Int.J.Curr.Microbiol.App.Sci (2022) *11*(07): 34-43

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 11 Number 07 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1107.005

Effect of Human Activities on the Population and Distribution of Fungi in Lobia Creek in Bayelsa State, Nigeria

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Keywords

Human activities, Lobia creek, health risk, fungi, *Aspergillus*, *Candida*

Article Info

Received:
10 June 2022
Accepted:
30 June 2022
Available Online:
10 July 2022

A B S T R A C T

Fungi are now gaining attention as a tool for water quality due to their implication in waterborne infections. The effect of human activities on the population, distribution and diversity of fungi in Lobia Creek was investigated. Water samples were collected from five designated stations along Lobia creek into separate sterile bottles. Samples were transported in ice packed coolers to the laboratory for analysis using standard mycological techniques. A total of 60 water samples were analyzed during the six months sampling period (August 2020 to January 2021). Results of fungal count for toilet, jetty, abattoir, drinking and control were $2.1\pm0.42\times10^4$cfu/ml, $1.1\pm0.14\times10^4$cfu/ml, $1.9\pm0.14\times10^4$cfu/ml, $7.0\pm0.04\times10^2$cfu/ml, and $7.0\pm0.01\times10^2$cfu/ml, respectively. The decreasing order of fungal population in the stations was; Toilet > Abattoir > Jetty > Drinking water > Control. Statistical analysis showed that, there was significant difference ($p<0.05$) across the sampled stations. Results also showed that the fungal counts recorded in the toilet station was not significantly different ($P>0.05$) from the counts recorded for the jetty and abattoir station despite being slightly higher but was significantly higher ($P<0.05$) than the counts recorded for drinking and control samples. Percentage occurrences for fungal isolates were: *Rhizopus* sp (2.3%), *Penicillium* sp (27.3%), *Mucor* sp (15.9%), *Candida krusei* (9.1%), *A. niger* (11.4%), *Saccharomyces* sp (4.5%), *Fusarium* sp (10.2%), *Aspergillus* sp (11.4%) and *Candida* sp (8.0%). The decreasing order of the types of fungi in the locations was; Toilet > Abattoir > Jetty > Control > Drinking water. The high fungal load and uneven distribution of fungal types in all stations is attributed to the type of human activity in respective station. The fungi reported are known potential pathogens that can cause respiratory, skin and soft tissue infections and poses health risk to the public.

Introduction

Water is an essential resource for human life. However, most of the surface water sources have been depleted due to pollution, climate change and poor solid waste management (Stephano, 2016) including other anthropogenic activities carried out on the water body and the surrounding area where the water is found. These pollutions have altered the quality of water making it unfit for human consumption. In a previous study, it was documented that out of the global water supplies

stored in glaciers, running surface water and groundwater, only about 0.6% freshwater is available for human consumption (Wurzbacher *et al.,* 2011). Water quality is a term used to describe particular water's physical, chemical, and biological characteristics for the intended use (Bhateria and Abdullah, 2015). These qualities are influenced by different factors. According to Iyerite *et al.,* (2021), the quality of rivers is influenced by numerous factors including weather, runoffs, waste discharges and other forms of human activities. A previous study documented those natural fluctuations and human activities (anthropogenic impacts including land use) are the major factors that influence the quantity and quality of water both at local, regional and global scale (Stephano, 2016). According to Attua *et al.,* (2014), the quality of water is influenced by seasonality and geographical location.

