
EFFECT OF GAS FLARE AND SEASONS OF THE YEAR ON FUNGAL DIVERSITY AND POPULATION IN THE NIGER DELTA AREA OF NIGERIA

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ABSTRACT

This study was carried out to identify the diversity and population of fungi in soils around three flow stations designated U, I and O of an oil exploration company in the Niger Delta. The effect of heat radiation was measured from the flare barriers using a pyrometer and impact on soil fungal diversity and population were determined using standard cultural methods. Top soil samples (0-15cm) were collected at 50m, 100m and 150m which were the same points where heat radiation was measured while the control samples were collected at a distance of 2 kilometers away from the flare during the dry and wet seasons being prevailing seasons of the region. Results showed that the fungi population of soils in the U, I and O flow stations ranged from 3×10^6 Cf/g in the dry season to 104×10^6 Cf/g in the wet season, 1×10^6 Cf/g in the dry season to 300×10^6 Cf/g the dry season and from 2×10^6 Cf/g in the dry season to 300×10^6 Cf/g in the wet season respectively. Fungi isolated in both seasons were *Aspergillus niger* aggregate, *Aspergillus* spp, *Penicillium chrysogenum*, yeast, *Mucor* and *Rhizopus* spp. The mean percentage occurrence ranged from 0.1% for *P. chrysogenum* to 72% for yeast cells in both seasons for soils around 'U' flow station. For 'I' flow station, 8% for *P. chrysogenum* to 100% for *Mucor* in both seasons and 'O' flow station ranged from *Botryodiplodia threobomae* (25%) and *Aspergillus* spp (25%) to

Rhizopus spp and yeast 100% in both seasons. Ambient temperatures in U, I and O flow stations ranged from 26.6°C in the wet season to 39.6°C in the dry season, 28.9°C in the dry season to 33.8°C in the wet season and from 29.4°C in the wet season to 34.1°C in the dry season respectively. While pH values in the U, I and O flow stations ranged from 4.3 in the wet season to 5.8 in the dry season, 4.6 in the wet season to 6.1 in the dry season and from 4.6 in the wet season to 6.0 in the dry season respectively.

Keywords: Crude oil, gas flare, soil, fungi diversity and population, heat radiation, pyronometer, *Aspergillus niger* aggregate.

INTRODUCTION

Nigeria is oil and gas producing nation and their production is carried out in the coastal area of the Niger Delta region. The produced gas is under-utilized, and about 10 to 40% is flared into the environment. Flaring is the usual trend in the Nigerian oil and gas industry and has adverse impacts on the environmental components and its associated biota (Enetimi and Sylvester, 2017). Soil fungi are part of the normal micro flora and are major actors in the nutrient cycling in the ecosystem. Their diversity is a good indicator of soil health especially in agriculture (Choi, 2003). Fungi are abundant and active in normal aerated soils and all the heterotrophic fungal mycelia in the soil spread over surfaces and helps bind mineral particles into aggregates. (Singer and (Munns, 1996). They are essential participants in the decomposition of many types of organic matter which are added to the

soils (Prescott *et al.*, 1999). Fungi are eukaryotes and possess cell walls like higher plants; however they do not contain photosynthetic pigment and are chemo-organo heterotrophs. Like many other heterotrophic organisms they obtain their cell carbon predominantly from organic matter which serve as both carbon and energy sources and are partially assimilated into the cell material and partially oxidized to provide energy. According to Lindley (1992), the widespread ability of yeast and filamentous fungi to transform hydrocarbons suggests that they may be involved in the recycling of naturally occurring hydrocarbons in the environment as well as in the bio deterioration of liquid fuels. The versatility of fungi in degrading hydrocarbon is due to the broad substrate specificity of their enzymes (Sutherland, 2001). Most filamentous fungi and yeast grow abundantly in soils contaminated by petroleum residues (Ekundayo and

Obuekwe, 2001). Research has shown that over 200 classes of fungi, yeast and bacteria can break down hydrocarbons such as methane. Yeast and fungi may also participate strongly in the degradation of petroleum hydrocarbon in fresh water environment (Cooney, 1984). The chemical composition of the soil affects the types and size of its microbial population. According to Alexander (1991) the abundance and physiological activity of the fungus flora of different habitats vary considerably, and the community and its biochemical activities undergo appreciable fluctuation with time at any single location. Temperature in gas flared areas ranges from 1300 – 1400°C. The use of shorter flare stacks causes pollution closer to ground level and deposition of substances closer to vegetation and soils (Akpojivi and Akumagba, 2003). Kalita *et al.*, (2012) also reported that the importance of soil temperature to plant growth and microbial activity is greater than that of air temperature. The soil is also regarded as a store for heat as well as a depot for energy in the day and a source to the top at night.

