Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2020; 1(2): 21-28

www.microbiojournal.com
Received: 14-01-2020
Accepted: 18-02-2020

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Assessment of mycological abattoir waste water contaminated soil in Port Harcourt

Victoria Ginika Awari, Aleruchi Owhonka and Loveday Peace Adaniella

Abstract
Due to numerous anthropogenic activities, such as the use of abattoirs, Nigeria's environment is suffering from major issues like excessive levels of waste generation and an insufficient disposal infrastructure. The release of untreated wastewater into nearby soils and water bodies has had a harmful impact on the ecosystem. The purpose of the study is to evaluate the effects of various abattoir wastewater on the population of soil fungi. Soil samples polluted with abattoir effluent were taken at random from three distinct abattoir locations in Port Harcourt, whereas a control sample of uncontaminated soil was taken from a farm at Rivers State University. The following day, all the samples were collected, and they were all subjected to mycological analysis. To isolate and count fungi, Sabouraud Dextrose Agar and lactophenol stain were utilized. The fungal counts in the contaminated soil ranged from 2.7 x 10^5 to 4.2x10^6 cfu/g, while the total heterotrophic fungi (THF) count in uncontaminated soil had 1.4x10^4 cfu/g. Fungi isolated from all the representative samples were identified as: *Candida* spp., *Trichoderma* spp., *Fusarium spp*, *Aspergillus fumigatus* spp., *Penicillium* spp. and percentage occurrence of isolates were determined. The result showed that the contaminated soil had higher fungal counts than the uncontaminated soil, reasons could be that the physicochemical constituents in the abattoir wastewater favours their growth.

Keywords: Abattoir, Environment, Fungi, Wastewater

Introduction
Untreated abattoir effluents are indiscriminately discharged into the ground or surface water bodies, which causes substantial surface and groundwater contamination, as the main cause of surface and groundwater pollution. Health risks and human mortality are being brought on by this decline in water quality. Although surface and ground water are the easiest to get, they are also the most contaminated due to human activity. Particularly in rural parts of developing nations like Nigeria, where the majority of the population relies on ground water sources like streams, rivers, ponds, lakes, wells, and boreholes, etc. However, as a result of the population's ongoing growth and the consequent rise in anthropogenic activity, the water supply situation is continuously getting worse. Over the years, this problem is aggravated by inadequate awareness, scarce financial resources, lack wastewater treatment facilities, and the inefficient ineffective environmental laws.

Since human activities like meat processing and animal production have been shown to have a negative impact on soil and natural water composition, resulting in pollution of the soil, natural water resources, and the entire environment (Olanike, 2002) [20], abattoir activities may be another source of pollution.

Abattoir operations in Nigeria are not well regulated. The main responsibilities of a slaughterhouse are to receive and hold cattle, kill animals, and prepare their bodies. According to Olanike (2002) [20], these processes are commonly ignored by untrained laborers or butchers who perform these operations in inadequate facilities in Nigeria. Additionally, there is a rise in demand for abattoir products that can fulfill the wants of the populace because Port Harcourt is a major hub for the processing of crude oil, a well-liked tourist destination, and a settlement for farming and fishing. This has caused the city of Port Harcourt to see an increase in the number of abattoirs and their haphazard placement. Concerns have lately been raised about the increasing and indiscriminate abattoir effluent discharges into the environment because to the harm they do to the ecosystem, aquatic life, plants, animals, and people (Otolorin *et al*., 2007) [11]. Canneries, milk dairies, sugar mills, breweries, distilleries, the meat and beverage industries, pulp and paper mills, tanneries, and yeast producers are just a few of the domestic and commercial sources of wastewater.

