Original Research Article

Fungal Population and Physicochemical Characteristics of Abattoir-Impacted soil in Iwofe, Rivers State

ABSTRACT

Aim: To determine the fungal population and physicochemistry of abattoir impacted soil in Iwofe, Rivers State.

Study Design: This study employs experimental design and statistical analysis of data and interpretation.

Place and Duration of Study: Abattoir impacted soil was collected from three points in an abattoir located in Iwofe, Rivers State while the unpolluted soil which served as control was collected from the Rivers State University, Port Harcourt in January, 2021.

Methodology: Standard microbiological techniques were used: the fungal population was determined by inoculating aliquots of an appropriate dilution resulting from a ten-fold serial dilution on prepared Sabouraud dextrose agar plates in duplicates. Plates were later incubated for 3-5 days after which colonies were enumerated and used in obtaining the fungal population in the soil samples while distinct colonies were subcultured for macroscopic and microscopic identification of fungi. The physicochemical parameters and heavy metals were analyzed using standard methods.

Results: Fungal load in the control and abattoir impacted soil were 1.09×10^5 and 3.9×10^4 CFU/g, respectively. The fungal load of the control soil was significantly higher (*P<0.05*) than the abattoir impacted soil. The fungal isolates identified in the abattoir impacted soil were *Microsporium* sp, *Aspergillus niger* and *Candida* sp while *Aspergillus niger, Aspergillus flavus, Fusarium sp, Penicillium sp, Mucor* sp and *Rhizopus* sp were identified from the control soil. The pH, temperature, nitrate and phosphate of the abattoir soil were 6.7, 28.33℃, 27.83($mgKg^{-1}$) and 1055($mgKg^{-1}$), respectively. The concentrations of Cadmium, Iron and Lead in the abattoir Impacted soil and control soil were 0.81, 563.35 and 7.12 $mgKg^{-1}$, 0.51, 582.0 and 3.18 $mgKg^{-1}$, respectively. The physico chemistry and heavy metals in the abattoir soil were within acceptable limits.

Discussion and Conclusion: The findings from this study showed that heavy metals in abattoir impacted soil had an impact in the fungal population which led to the isolation of only three fungal isolates belonging to *Microsporium* sp, *Candida* sp and *Aspergillus niger.* More so, despite the presence of heavy m etals in the abattoir impacted soil, the metals were all within permissible limits. Thus, the abattoir impacted soil was not heavily polluted.

Keywords: Abattoir impacted soil, Fungal population, heavy metal concentrations, Physicochemistry

1. Introduction

Abattoir waste is an emerging solid waste whose rate of generation is becoming alarming. Meat processing is usually carried out in a specialized environments known as abattoirs or slaughter houses. According to [1] an abattoir or slaughter house is a specialized environment where meat processing is usually carried out (i.e. It is a building where animals are killed for their meat). Several activities are involved in the operation including receiving and holding of livestock, slaughter carcass, dressing of animals, chilling of carcass products, carcass boning and packaging, drying of animal skins [2]. However, in Nigeria, meat processing activities are mostly carried out in unsuitable places or buildings by butchers who have little or no idea of sanitary principles. These activities are usually accompanied by the generation of large amount of wastes like blood, fat, organic and inorganic solids, salts which are hitherto discharged into soils and water bodies around the abattoir premises [3,4].

Abattoir wastes have complex compositions and can be very detrimental to any environment where they are discharged. Various organs of cattle like muscles, blood, liver and kidney have been reported to contain trace metals, faeces of livestock consist of mucus, bacteria, cellulose fibre, manure which is very acidic in nature and others [5].

Additional reports have been made on the effect of abattoir wastes on soil including increased concentration of trace metals, increase population of decomposers, loss of aesthetic value, excessive soil nutrient enrichment and increased toxin accumulation as well as large accumulation of sulphides, mercaptans, amines and organic acids [6, 7].