One of the major challenges faced in the availability of drinking water in respect to its quality is the contamination resulting from pathogenic microorganisms. These microorganisms such as bacteria, fungi, viruses, and parasites are known contaminants which have been associated with serious and mild waterborne diseases (Gunhild *et al.,* 2009). The occurrence of microorganisms with potential risks to human health especially to immunocompromised individuals in water bodies is well documented in previous studies (Babič *et al.,* 2017; Magwaza *et al.,* 2017). The microbiological quality of water is usually determined by determining the presence of *E. coli* which is an indicator microorganism suggesting the possible presence of pathogens in the water body but the over dependance on *E. coli* as a yard stick to determine potable water is challenging since other microorganisms such as enteroviruses and protozoa are more resistant to disinfection than *E. coli* (Ntombie *et al.,* 2019). This could imply that the absence of *E. coli* might not clearly express total microbiological quality of the water (WHO, 2011a). Pathogens such as bacteria, viruses and parasites have been established as contaminants of (Ntombie *et al.,* 2019), however, inclusion of fungi in the drinking water regulations is scarce and most national and international guideline documents (including the World Health Organization) list fungi among the "nuisance organisms" causing odour problems, and do not consider regular monitoring necessary (WHO, 2011; NHMRC, 2011). Gunhild *et al.,* (2009) opined that one of the reasons for this omission is the dearth of information on acute diseases occurring as a result of consumption of fungi contaminated water unlike those reported for bacteria, viruses and parasites. Although fungi have not been widely considered when discussing waterborne pathogens, recent studies now consider it as an emerging chronic water quality problem that require attention (Sonigo *et al.,* 2011; Ashbolt, 2015). With the unending human activities such as disposal of untreated wastes (domestic and industrial) and other factors influenced by anthropogenic activities on rivers or streams, the water body could experience fluctuations ranging from addition or removal of microorganisms. This study is therefore aimed at investigating the effect of human activities in the population and disribution of fungi in Lobia Creek, Bayelsa State, Nigeria.

Materials and Methods

Description of the Study Area

This research was carried out on surface water samples collected from Lobia Creek in Southern Ijaw region of Bayelsa State located in the central Niger Delta in Nigeria. The Lobia Creek is about 85km long with several communities located along its banks. The inhabitants are indigenous human population solely engaged in the occupation of fishing activities and a few are traders who travel to larger cities to buy foodstuff and other commodities for sale in Lobia. The communities along the creek engage in similar economic activities and so they generate similar waste and adopted the same method of disposal (Iyeritei *et al.,* 2022).

The geographic coordinates of Lobia are Latitude (width): 4°39'23.8"N (4.6566100°) and Longitude (length) 5°48'38.9"E (5.8108100°). Distances from Lobia to equator (0° lat) is 516 km north of the

equator to prime meridian (0° lon) is 645 km east of the prime meridian. The sampling stations were along the Lobia Creek area where Lobia communities are located.

Description of Sample Stations on Lobia Creek

The peculiar nature and sources of pollution from human activities along the study creek was the major factor that influenced the choice of sampled stations in this study. Five stations were chosen and designated as station 1, station 2, station 3, station 4, and station 5 respectively, for the purpose of this study. The choices of stations were based on sites where waste materials from human activities are channeled into or directly deposited into the creek. Station 1 is a toilet on wood planks where raw human feaces and urine are directly discharged by residents around the creek into the surface water without treatment, station 2 is a jetty where marine boats related and other anthropogenic activities are being carried out along the creek, station 3 is a fish abattoir dumpsite point where fishes are being slaughtered and many organic wastes are deposited, station 4 is the drinking water point where people around the creek use canoes to get water for domestic consumption. Station 5 is the last station and is located downstream to all other stations. It is located about 300 meters away from station 4 and is free of any human activities hence use as a control station. The Map of the study area is shown in Figure 1.

Collection of Water Samples from Stations

Prior to sample collection, sample bottles were sterilized by autoclaving at 121ºC for 15 minutes at 15psi. During collection of the samples, the necks of the bottles were slightly tilted upwards towards the water current. The bottle was allowed to get filled and the cover was replaced while still under water (Cheesbrough, 2006). Methods adopted in the collection of water samples were in accordance with APHA (2012). Collected sample in sample bottles were appropriately labelled with the station code number immediately after collection at each station, and stored in a portable cooler box containing ice pack before transporting to the laboratory for analysis. Two (2) samples were collected from each station and a total of 10 samples were collected during each visit from the five stations. A total of 60 creek water samples were collected and analyzed during the six months sampling period (August 2020 to January 2021).

Microbiological Analyses of Water Samples

Serial dilution

One millilitre each of the water samples was separately added to 9 ml of normal saline (diluents). After thorough shaking, further serial 10-fold (v/v) dilutions were made by transferring 1 ml of the diluted water sample to another tube of sterile normal saline (diluents) to a range of 10^{-3} dilutions (Prescott *et al.*, 2011).