According to Liu and Haynes (2011) [21], untreated wastewater discharges frequently contain significant quantities of pollutants, nutrients, and pathogens. The meat business is one of the industries that produces a substantial amount of wastewater due to the rise in human meat consumption. Agriculture and allied industries consumed the most water in Australia in the years 2009 to 2010, accounting for more than 50% of all water use (Choudhary *et al*., 2011) [22]. These industrial operations need a lot of water to make products of high quality and please customers. Through point sources or nonpoint sources, abattoir effluents infiltrate water bodies, degrading ecosystems and lowering oxygen levels in the water, harming aquatic life and perhaps having fatal consequences. According to Adesemoye (2006) [2], the increased microbial growth brought on by the organic fertilizers added to groundwater causes the water from this source to taste and smell foul. Various environmental challenges affect the industrial sector globally, notably in terms of sustainable management. Due to the rigorous environmental regulations in the majority of industrialized nations, businesses were compelled to use pricey remediation methods like phytoremediation, land treatment, and constructed wetlands (Hoekstra and Chapagain, 2007) [23]. Low-cost wastewater management technology development is necessary to treat wastewater from various sources. However, regular effluent discharge increases the levels of nutrients, organic matter, and heavy metals, posing a number of dangers to the environment, surface and underground water resources, as well as land degradation (Choudhary *et al*., 2011) [22]. Abattoir wastewater that has not been treated is not acceptable for reusing or releasing into the environment. It will result in serious environmental risks in the receiving environment, including eutrophication, land degradation, nutrient leaching, ground water contamination, greenhouse gas emissions, and effects on ecosystem value (Tjandraatmadja *et al*., 2012) [24]. As a result, it is crucial to properly reduce pollutant levels in the prior stage. Wastewater contains a variety of bacteria that can contaminate water sources and cause the spread of infections (Mittal, 2004) [25]. Numerous illnesses, including cholera, typhoid, and dysentery, may result from this (Idika, 2002) [26]. According to the Organization for Economic Co-operation and Development and Food and Agricultural Outlook (2011), large populations of E. coli and Salmonella sp. in wastewater released from slaughterhouses cause the occurrence of meat-based infections. Poorly treated wastewater discharges that may contain heavy metals, organic chemicals, inorganic compounds, soluble salts, and pathogens pose major risks to the environment and to people (Anderson *et al*., 2006) [27] are the main cause of soil contamination. Continuous abattoir effluent outflow causes soil pollution, which has an impact on soil biodiversity and production. The following problems have a negative impact on the availability of productive land and clean water resources. According to Lui and Haynes (2011) [21], abattoir waste water also contains a sizable number of pollutants that, if not adequately handled, could induce disease outbreaks, decrease the value of the land, and destabilize the soil microflora. The amount of physiochemical elements like nitrogen and phosphorous in soil and water may be considerably increased by abattoir waste water. While these elements may be good for the soil, they represent serious health risks in drinking water. The soil and environment are known to be contaminated by slaughterhouses either directly or indirectly as a result of their many processes. The majority of the waste produced by abattoirs can pose risks to the environment and human health, including oil, minerals, organic solids, salts, and chemicals added during operation. According to Tamenach and Tamirat (2007), animal waste such as feces, urine, and blood typically include considerable amounts of harmful microbes, with fungi being one of the most prevalent. Fungi also thrive in the damp conditions that are typical of abattoirs, which explains why they are abundant in both the atmosphere and the waste products of these establishments. The need for this research stems from the fact that pathogenic fungus present numerous health risks if left unchecked. Research on the microbiological quality of soil and water contaminated by abattoir effluents has been conducted by a number of researchers. Other researchers have looked into the effects of poor abattoir management on the well-being of local residents and the health of their communities. In order to raise awareness about the detrimental effects to the environment, the current study aims to evaluate the mycological effects of abattoir wastewater discharge in the Port Harcourt environment with an emphasis on quantifying the fungal load associated with abattoir wastewater.

Materials and Method

Study area

Port Harcourt is the state capital of Rivers State, Nigeria. It lies along the Bonny River. The city is located in the Niger Delta, at approximately latitude 4.8472^{o}N and longitude 6.9746K^{2}, with a population of about 1,865,000 according to 2006 population census.

The sampling sites of the three abattoirs and the university farm and the coordinates of their geographical positioning system (GPS) locations are presented in Table 1.

Table 1: GPS coordinates of sampling sites

Sampling sites	Coordinate of sampling sites
Rumuokoro Abattoir	(Latitude: 4.8697784. Longitude: 69992025).
Fimie Abattoir	(Latitude: 4.7872057, Longitude: 7.036542).
Mgboshimini Abattoir RSU Farm site	(Latitude: 4, 8011377, Longitude: 6970704). Latitude 6,583912, Longitude 4,481850.