Soil temperature is the most essential factor that influences important physical, chemical and biological activity of plant and soil because it activates the rate of these processes in soils (Venkateswarlu, 1998). Crude oil exploration in the Niger Delta cannot be

separated from gas flaring which is the most notorious activity of the oil companies. Enormous amount of heat is also emitted in the process which causes varying degrees of environmental pollution. The magnitude of these effects on soil microbiological, meteorological, soil chemical and physical parameters have been adequately documented Giovannini *et al* (1988). To grow and function, fungi need favorable physical conditions which include, available space, usually on a surface. Space is probably adequate in nearly all conditions because microbial growth is usually limited by unsuitable temperature or inadequate water or nutrition (Tortora *et al.*, 2002). The fragile nature of the Niger Delta environment and ecosystem, the existing stress posed by oil exploration has necessitated the need for this research. Therefore this research aims at determining population and identifying fungal diversity at various distances away from flare stack and in the two prevailing seasons of the year on soils of three communities flow stations in the Niger Delta region of Nigeria.

MATERIALS AND METHODS

Description of Study Locations

Three flow stations owned by an oil exploration company where soil samples were collected in Rivers, Delta and Bayelsa States in the Niger Delta Area

of Nigeria, for the purpose of this study were designated 'U', 'I' and 'O' respectively. There are two main climatic seasons of the year in the entire region and these are the dry and wet seasons.

Collection of Soil Samples

Sampling was done twice, in dry season (late November) and raining season (early August) the following year. Soil samples were taken from 0-15cm depth at 50m intervals; 50m, 100m and 150 m away from flare barrier and the control about 2km away. Thus, twelve (12) soil samples were taken from each station; giving a total of 36 samples for each season and 72 for both dry and wet seasons. These soil samples were kept in sterile bottles at room temperature to maintain the samples before analysis in the laboratory since they were taken around the flare stacks.

Apparatus used during the Study

Pyronometer (UVX radiometer) was used to measure heat radiation. The radiation was taken from wind-ward direction and was measured at 50 meters interval i.e. 50m, 100m 150m away and about 2km away from flare for the control. Soil thermometer was used to determine the temperature of the soil at any sampling point from the flare barrier to the control. A mercury thermometer was used to measure the atmospheric or ambient temperature on site. It measured the degrees of hotness or coldness in a particular

sampling point. A measuring tape was used to measure the distances. A hand-trowel was used to collect soil samples into sterile bottles before analysis.

Microbial analysis

The diluted soil samples were cultured on Sabouraud and Potato Dextrose Agar for the isolation of fungi and yeast respectively. The morphologies of the isolated fungi were examined macroscopically and recorded accordingly as they were sub cultured. Wet preparations were made from the sub cultured fungi on clean grease free slides, covered with slides, examined under the microscope and identified based on their cultural and microscopic characteristics using the methods described by Barnett and Hunter (1998) and Nyongesa *et al.* (2015).

Determination of Microbial Load

The samples were determined visibly by counting the colony forming unit after 24 hours. The microbial load/ml was determined by the formula of Cheesbrough (2002) as:

$$\text{Count/ml} = \frac{\text{No of counted colonies on plate} \times \text{vol. used} \times \text{dilution factor}}{\text{No of counted colonies} \times 0.1 \times 10^3 \text{ colony forming unit per ml (Cfu/ml)}}$$

Determination of soil pH

Soil reaction (pH), which was done according to Mclean (1982) by electro-metric method in a soil/water and 0.01

CaCl₂ suspension ratio of 1:2.5 using glass and a calomel combination electrode digital pH meter (Corning Model 7).