Enumeration and Isolation of Fungi

The presence of fungi in water samples collected from the different stations were enumerated by inoculating aliquot (0.1ml) of 10^{-2} dilutions on Sabouraud Dextrose agar (SDA) plates in duplicates. The inoculated plates were incubated at 25^{o}C for 5-7 days (Oyibo and Obire, 2022). After incubation, the fungal plates were observed for growth, the colonies on the plates were counted and recorded and used to determine the fungal populations of the water samples while discrete colonies on plates were also sub-cultured on freshly prepared SDA plates.

Purification of Isolates

After incubation, pure isolates were obtained by picking (with a sterile inoculating needle) discrete culturally and morphologically different colonies from the various plates. These colonies were cultured on SDA plates and incubated at required at 25^{o}C for 3-5 days. This was done repeatedly until pure colonies void of contaminants and mixed cultures were obtained. Pure fungal isolates were preserved refrigerated in SDA slants.

Identification of Fungal Isolates

Spores of the fungal isolate were picked using a flamed inoculating needle and placed on a clean glass slide containing a drop of lactophenol cotton blue. The preparation was covered with a coverslip and then observed with the aid of a microscope. The presence or absence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata, and other microscopic characteristics, as well as cultural characteristics, were used in the identification of the fungal isolates (Kidd *et al.*, 2016).

Statistical Analyses

Statistical analyses were carried out using one-way ANOVA and mean separation was done using Tukey-Kram. The mean and standard deviation of the microbial counts including the percentages of occurrence and percentage susceptibility to antibiotics were obtained through descriptive statistics. All analysis was done on SPSS (version 25).

Results and Discussion

Results of the fungal counts obtained from five (5) sampled stations within Lobia Creek are presented in Table 1.

Results showed that the fungal count for toilet, jetty, abattoir, drinking and control station was 2.1± 0.42×10^4 CFU/ml, 1.1±0.14×10^4 CFU/ml, 1.9± 0.14×10^4 CFU/ml, 7.0±0.04×10^2 CFU/ml, and 7.0± 0.01×10^2 CFU/ml, respectively. The water samples collected from the toilet station had the highest fungal count while the control recorded the least. Generally, the decreasing order of the fungal population in the various locations of Lobia creek was; Toilet > Abattoir > Jetty > Drinking water > Control. Statistically, the varied fungal counts showed evidence of significant difference ($p<0.05$) across the sampled stations. Results also showed that the fungal counts recorded in the toilet station was not significantly different ($P>0.05$) from the counts recorded for the jetty and abattoir station despite being slightly higher but was significantly higher ($P<0.05$) than the counts recorded for drinking and control samples. Similarly, the fungal counts recorded for the jetty samples despite being higher than those recorded in the drinking and control samples, there was no significant difference ($P>0.05$) recorded.

The distribution and frequency of occurrence of the types of fungi isolated from the various sampled stations of Lobia Creek is shown in Table 2.

A total of seven (7) fungal genera were identified in this present study and they were; *Aspergillus*, *Candida*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Saccharomyces* The types of fungi that were isolated varied in their occurrence across the different stations. In the toilet station, six (6) genera of fungi; *Aspergillus*, *Candida*, *Fusarium*, *Mucor*, *Penicillium* and *Saccharomyces* were isolated while the jetty samples only had three (3) which include *Aspergillus*, *Mucor*, and *Penicillium*. The abattoir station recorded five (5); *Aspergillus*, *Candida*, *Fusarium*, *Mucor*, and *Penicillium*; Drink water station recorded (3) *Aspergillus*, *Fusarium* and *Rhizopus* while the control station also recorded three (3) fungal genera of *Aspergillus*, *Candida* and *Mucor*. The decreasing order of the types of fungi in the various locations was; Toilet > Abattoir > Jetty > Control > Drinking water.

Results of the distribution of fungal isolates across the sampled stations showed that a total of eighty-eight fungal isolates were isolated and these fungal isolates were not evenly distributed across the sampled stations. This implied that while some fungal isolates which occurred in certain stations were not isolated in other stations. For instance, *Aspergillus* was isolated from all the stations while *Mucor* sp was isolated from all except the drinking water station. *Penicillium* sp was isolated from all except the drinking water and control stations. *Fusarium* sp was isolated from all except the jetty and control stations while *Candida* species was isolated from the toilet, abattoir and control stations.