Collection of Soil Samples

From a total of four (4) different sampling locations, soil samples were collected. In order to sample the soils polluted with abattoir wastes, samples were taken at three (3) separate locations. These samples were bulked and thoroughly mixed to create composite representative samples for each sampling site, which were then used to determine the microbiological analysis of the soils. Reference point A was designated at the point of discharge of the effluent from the abattoir into the soil, point B was designated at a distance of about 30m from the reference point, within the point of discharge, and point C was designated at approximately 120m from point B. The abattoir soil samples were collected using sterile soil anger

at a depth of 0-20cm and sterile sample bottles to minimize contamination according to (Cheesbrough, 2005) [5].
Soil samples were also collected from Rivers State University, school farm observing protocols without contamination to serve as control. All the samples were properly labeled accordingly, aseptically bagged, placed in sterile ice packs and transported immediately to the microbiology laboratory, Rivers State University, for further analysis (Cheesbrough, 2005) [5].

Microbiological analysis of the soil samples
One gram of the representative soil samples were diluted, and then, the appropriate dilution factor was obtained and used for further microbiological analysis according to (Cheesbrough, 2005) [5].

1- Isolation and enumeration of fungi
The total heterotrophic fungi count (THFC) was determined by spread plate technique. An aliquot of 0.1ml was aseptically placed in triplicates from 10^{-3} and 10^{-4} dilutions onto freshly prepared sterile Sabouraud Dextrose Agar (SDA) plates. The inoculated plates were incubated at room temperature for 3-5 days. The fungi mixed culture obtained after incubation were well-labeled accordingly and counted as colony forming unit per gram. The fungi colonies were counted based on their colour on the surface and reverse side of each plate and reported as frequency of recurrence of isolated fungi per sample.

2- Sub-culture and preservation of fungal isolates
Fungal isolates were sub-cultured on freshly prepared Sabouraud Dextrose Agar plates for each of the distinct colonies formed. A sterile inoculating loop and pin was used to make streaks of each of the colonies on the medium and incubated at 37 ºC for 72 hours to obtain pure cultures of the various isolates while, the distinct fungal colonies were preserved and maintained on Sabouraud Dextrose Agar slants in bijou bottle, separately and properly labeled, stored in the refrigerator at 4 ºC for further and used as stock culture.

3- Identification of fungi isolates
The fungal cultures were identified on the basis of macroscopic (colonial morphology, colour, texture, shape, diameter and appearance of colony) and microscopic septation in mycelium, presence of specific reproduction structure, shape and structure of cordial and presence of mycelium characteristics pure cultures of fungi isolate were identified with the help of normal of fungi atlas (APHA, 2005; Watanabe, 2010) [3, 16].

Results

The Results of the Fungal Counts (CFU/ml) of the Different Soil Samples
The results of the fungal counts (CFU/ml) of the different soil samples are presented in Table 2. The total heterotrophic fungal (THF) counts in all the representative samples (contaminated and uncontaminated soils) ranged from 2.7 x 10^5 to 4.2x10^6 cfu/g. The total heterotrophic fungi (THF) count in uncontaminated soil had 1.4x10^4 cfu/g. Fimie abattoir contaminated soil sample had the highest microbial (Fungal) count of 4.2 x 10^6 CFU/g, followed by Rumuokoro Abattoir contaminated soil with 3.0x10^6 CFU/g and then, Mgbuoshimini Abattoir contaminated soil had the least fungal count of 2.7 x10^6 CFU/g while, the control sample had fungal counts of 1.4x10^4 CFU/g.