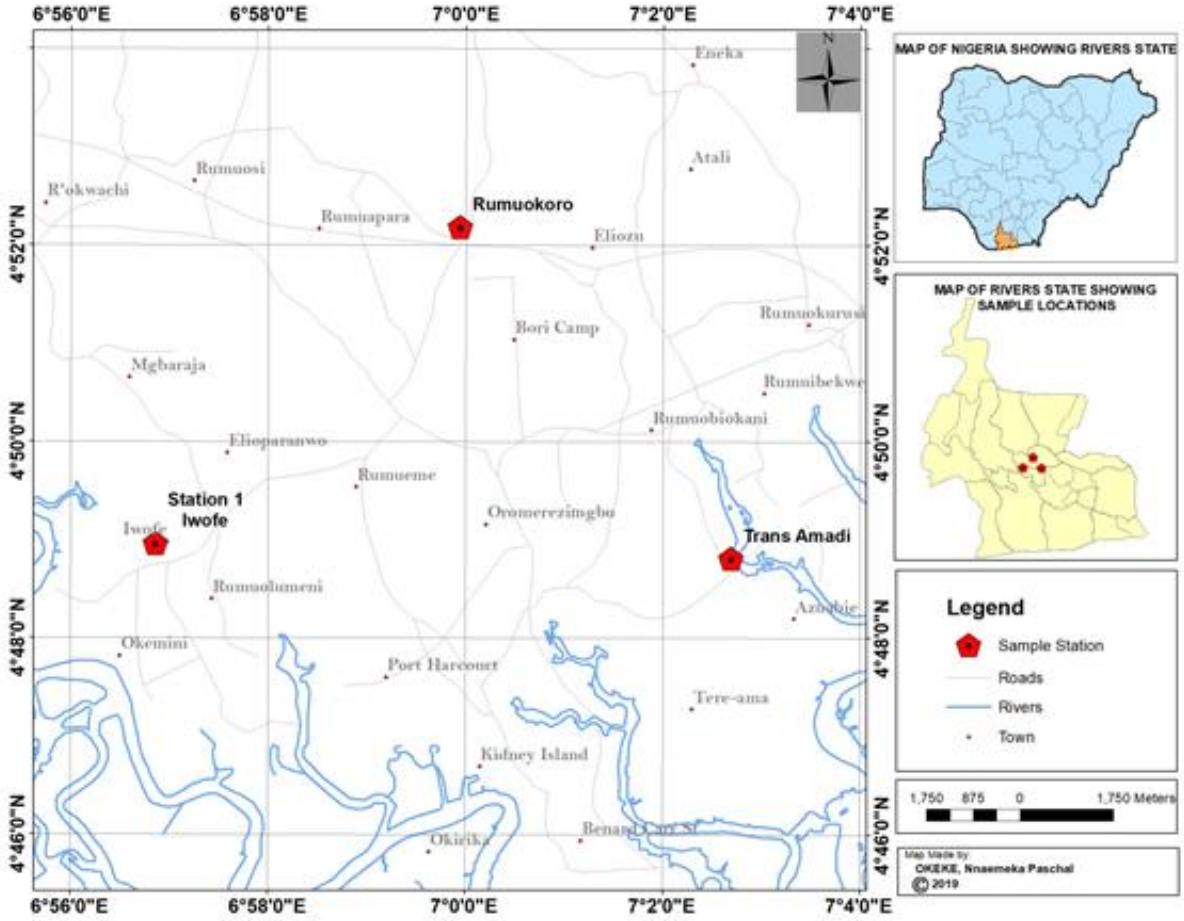
[8] reported the microbiological and physicochemical assessment of soil contaminated with abattoir effluents in Sokoto metropolis, Nigeria. The result showed high microbial loads and microbial diversities most of which are pathogenic in the soil contaminated with the abattoir effluents. In Nigeria, some abattoirs are surrounded by farmlands and these farmlands are usually cultivated by local farmers living in the vicinity of these abattoirs. These soils are used to grow crops mostly vegetables and legumes which are consumed by humans due to good crop yields from these abattoir soils without any assessment of its sanitary nature [9].

The aim of this study is to evaluate the fungal population and physicochemical characteristics of abattoir effluent impacted soil in Iwofe, Rivers State.