On the other hand, *Rhizopus* and *Saccharomyces* was isolated only from the drinking water and toilet station respectively. Results showed that the percentage occurrences for the isolates were: *Rhizopus* sp (2.3%), *Penicillium* sp (27.3%), *Mucor* sp (15.9%), *Candida krusei* (9.1%), *A. niger* (11.4%), *Saccharomyces* sp (4.5%), *Fusarium* sp (10.2%), *Aspergillus* sp (11.4%) and *Candida* sp (8.0%).

The results of percentage occurrence of the different genera of fungi isolated are presented in Figure 1. The decreasing order of the types of fungal genera was; *Penicillium* > *Aspergillus* > *Candida* > *Mucor* > *Fusarium* > *Saccharomyces* > *Rhizopus*.

This present study has revealed the population and types of fungi in the Lobia creek. The fungal load in all the stations including drinking water station was generally high. Water samples from the toilet station recorded the highest fungal counts while the least was recorded by the control station. Generally, the decreasing order of the fungal population in the various locations of Lobia creek was; Toilet > Abattoir > Jetty > Drinking water > Control. Statistical analysis showed that, there was significant difference ($p<0.05$) across the sampled stations. Although there is no regulation by the WHO or the Nigeria standard for drinking water quality (NSDWQ, 2008) of the acceptable fungal load in surface or drinking water as contained for bacteriological parameters but comparing with the Swedish Drinking Water Guidelines which specified a criterion of 100 CFU of micro-fungi per 100 mL in treated water as being fit for human consumption (Sammon *et al.,* 2010), it implies that the fungal load in the Lobia creek stations are above limits.

The high fungal loads in the water samples from toilet and abattoir stations could be attributed to the perceived high nutritional composition and favourable physiochemistry in these areas which is contained in the waste products of human and animals. The raw human faeces and slaughtered fish wastes in these stations served as nutrients to the fungi which proliferated in these stations of the creek. According to Magwaza *et al.,* (2017), terrestrial fungi are capable of migrating from soil into fresh water systems through animals, plants and soil. Fungi are ubiquitous and as such can be found in different environments especially as they are natural inhabitants of soil, water and decomposing plants (Korzeniewska, 2011; Calvo-Polanco *et al.*, 2016). More so, the availability or presence of these fungal isolates in all the stations might have been due to anthropogenic activities such as dumping of wastes into water bodies and other domestic and recreational activities that may have released the fungal spores into the water body. Fungal deposition in water distribution systems is attributed to spores and not hyphae growth. Other forms through which fungi could get into the water body could be through natural activities like erosion or other materials habouring these isolates that are in close contact with water bodies (Mholongo *et al.,* 2019). The persistence of fungal isolates and other microorganisms in the aquatic environment is as a result of direct contact with soil, wood and decomposing materials (Fox *et al.,* 2016).

The identified fungal genera in this present study were; *Aspergillus*, *Candida*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Saccharomyces*. Furthermore, the fungal isolates in the present study have also been reported in previous studies. *Aspergillus, Byssochlamys, Candida, Cephalosporium, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Saccharomyces, Sporobolomyces* and *Trichoderma* were reported by Obire *et al.*, (2009). Arroyo *et al.,* (2020) also reported the presence of *Aspergillus* sp, *Fusarium* sp and *Penicillium* sp from hospital water which are also similar to the fungal isolates in the present study. Although in their study, *Cladosporium* sp was the most common fungal isolate with wide distribution followed by *Penicillium* sp while in the present study, *Penicillium* sp was the most common fungal isolates followed by *Aspergillus*.

Table.1 Microbiological Load (CFU/mL) of the Lobia Creek

Parameter	Microbial Load Sampling Station				
	Toilet	Jetty	Abattoir	Drinking	Control
Fungal count	$2.1\pm0.42 \times 10^{4cd}$	$1.1\pm0.14 \times 10^{4abc}$	$1.9\pm0.14 \times 10^{4cd}$	$7.0\pm0.04 \times 10^{2a}$	$7.0\pm0.01 \times 10^{2a}$

Mean with different superscript (^{ab}) shows Significant Difference along columns ($P \leq 0.05$)

Table.2 Distribution, Diversity and Frequency of Fungi in Stations of Lobia Creek