Table 2: Mean Fungal Counts of soil samples from abattoir wastewater discharge and Rivers state University farm

Sample site	Fungal counts (cfu/g) contaminated soils	Fungal counts (cfu/g) uncontaminated soils
Rumuokoro	3.0 x 10^6	1.4 x 10^4
Fimie	4.2 x 10^6	1.4 x 10^4
Mgbuoshimini	2.7 x 10^5	1.4 x 10^4

The Result of the Frequency/Percentage Occurrence of Fungal Isolates
Tables 3. show the frequency of occurrence and the percentage occurrences of fungal isolates from the various contaminated soils in increasing order as follows: *Mucor* spp. (12.5%)/15 < *Fusarium* spp. (13.3%)/16 < *Penicillium* spp. (20.9%)/25 < *Candida* spp. (23.3%)/28 < *Aspergillus* spp. (30.0%)/36. From the results, *Aspergillus* spp. recorded the highest percentage and frequency of occurrence whereas, *Mucor* spp. had the least percentage and frequency of occurrence.

Table 3: Percentage/Frequency of occurrence of fungal isolates from contaminated soil samples

Isolate code	Fungal isolates	Frequency of occurrence	Percentage (%) occurrence
CSAS	*Aspergillus* spp.	36	30.0
CSMS	*Mucor* spp.	15	12.5
CSPS	*Penicillium* spp.	25	20.9
CSCS	*Candida* spp.	28	23.3
CSFS	*Fusarium* spp.	16	13.3
Total		120	100

Tables 4. show the frequency of occurrence and the percentage occurrences of fungal isolates from the uncontaminated soil sample in increasing order as follows: *Trichoderma* spp. (10.6%)/2 < *Penicillium* spp. (21.0%)/4 < *Fusarium* spp. (26.3%)/5 < *Aspergillus* spp. (42.1%)/8. From the results, *Aspergillus* spp. recorded the highest percentage and frequency of occurrence whereas, *Trichoderma* spp. had the least percentage and frequency of occurrence.

Table 4: Percentage/Frequency of occurrence of fungal isolates from uncontaminated soil sample

Isolate code	Fungal isolates	Frequency of occurrence	Percentage (%) occurrence
USTS	*Trichoderma* spp.	2	10.6
USFS	*Fusarium* spp.	5	26.3
USAS	*Aspergillus* spp.	8	42.1
USPS	*Penicillium* spp.	4	21.0
Total		19	100

The Result of the Fungal Population of the Soil Samples
Table 5. Shows the fungal population isolated from then representative soil sample. The fungi common to both contaminated soil samples and the uncontaminated soil sample were *Aspergillus* spp.(CSAS) and *Penicillium* spp.(CSPS) Also, *Candida* spp.(CSCS) and *Mucor* spp.(CSMS) were common to both Fimie Abattoir contaminated soil and Rumuokoro abattoir contaminated

soil. Plates 1, 5, 2 and 4 show their culture plates on sabouroud dextrose agar (SDA) respectively, while, in addition, *Fusarium* spp. was present in both Fimie Abattoir contaminated soil Mgbuoshimini abattoir contaminated and Rivers State University farm used as the uncontaminated soil samples, plates 3 shows the culture plate of *Fusarium* spp. (USFS) on sabouroud dextrose agar (SDA) accordingly. Whereas, *Trichoderma* spp. was isolated in addition only, from the Rivers State University farm used as the uncontaminated control soil sample. Plates 6 also shows the culture plate of *Trichoderma* spp. (USTS) on Sabouroud dextrose agar (SDA) accordingly.

Table 5: Fungal population from the different sample sites

Sample Site	Suspected Fungal Organisms
Rumuokoro abattoir contaminated soil	Aspergillus fumigatus Mucor spp. Candida spp. Penicillium, Aspergillus niger, Aspergillus flavus
Fimie Abattoir contaminated soil	Aspergillus flavus, Mucor spp., candida spp. Penicillium spp. Aspergillus niger, Fusarium spp.
Mgbuoshimini abattoir contaminated soil	Aspergillus niger, Penicillium spp. Fusarium spp.
Rivers State University farm pristine soil	Penicillium oxysporum, Trichoderma spp. Aspergillus fumigatus