2. Materials and Methods

2.1 Study Area / Collection of Samples

Soil samples were collected from three (3) points from abattoir impacted soil in Iwofe., Rivers State and a composite sample was made The Iwofe abattoir has only existed for about five years and it is located at a latitude 4 59'14.0N and longitude 7 16' 12.0 E. Prior to soil collection, wastes on the abattoir impacted soil was cleared. Sterile auger which was sterilized in the hot air oven at 160 ℃ for two hours [10] was used to collect soil sample from the abattoir at a depth of 0-15 cm. The soil samples were collected into black clean polythene bag. Soil samples were collected from three (3) points from the back of the Postgraduate Microbiology laboratory, Rivers State University (a place devoid of abattoir contaminant) and a composite sample was made and it was used as control. The map of the slaughter houses in Port Harcourt metropolis is shown in Fig. 1. The area marked station 1 was the area under study (Iwofe Abattoir).



Key note: Station 1- Area of study (Iwofe Abattoir)

Fig.1: Slaughter Houses/ Abattoirs in Port Harcourt metropolis, Rivers State.

2.2 Medium used

Sabouraud Dextrose Agar (SDA) was used for the enumeration and isolation of fungal load.

2.2.1 Media Preparation

Sabouraud Dextrose Agar (SDA)

Sabouraud Dextrose Agar (SDA) was prepared according to Manufacturer's specifications. The method used was the 10-fold dilution method of [11]. Ten grams (10 g) of composite soil samples from abattoir impacted and control soil were aseptically transferred into 90 ml of sterile normal saline in 150 ml conical flasks. The flasks were shaken vigorously to dislodge the microbial flora. Further 10-fold dilutions were carried out by adding 1.0 ml of the penultimate dilution to 9 ml of fresh diluents.

The diluent used was normal saline and it was prepared by dissolving 8.5grams of sodium chloride ($NaCl_2$) in 1000ml of distilled water. Nine milliliter (9 ml) each of the prepared diluents was dispensed into six clean test tubes using a 10ml pipette. The test tubes were plugged with cotton wool and were sterilized in an autoclave at 121^{o}C at 15 psi for 15 minutes. The diluents were used to carry out serial dilution of each soil sample [12].

2.3 Enumeration and Isolation of Fungi

Tenfold serial dilution was carried out on the soil samples. This was done by transferring one gram of the soil sample using a sterile spatula into test tube containing 9 ml sterile diluent which served as the stock. The mixture was agitated and allowed to settle. After the soil sample had settled in the bottom of the test tubes, one milliliter (1ml) sterile pipette was used in transferring 1ml of the stock (mixture of soil and diluent) into another test tube containing 9 ml sterile diluent. This was done serially until 10^{-6} dilution was obtained. Aliquots (0.1 ml) of 10^{-1} dilutions were inoculated on Petri dishes containing freshly prepared SDA containing streptomycin (1 mg / 100 ml) in triplicate. Plates were incubated at room temperature for 5 days. After incubation, ensuing colonies which developed on the plates were counted and the counts were used to calculate the spore forming units. Distinct colonies were characterized

macroscopically and sub-cultured by streaking fungal spores/ colonies unto freshly prepared SDA plates.

2.4 Identification of Fungal Isolates

The fungal isolates were identified based on their macroscopic and microscopic characteristics. The macroscopic characteristics include; colony size, texture of the colony, spore colour and the reverse colour while the microscopic characteristics involved the arrangement of hyphae, conidia, or sporangium. The method described by [13] was adopted for the microscopic identification of the fungal isolates. In this method, a drop of the lactophenol blue stain was placed on a clean microscopic slide with the aid of a Pasteur pipette and a small portion of the aerial mycelia from the representative fungal culture was removed using sterile forceps. The spores were placed in the drop of lactophenol on the slide. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The macroscopic and microscopic characteristics of fungal isolates were matched with similar isolates in the book of descriptions of medical fungi by [14].

2.5 Analysis of Physicochemical Parameters

The physicochemical parameters analyzed in this study were pH, Temperature, Nitrate and Phosphate. The analysis was carried out as described [15].

The pH of the soil was determined using the Jenway pH meter (3015 model). The mercury thermometer was used to determine temperature. Other parameters like nitrate and phosphate were monitored as described in [16].