Fungal Isolate	Occurrence of Fungi in the sampling Stations							
	Toilet	Jetty	Abattoir	Drinking	Control	Total Frequency	(%)	Total (%)
Aspergillus niger	6	0	3	1	0	10	11.36	
***Aspergillus* sp**	0	7	0	0	3	10	11.36	**22.72**
Candida krusei	8	0	0	0	0	8	9.10	
***Candida* sp**	0	0	5	0	2	7	7.95	**17.05**
***Fusarium* sp**	4	0	3	2	0	9	10.23	**10.23**
***Mucor* sp**	5	3	4	0	2	14	15.90	**15.90**
***Penicillium* sp**	7	5	12	0	0	24	27.27	**27.27**
***Rhizopus* sp**	0	0	0	2	0	2	2.27	**2.27**
***Saccharomyces* sp**	4	0	0	0	0	4	4.55	**4.55**
Total	**34**	**15**	**27**	**5**	**7**	**88**		
(%)	38.64	17.05	30.68	5.68	7.95			

Fig.1 Percental occurrence of fungal genera

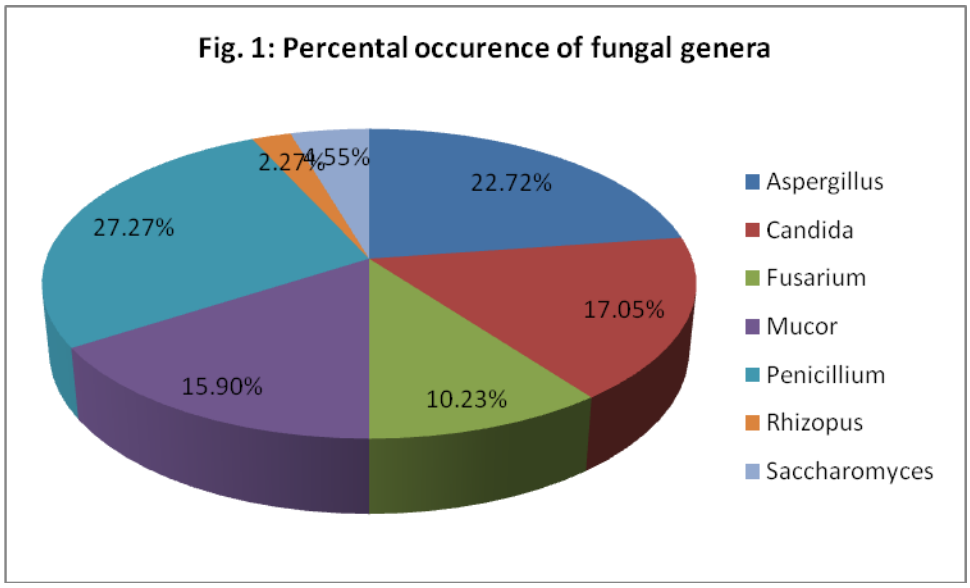
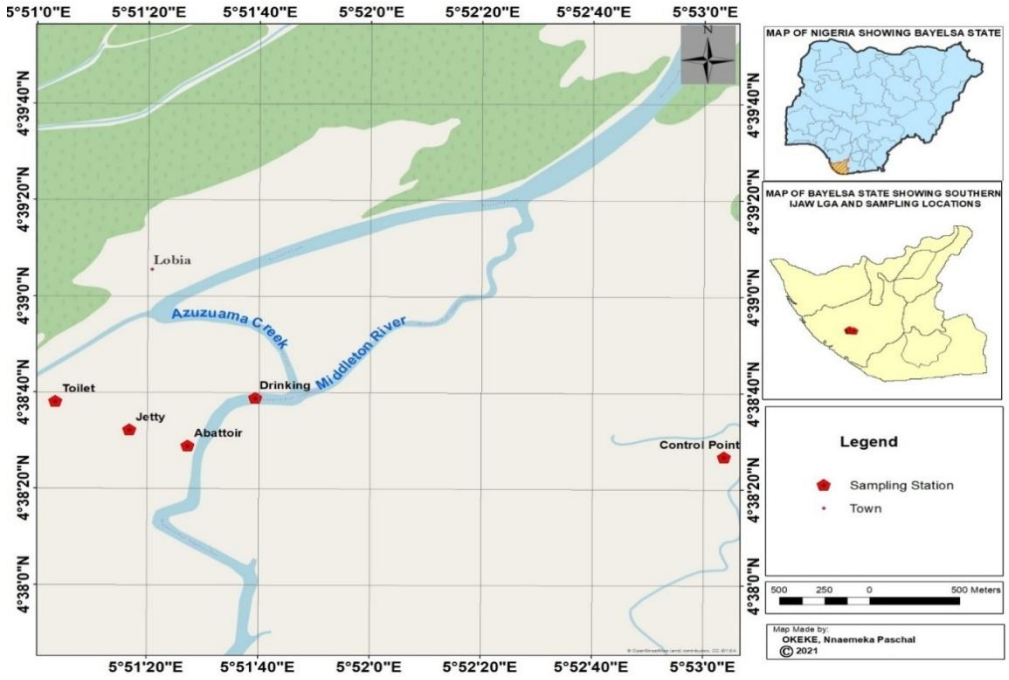