RESULTS

Fungal enumeration and Identification in Soils of 'U' flow stations at various Distances from Flare in the Dry and Wet seasons.

Fungal enumeration and identification in soils of the 'U' flow station with distances away from flare in the dry and wet seasons are presented in table 1. The table showed that values pH values ranged from 4.3 at 50 m in the wet season to 5.8 at 50 m in the dry season. Ambient temperature ranged from 26.6°C at 50 m to 39.9°C at 100 m in the dry season, soil temperature ranged from 25.7°C at 100 m to 31.6°C at 50 m and 100 m in the dry season respectively. Radiation ranged from 180 ($\mu\text{w}/\text{cm}^2$) at the control in both seasons to 280 ($\mu\text{w}/\text{cm}^2$) at 50 m in the wet season. The diversity of fungi isolated in 'U' flow station, in the dry season at 50 m revealed the presence of yeast cells (72%), *Aspergillus sp* had (20%) and *Penicillium chrysogenum* (8%). In the wet season at the same distance from the flare the diversity of fungi isolated includes; *Penicillium chrysogenum* (0.1%), *Aspergillus niger* (aggregate) (1.9 %), *Aspergillus niger* (3.9%), *Mucorsp* (1.9%) and Yeast cells

(92.2%). At the 100 m, the isolated fungi were *Mucor spp* (50%), *Aspergillus spp* (49.1%) and *Rhizopus sp* (0.9%) while in the wet season at same distance the fungi isolates were *Aspergillus sp* (1.9%), *A. niger* (0.9%), *Rhizopus sp* (0.9%) and Yeast cells (96.1%). At the 150 m distance away from the flare in the dry season, the following fungi were isolated, Yeast cells (82%), *Aspergillus spp* (50%) *P. Chrysogenum* (8%) and in the wet season at the same distance, *P. chrysogenum*, (1.3%) *A. niger* (aggregate) (3.8%), *A. niger* (2.5%) and Yeast cells (92.4%) were isolated. The control in the dry season recorded the presence of *Aspergillus spp* (50%), *A. niger*, (92.5) *P. chrysogenum*. (6.5%) and the wet season at same distance, had *A.niger*, (92.5%) and *P. chrysogenum* (7.5%) isolated. The 'U' flow station revealed the leastcfu for fungi at 50 meters away from the flare during dry season and the highest during wet season at 100 meters away from flare. In the dry season, the prevalent fungi were the *Aspergillus* group (*A. niger* (aggregate), *A. niger*, *Aspergillus spp.*) while yeast cells also occurred at the 50 and 150 meters away from the flare. Control had percentage occurrences of 72% and 42.0% respectively. *A. niger* was the prevalent fungi in the wet season and occurred in all the distances away from the flare. Yeast cells had percentage occurrences of 92.2%, 96.1%, and

92.4% at 50, 100, 150 meters respectively from flare but none was seen at the control.

Fungal enumeration and Identification in Soils of 'I' flow stations at various Distances from Flare in the Dry and Wet seasons.

Fungal enumeration and identification in soils of the 'I' flow station with distances away from the flare in the dry and wet seasons are presented on table 2. The values for pH ranged from 4.6 at the control in the wet season to 6.1 at 100 m in the dry season, ambient temperature ranged from 28.9 °C in the dry season at the control to 33.8 °C at 50 m in wet season, soil temperature ranged from 27, 8°C in the dry season at the control to 34.2 at 150 m in the dry season and radiation ranged from 186.7 μ /w/cm² in 50 m and 150 m in the dry season to 233.7 μ /w/cm² at 50 m in the wet season. The isolated fungi were *Aspergillus niger* aggregate (92%) and *P. chrysogenum* (8%) in the dry season at 50 m, in the wet season and same distance, *A. niger* aggregate (12.0%), Yeast cells (75.5%) and *A. Niger* (12.5%) were isolated. At 100 m, in the dry season, only *Mucor* spp. (100%) was isolated. And in the wet season, *A.niger* alone was isolated. The distance of 150 meters away from the flare in 'I' flow station in the dry season had *Aspergillus* spp. (100%) isolated while in the wet

season, *A.niger* (17.60%) and Yeast cells (82.40%) were isolated. At the control, *Aspergillus* spp (100%) were isolated and at the same distance in the wet season, yeast cells (100%) were found. In 'I' flow station, the least colony forming unit were observed at 100 meters distance away from the flare in the dry season and the highest at 50 m in same season. In 'I' flow station, the *Aspergillus* group were also dominant but was completely absent at the 100 meters away from the flare, which had *Mucor* spp.100% and in the wet season, the yeast cells were predominant except at the 100 meters away from the flare where *A. niger* had 100% occurrence.