2.6 Heavy metal Analysis

The heavy metals were analysed using the method of [17] . The soil samples were analyzed for the following heavy metals; Iron (Fe), Zinc (Zn), Cadmium (Cd) and Lead (Pb). Each metal ion was calculated in milligrams per litre by referring to the appropriate calibration curve.

2.7 Statistical Analysis

2.8

Results were subjected to statistical analysis employing the student t-test at 95% probability levels using SPSS (VERSION 14.0) statistical package.

3 Results

3.1 Fungal Load

The result of the fungal load of both the abattoir impacted and control soil is presented in Table 1. The result showed that the fungal load in the control and abattoir impacted soil were 1.09×10^5 and 3.9×10^4, respectively. The unpolluted (control) soil had a higher fungal load.

3.2 Fungal Isolates and Distribution of Occurrence

The result of the macroscopy and microscopy of the fungal isolates from this study is presented in Table 2. A total of eight fungal isolates were isolated. The fungal isolates identified in this study were *Microsporium* sp, *Aspergillus niger*, *Aspergillus flavus*, *Candida sp*, *Fusarium* sp, *Penicillium* sp, *Mucor* sp and *Rhizopus* sp. The distribution of fungal isolates presented on Table 3 showed that out of the eight fungi isolated, only three: *Microsporium* sp, *Aspergillus niger* and *Candida* sp were isolated from the abattoir impacted soil. Some pure cultures of fungal isolates are presented in Plate 1.

Table 1: Fungal Counts (sfu/g) of the Soil Samples

Soil Sample	Fungal Count (TFC)
Control	1.09×10^{5}
Abattoir Impacted	3.9×10^{4}

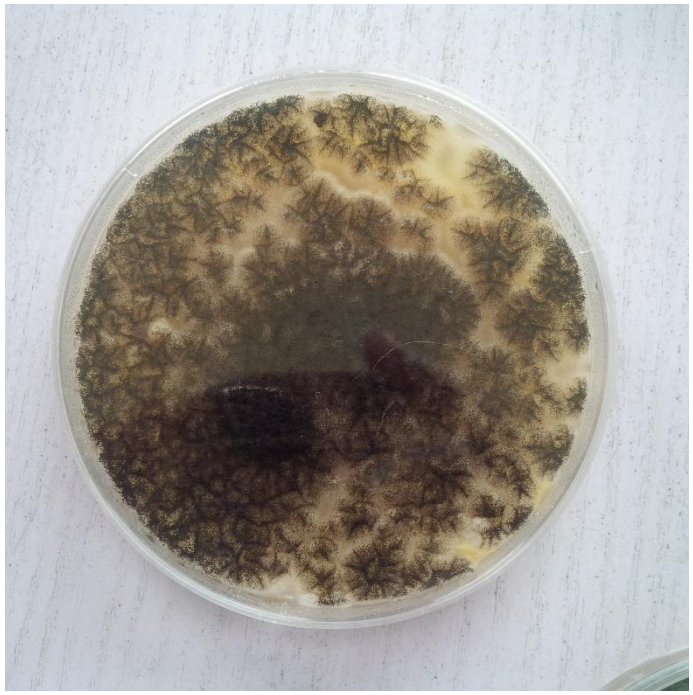
Table 2: Macroscopy and Microscopy of Fungal Isolates

Isolates	Macroscopy	Microscopy	Probable Identity
a.	Fluffy white cottony, white reverse	Aseptate hyphae bearing round sporangiospores	*Mucor* sp
b.	waxy, glabrous, convoluted thallus with a cream to buff-coloured surface and no reverse pigment	Absence of conidia. Presence of irregular branching hyphae with prominent cross walls	*Microsporium* sp
c.	Orange small round raised	Spherical budding blastoconidia	*Candida* sp
d.	Green powdery surface surrounded by white lawn, brown reverse	Septate hyphae with septate conidiophores bearing conidia	*Penicillium* sp
e.	Black spores surrounded by white lawn-like growth	Aeseptate conidiophores bearing conidia	*Aspergillus niger*
f.	Light green lawn surrounded by white lawn-like growth	Septate hyphae with aseptate conidiosphore bearing conidia	*Aspergillus flavus*
g.	Fluffy white to grey spores, brown reverse	Aseptate banana hyphae	*Rhizopus* sp
h.	Slightly whitish to orange colony	Slightly curved and pointed macroconidia with septate basel cells	*Fusarium* sp