Fig.2 Map of the Study Area (Source: Iyerite *et al*., 2021)



The presence of *Mucor* sp, *Fusarium* sp, *Penicillium* sp, *Candida* sp, *A. niger* and other *Aspergillus* sp in drinking water, groundwater and surface water in a previous study (Babič *et al.*, 2017) corroborates the findings in the present study. The health implications of fungi as well as the activities of the mycotoxins especially in immunocompromised individuals are well documented (Prescott *et al.*, 2011). Pfaller and Diekema (2004) opined that these dematiaceous fungal isolates are the major cause of mycoses and other types of infections including cutaneous, subcutaneous, invasive, and contagious infections. More so, fungal genera such as *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, etc are known mycotoxin producers (Prescott *et al.*, 2011) and consumption of mycotoxins could lead to mycosis and vascular diseases (Arroyo *et al.*, 2020). Most of the isolated fungal genera contain species that are potential pathogens or opportunistic pathogens. The main hazardous species belong to *Aspergillus, Fusarium. Mucor*, and *Penicillium* (Alonso *et al.*, 2013). Various strains of these families of molds have been implicated in being causative agents in asthma, hypersensitivity pneumonitis and pulmonary mycosis. The high occurrence of *candida* species in the toilet and Jetty stations is of considerable concern as the genera can cause candidiasis, endocarditis, septicemia, protracted urinary tract infections, kidney and lung infections, esophagitis and other soft tissues infections. *Fusarium* species are common plant pathogens and causative agents of superficial and systemic infections in humans (Tupaki-Sreepurna, 2017; Askun, 2018). The presence of these organisms is a clear indication of the need to constantly monitor the water quality of the Lobia creek.

The proliferation of fungal isolates of *Fusarium* sp and *Aspergillus* sp in water reservoirs have been linked with waterborne infections (Kanzler *et al.*, 2007). In this present study, *Aspergillus*, *Fusarium*, and *Rhizopus* were the fungi isolated from the drinking water station. The presence of these fungal isolates in water bodies could also affect the water quality by altering the taste and producing offensive odour. This statement corroborates with Mhlongo *et al.*, (2019) who documented that vegetative growth of fungi in water could lead to taste and odour problem in water due to the presence of mycotoxins.

Although fungi were not included amongst the routine parameters for determination of water quality by the WHO, it was however labelled as nuisance organisms because it causes taste and odour problems in water (Sonigo *et al.*, 2011). This implied that fungi should not be found in drinking water. The transmission of pathogenic microorganisms by drinking water has continued to be a major cause of water-related diseases, as confirmed by the frequencies of outbreaks reported around the world (WHO, 2011b). The mycotoxins such as aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids produced by fungal isolates are said to be carcinogenic and have the ability to impair the immune system (Sonigo *et al.*, 2011; Magwaza *et al.*, 2017; Zain, 2011).

The fungal load or populations in the different stations were generally high and the disparity in fungal types across the stations is attributed to the different forms of human activities in each. Thus, human impacts could be the major influence on the fungal load and distribution in Lobia creek. More so, the fungal isolates included known potential pathogens which are serious public health problem not just only to those who consume the water directly without any form of treatment but also to those in close proximity to the creek who might inhale them as disturbances in the water could cause the release of fungal spores into the surrounding atmosphere.

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How to cite this article:

Iyeritei, Flora and Obire, Omokaro. 2022. Effect of Human Activities on the Population and Distribution of Fungi in Lobia Creek in Bayelsa State, Nigeria. *Int.J.Curr.Microbiol.App.Sci.* 11(07): 34-43.
doi: https://doi.org/10.20546/ijcmas.2022.1107.005