S. ONIONS, D. ALLSOPP, H. O. W.

EGGINS. Publ. Pitman Press, Bath. Sjöstrom, (1981). Pp.125-152.
[14]. GUTER, R. L., KOSHLAND JR. D. E. The molecule of the year. *Science*, **246**: 1543-1544. (1990).
[15]. SOLANKI, G. Polymerase Chain Reaction. *International Journal of Pharmaceutical Research* **2**(3):98-102. (2012).
[16]. PINCUS, D. H., ORENGA, S., CHATELLIER, S. Yeast identification-past, present and future methods. *Medical Mycology* **45**(2):97-210. (2007).
[17]. WANG, X., FU, Y. F., WANG, R. Y., LI, L., CAO, Y. H., CHEN, Y. Q., ZHU, L. P. Identification of clinically relevant fungi and *prototheca* species by rRNA gene sequencing and multilocus PCR coupled with electrospray ionization mass spectrometry. PLoS One, **9**(5): e98110. (2014).
[18]. TAMURA, K., STECHER, G., KUMAR S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* **38**:3022-3027. (2021).
[19]. R CORE TEAM R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. (2021).
[20]. WICKHAM, H. Reshaping Data with the reshape Package. *Journal of Statistical Software*, **21**(12): 1-20. URL http://www.jstatsoft.org/v21/i12/. (2007).
[21]. WICKHAM, H., FRANÇOIS R., HENRY, L., MÜLLER, K., dplyr: A Grammar of Data Manipulation. R package version 1.0.7. https://CRAN.R-project.org/package=dplyr (2021).
[22]. WICKHAM, H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. (2016).
[23]. KASSAMBARA, A. ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0. https://CRAN.R-project.org/package=ggpubr (2020).
[24]. SPIESS, A. qpcR: Modelling and Analysis of Real-Time PCR Data. R package version 1.4-1. https://CRAN.R-project.org/package=qpcR (2018).
[25]. XIE, Y. knitr: A Comprehensive Tool for Reproducible Research in R. In: *Victoria Stodden, Friedrich Leisch and Roger D. Peng, (editors) Implementing Reproducible Computational Research*. Chapman and Hall/CRC. ISBN 978-1466561595. (2014).
[26]. XIE, Y. Dynamic Documents with R and knitr. (2nd edition). Chapman and Hall/CRC. ISBN 978-1498716963. (2015).
[27]. XIE, Y. knitr: A General-Purpose Package for Dynamic Report Generation in R. R package version 1.36. (2021).
[28]. XIAO M, CHEN SC-A, KONG F, FAN X, CHENG J-W, HOU X, *et al.* Five-year China Hospital Invasive Fungal Surveillance Net (CHIF-NET) study of invasive fungal infections caused by non-candidal yeasts: species distribution and azole susceptibility. *Infect. Drug Resist.* **11**:1659–67. (2018).
[29]. JARROS, I. C., VEIGA, F. F., CORRÊA, J. L., BARROS, I., GADELHA, M. C., VOIDALESKI, M. F., PIERALISI, N., PEDROSO, R. B., VICENTE, V. A., NEGRI, M., SVIDZINSKI, T. Microbiological and virulence aspects of *Rhodotorula mucilaginosa*. *EXCLI Journal*, **19**: 687–704. (2020).
[30]. GOMEZ-LOPEZ, A., MELLADO, E., RODRIGUEZ-TUDELA, J. L., CUENCA-ESTRELLA, M. Susceptibility profile of 29 clinical isolates of *Rhodotorula* spp. and literature review. *J Antimicrob. Chemother.* **55**: 312–316. (2005).
[31]. FERNANDA, W., LUCIANO, G. Epidemiology of *Rhodotorula*: an emerging pathogen. *Interdisciplinary Perspectives on Infectious Diseases*, **465717:**7. (2012).
[32]. CENTERS FOR DISEASE CONTROL AND PREVENTION FUNGAL DISEASES: Aspergillosis. Retrieved on August 10, 2021 from: *https://www.cdc.gov/fungal/diseases/aspergillosis/index.html*. (2021).
[33]. HONG, C. X., MOORMAN, G. W. Plant pathogens in irrigation water: challenges and opportunities. *Critical Reviews in Plant Sciences*, **24**: 181-208. (2005).
[34]. AYODE, O., OLAYIOYE, E. O. Microbiological Assessment of Housekeeping Practices and Environmental Impact of Selected Abattoirs in Lagos and Ogun States of Nigeria. *Journal of Applied Biosciences.* **99**: 9363 – 9372. (2016).
[35]. COOK, E. A. J., DE GLANVILLE, W. A., THOMAS, L. F., KARIUKI, S., BRONSVOORT, B. M., FEVRE, E. M. Working conditions and public health risks in slaughterhouses in western Kenya. *BMC Public Health* **17:** 14. (2017).
[36]. UNITED NATIONS ENVIRONMENT PROGRAM MAKING PEACE WITH NATURE: *A scientific blueprint to tackle the climate, biodiversity and pollution emergencies.* *https://www.unep.org/resources/making-peace-nature* (2021).
[37]. U.S ENVIRONMENTAL PROTECTION AGENCY. Science and Ecosystem Support Division (SESD) Operating Procedure Waste Water Sampling. Athens, Georgia https://www.epa.gov/sites/default/files/2015-06/documents/Wastewater-Sampling.pdf (2013).