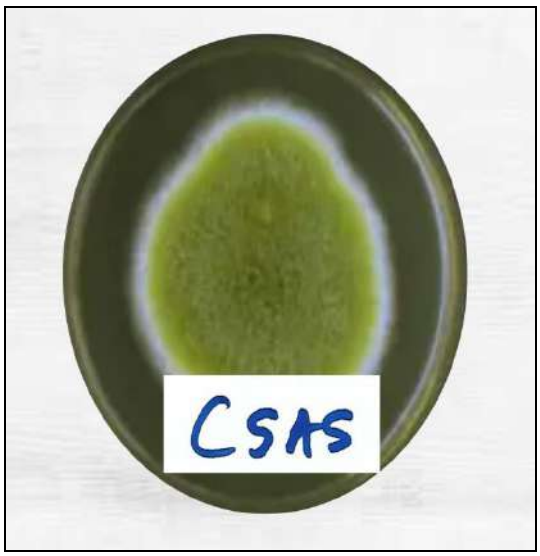

Plate 1: Culture plate of *Aspergilus* sp. on SDA (CSAS: contaminated soil *Aspergilus* sp.)

Plate 3: Culture plate of *Fusarium* sp. on SDA (USFS: uncontaminated soil *Fusarium* sp.)

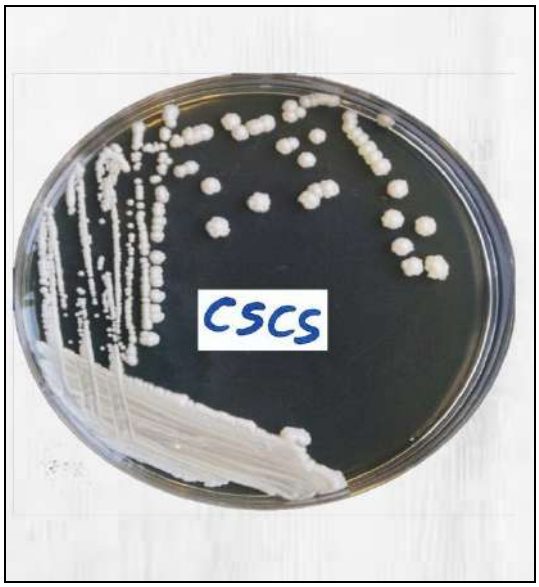

Plate 2: Culture plate of *Candida* sp. on SDA (CSCS: contaminated soil *Candida* sp.)

Plate 4: Culture plate of *Mucor* sp. on SDA (CSMS: contaminated soil *Mucor* sp.)

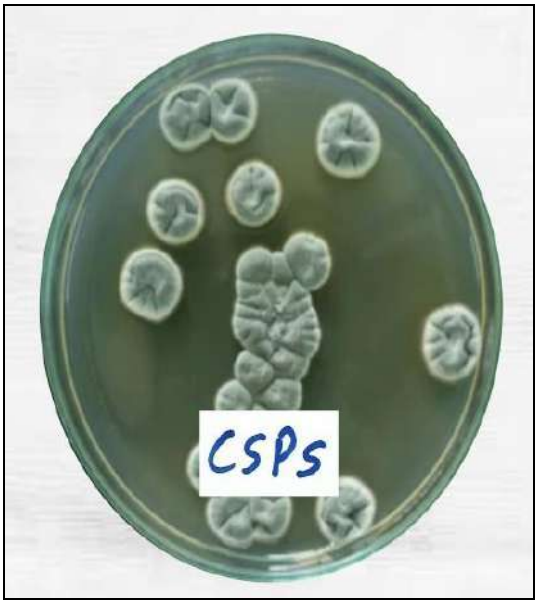

Plate 5: Culture plate of *Penicilium* sp. on SDA (CSPS: contaminated soil *Penicilium* sp.)

Plate 6: Culture plate of *Trichoderma* sp. on (USTS: uncontaminated soil *Trichoderma* sp.)

The Result of the Macroscopic and Microscopic Characteristics of Fungal Population

The macroscopic and microscopic characterizations of fungal isolates from the different abattoir sites are presented in Table 6. Isolates CSAS and USAS showed similar microscopic and macroscopic characterization of *Aspergillus* spp. Isolate CSCS matched macroscopic and microscopic characterization of *Candida* spp., Isolates CSFS and USFS matched that of *Fusarium* spp., isolate CSMS was similar to characteristics of *Mucor* spp. *fungi with isolate codes* CSPS and USPS matched macroscopic and microscopic characterization of *Penicillium* spp. *whereas,* USTS was similar to characteristics of *Trichoderma* spp.