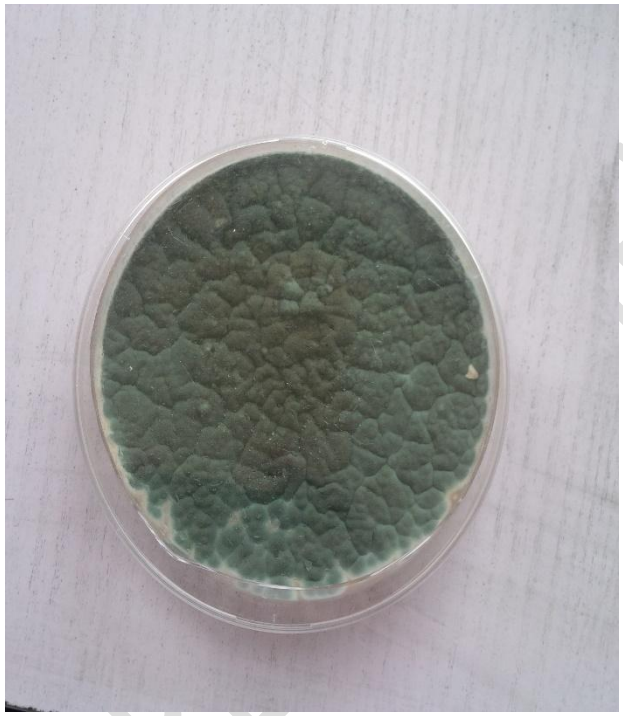
Table 3: Distribution of Fungal Isolates in the Soil Samples

Fungal Isolates	Soil	
	Abattoir Impacted	**Control**
Microsporium sp	+	-
Aspergillus niger	+	+
Aspergillus flavus	-	+
Candida sp	+	-
Fusarium sp	-	+
Penicillium sp	-	+
Mucor sp	-	+
Rhizopus sp	-	+

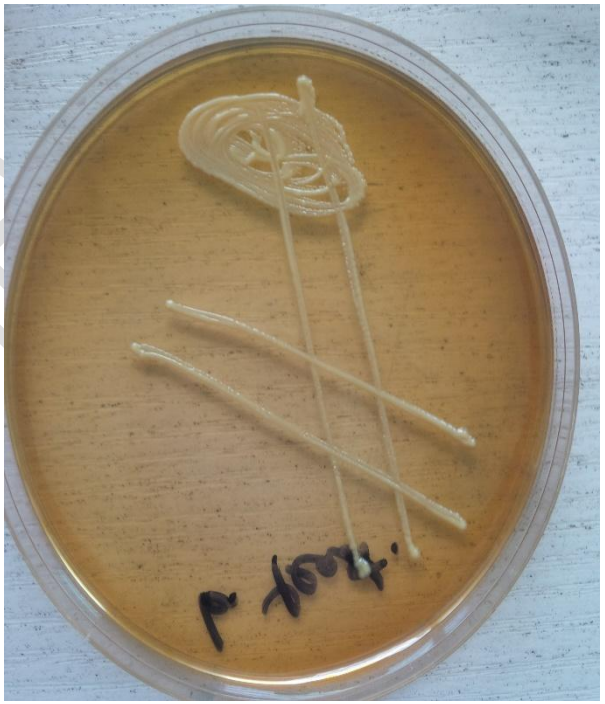
Key: +: present, -: absent



***Aspergillus* sp**



***Penicillium* sp**



***Candida* sp**

Plate 1: Pure cultures of some of the fungal isolates identified in this study

The result of the physicochemical parameters of the soil samples is presented in Table 4 while the result of the heavy metals analysis is presented in Table 5. The values for pH, temperature, nitrate and phosphate of the abattoir impacted soil were 6.7, 28.33℃, 27.83 $mgKg^{-1}$ and 1055 $mgKg^{-1}$, respectively. The values for pH, temperature, nitrate and phosphate of the control soil were 7.1, 28.27℃, 65.84 $mgKg^{-1}$ and 1735 $mgKg^{-1}$, respectively.