Table 6. Macroscopic and microscopic characteristics of fungal population

Macroscopic	Microscopic	Identified Fungi
The colonies were widely spread, black, with smooth white edges and spongy surface densely packed and brown on the reverse side	The conidiophore was long, erected from the base to the vesicle, smooth walled, hyaline with globes conidial head	*Aspergillus niger*
The upper surface of colonies was olive green with white edge, granular surface and green coloration on the reverse side	The conidiophore was thick walled, hyaline and slightly rough head, event, and long aseptate with a vesicle at the top with phialides and with short conidial chains.	*Aspergillus flavus*
The colony was widely spread, dark green with smooth white edge spongy surface and brown on the reverse side	The conidiophores were long, narrow at the base and broad near the vesicle, smooth walled hyaline	*Aspergillus fumigatus*
The colony is white with smooth edge and tart surface and in the reverse side is brown in coloration	The macroconidia are canoe shaped, multi septate which contain 3-6 septations and slightly pointed at the end	*Fusarium oxysporium*
White fluffy growth, with reverse white colour cream	Pseudo-hyphae, oval shaped cells	*Candida* spp.

coloured, smooth round glistering colonies. Creamy white dull, dry irregular and heaped up.		
Radially furrowed blue-green velvety growth with white periphery, with reverse white colour	Septate hyphae, with branched conidiophores bearing phialides. Conidia are arranged in chains on the phialides. Conidiophores are smooth	*Penicclium* spp.

Discussion

Continuous discharge of abattoir effluent into the environment can contaminate the soil and can lead to a decline in the quality and texture of the soil, making it less available for plant productivity and soil fertility, which may then cause low productivity in the nearby farmlands (Roberts *et al*., 2009) [13]. In comparison to the uncontaminated soil sample (control), the fungal numbers isolated from the contaminated soil samples were greater. This may be due to the higher levels of organic matter in the contaminated soil samples as well as the fact that abattoirs' usual damp settings favor the growth of fungi. These findings support the findings of Rabah *et al*. (2010) [28] and Eze *et al*. (2013) [29] studies. Aspergillus spp., Mucor spp., Penicillium spp., Candida spp., and Fusarium spp. were among the fungi isolated from the contaminated soil in this investigation, while Trichoderma spp., Fusarium oxysporium, Aspergillus fumigatus, and Penicillium spp. were found in uncontaminated soil. This is in accordance with the findings of Adesemonye *et al*. (2006) and Ezeronye and Ubalua (2005) [30], who discovered related organisms as well. The control sample of uncontaminated soil had fewer bacteria than the contaminated ones. More organic material in the contaminated samples may be the cause of this. According to Atlas and Bartha (2007), fungi are microorganisms that live in soil and are frequently related with the cattle sector as spoiling organisms. Opportunistic fungus describe them. The Aspergillus spp., which can cause aspergillosis in humans, livestock, and poultry, are typically found in areas where organic detritus is abundant. As a result, the Aspergillus isolation in this investigation was consistent with the findings of (Emmanuel *et al*., 2018) [31]. The fungal load of the control soil was substantially greater (P 0.05) than the abattoir impacted soil, according to Ediene *et al*. (2016) [5], who recorded fungal loads of 1.09× 10^5 CFU/g and 3.9× 10^4 CFU/g in their control and abattoir impacted soils, respectively. A*spergillus Niger, Aspergillus flavus*, *Fusarium* sp., *Penicillium* sp., *Mucor* sp., and *Rhizopus* sp. were found in the control soil, whereas *Microsporium* sp., Aspergillus Niger, and Candida sp. were found in the abattoir-impacted soil. Inhalation of *Aspergillus* sp. can result in Asthma with difficulty in breathing. A large Aspergilloma in the lungs can block respiratory gas exchange and cause death due to asphyxiation. The microbial contamination observed in this study is an indication of possible pollution and this may have effect on the ecological balance of the soil (Ogbonna and Igbenijier, 2006) [10]. Contaminated abattoir effluent is neither good for domestic use nor is it supposed to be discharged directly into the environment without treatment (APHA, 2005) [3] Abdullahi *et al*., (2015) [1] also reported cases of food poisoning and prevalence of diseases experienced by the residents around abattoirs were typhoid fever, diarrhoea, coughing, asthma, foot and mouth disease and dengue among the residents. Hassan *et al.,* (2014) [8] stated clearly, that the results from their study, there was significant association between abattoir effluents and microbial diseases affecting abattoir neighboring community as the microbial loads of the soils around all the abattoir areas were far higher in counts than the soils analysed farther than the abattoir areas. According to Nafarnda *et al.,* (2012) [9], it has been reported that abattoir effluents contain a lot of disease causing organisms. Similarly, medical experts including: Wing and Wolf, (2000) [17]; Sobsey *et al.,* (2002) [14], Jegede *et al.,* (2022) [32], have reported cases of population residing in close proximity to abattoir environments to be associated with some diseases as a result of abattoir activities, which include: pneumonia, diarrhoea, typhoid fever, asthma, wool sorter diseases, respiratory and chest diseases. Hassan, *et al*., (2014) [8], investigated on the effect of abattoir effluent on surrounding underground water quality where, a total of forty water samples were collected from five sampling points situated at distance of 0m, 10m, 50m, 100m downstream and 10m upstream (to reflect the ambient condition of the river prior to pollution with abattoir effluent). The results of the bacterial counts ranged from 1.3 x 106 cfu/ml to 9.0 x 106 cfu/ml of total aerobic heterotrophic bacterial count, and the total coliform count ranged from 9 x 104 to 3.5 x 106 MPN/100ml and total fungal count ranged from 0.5 x 106 cfu/ml to 1.1 x 106 cfu/ml. The results revealed that the effluent discharge point had the highest microbial load which was evident in the total aerobic heterotrophic bacterial count of 9.0 x 106cfu/ml and total fungal count of 1.1 x 106cfu/ml. The bacteria, *Salmonella* species (19.51%), were mostly enteric organisms and their frequency of isolation included; Salmonella species (19.51%) while, *Aspergillus Niger* (33.33). These abattoir effluents have negative microbiological impact on the quality of the rivers, exposing the health of those who directly use the water for various purposes to hazard (Hassan, *et al*., 2014) [8]. Effective abattoir wastewater treatment methods should remove the pollutants, nutrients, organic load, fat, oil and grease, blood and pathogens from the wastewater to ensure the low level of toxicants in the final discharge effluent (DOW, 2007).

According to the current study, the abattoir effluent-contaminated soil had a significant level of contamination. This finding supports previous research that has warned against dumping untreated waste water into nearby soils and waterways (Akinnibosun and Ayejuyoni, 2015) [33]. Given the current demand for livestock as a result of the expansion in population in Port Harcourt metropolis and many other states in Nigeria, proper treatment of abattoir effluent is therefore required to assure decontamination. Given how sustainability in meat production affects both public health and economic growth, it should be given top consideration.

Conclusion

Poor abattoir facility location and management lead to environmental and water pollution, especially for the areas nearby. The purpose of the study was to look at how poorly managed abattoir operations affected the water quality, as well as the life and health of the surrounding ecosystems and population.

The results of this study have highlighted the environmental effects of the fungus mycology of abattoir effluents in Port

Harcourt, Rivers State, Nigeria. *Aspergillus, Candida, Fusarium, Penicillium, Mucor,* and *Trichoderma* species were among the genera from which the following fungus were isolated. In conclusion, the study's findings showed that contaminated soil had much higher abattoir wastewater contamination than uncontaminated soil. The amount and types of contaminants and microorganisms found in the soil samples as a result of the abattoir effluent that the slaughterhouse released into the soil were largely revealed by the fungal population studied in this study.

Conflict of Interest
Not available

Financial Support
Not available

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How to Cite This Article
Awari VG, Owhonka A, Adaniella LP. Assessment of mycological abattoir waste water contaminated soil in Port Harcourt. Journal of Advances in Microbiology Research. 2020;1(2):21-28.