The concentrations of Cadmium, Iron and Lead in the abattoir Impacted soil and control soil were 0.81, 563.35 and 7.12 $mgKg^{-1}$, 0.51, 582.0 and 3.18 $mgKg^{-1}$, respectively. The cadmium concentration of the control soil ,0.51 $mgKg^{-1}$ was less than the concentration of 0.81 $mgKg^{-1}$ of the abattoir Impacted soil. The iron concentration present in the abattoir Impacted soil was less than the value obtained in the control while the Lead concentration of the abattoir Impacted soil was higher than value obtained from the control soil.

Table 4: Physicochemical Parameters of the Soil Samples

Parameters	**Soil**	
	Abattoir Impacted	**Control**
pH	6.7	7.1
Temperature (℃)	28.33	28.27
Nitrate ($mgKg^{-1}$)	27.83	65.84
Phosphate ($mgKg^{-1}$)	1055	1735

Table 5: Heavy metal Concentrations in the Soil samples

Heavy metals	Abattoir Soil	Control	FEPA limit
Cadmium (Cd) ($mgKg^{-1}$)	0.81	0.51	2.0-3.0
Iron (Fe) ($mgKg^{-1}$)	563.35	582.0	1000-5000
Lead (P) ($mgKg^{-1}$)	7.12	3.18	15.0-25.00

4 Discussion

4.1 Fungal Population

The fungal population of the unpolluted soil (control) was higher than that from the abattoir impacted soil. T-test showed that there was a significant difference ($P<0.01$) between the fungal load of the control which was higher than the abattoir impacted soil. The reason for the low fungal count in the abattoir impacted soil could be due to the presence of contaminating substances which might have interfered with fungal growth as well as being selective for the type of fungi found in this location. The fungal load in this study (3.59 ± 0.40 Log_{10}SFu/g) is lower than the 4.94 ± 0.26 to 5.79 ± 0.3 log_{10}SFU/g reported by [3]. The fungal isolates isolated in this current study were *Microsporium* sp, *Aspergillus niger* and *Candida* sp. In a study by [3],

Aspergillus and *Penicillium* sp were the two fungal isolates isolated from the abattoir contaminated soil. Thus, the isolation of *Aspergillus* in this current study agreed with findings of [3]. [18] isolated *Aspergillus niger Fusarium sporotrichoides* and *Mucor pusillus* from abattoir impacted soil. The presence of *Microsporium* sp and *Candida* sp in this current study could be attributed to the type of nutrient and other environmental factors that favored their growth. This is unlike the control soil sample which favored the proliferation of more fungal genera. This agreed with [18] who opined that the diversity of microbial community in abattoir impacted soil could be attributed to the availability of substrates as well as favorable pH.

4.2 Physicochemical and Heavy Metal Parameters

The pH of the abattoir impacted soil was slightly acidic while the pH of the unpolluted soil was slightly alkaline. Increased rate of decomposition has been linked with low pH while accumulation of particles and dissolved materials contributes to increased pH [19]. However, both the pH of the abattoir impacted soil and the control are within the 6.4-8.5 pH limits [20]. The pH values in this current study are within the values of pH that was reported by [21] but higher than 4.74-5.30 reported by [3]. The pH is an important abiotic factor that plays key role in microbial activities as it could affect enzyme functions and other metabolic activities as well as influence the type of microbes within that environment. This agreed with [22] opined that the pH plays a great role in determining the availability of nutrients in soil to plant and the type of organism found in the soil. The temperature, nitrate, phosphate, cadmium, Iron and lead in the abattoir impacted and control soil were all within the FEPA and WHO permissible limits and similar values have been reported of abattoir impacted soil [21,3 and 18].

5 Conclusion

The findings from this study showed that heavy metals in abattoir impacted soil had an impact in the fungal population which led to the isolation of only three fungal isolates belonging to *Microsporium* sp, *Candida* sp and *Aspergillus niger*. More so, despite the presence of heavy metals in the abattoir impacted soil, the metals were all within permissible limits. Thus, the abattoir impacted soil was not heavily polluted.

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