Fungal enumeration and Identification in Soils of 'O' flow stations at various Distances from Flare in the Dry and Wet seasons.

Fungal enumeration and identification in soils of the 'O' flow station with distances away from flare in the dry and wet seasons are as presented on table 3. pH ranged from 4.6 in the wet season at the control to 6.0 at 150 m in the dry season, ambient temperature ranged from 26.1 at 100 m in the dry season to 34.1 at the control in the same season, soil temperature ranged from 25.6 °C in the dry season at 150 m to 37.8°C in the wet season at 50 m and radiation ranged from 213.3 (μ /w/cm²) at the control in the wet season to 346.7 (μ /w/cm²) at 50 m in

Table 1 Fungal enumeration and Identification in Soils of ‘U’ Flow Station at various Distances from Flare in the Dry and Wet Season

Distance from Flare	Dry season						Wet season						
	pH	AT (°C)	ST (°C)	Rad (μ/w/cm ²)	Cfu/g	Fungi	pH	AT (°C)	ST (°C)	Rad (μ/w/cm ²)	Cfu/g	PO (%)	Fungi
50 m	5.8	37.7	31.6	206.7	3x10 ⁶	72.0	4.3	26.6	25.8	280.0	51x10 ⁶	92.2	Yeast cells
						20.0						1.9	<i>A. niger aggregate</i>
						8.0						3.9	<i>A. niger</i>
100 m	5.2	39.6	31.6	200.0	10x 10 ⁶	50.0		26.7	25.7	273.3	10 ⁴ x10 ⁶	96.1	Yeast cells
						49.1			4.4			0.9	<i>A. niger</i>
						42.0						1.9	<i>Aspergillus sp.</i>
												0.9	<i>Rhizopus sp</i>
150 m	5.4	36.7	28.2	186.7	79x10 ⁶	42.0	5.1	27.1	27.2	246.7	79 x10 ⁶	92.4	Yeast cells
						8.0						3.8	<i>A. niger aggregate</i>
						50.0						2.5	<i>A. niger</i>
Control	4.8	35.4	29.8	180.0	71x10 ⁶	92.5	4.6	27.5	27.0	180.0	79 x10 ⁶	92.5	<i>A. niger</i>
						7.5						7.5	<i>P. chrysogenum</i>

Key: AT = Ambient Temperature, ST = Soil Temperature, Rad = Radiation, Cfu = Colony forming unit, PO = Percentage occurrence

Table 2 Fungal enumeration and identification of soils of 'I' stationat various Distances from flare in the dry and wet seasons

Distance from Flare	Dry season						Wet season							
	pH	AT (°C)	ST (°C)	Rad (µw/cm ²)	Cfu/g	PO (%)	Fungi	pH	AT (°C)	ST (°C)	Rad (µw/cm ²)	Cfu/g	PO (%)	Fungi
50 m	5.5	33.4	31.0	186.7	300x 10 ⁶	92	<i>A. niger aggregate</i>	5.3	33.8	32.5	233.7	49x10 ⁶	12.5	<i>A. niger</i>
100 m	6.1	32.4	30.8	200.0	1x 10 ⁶	8	<i>P. chrysogenum</i>						12.0	<i>A. niger aggregate</i> Yeast cells
150 m	5.6	30.7	34.2	186.7	17x10 ⁶	100	<i>Mucorsp</i>	5.8	33.0	31.6	220.0	33x10 ⁶	100	<i>A. niger</i>
Control	5.2	28.9	27.8	220.0	5x10 ⁶	100	<i>Aspergillus spp</i>	5.5	32.8	31.8	220.0	51 x10 ⁶	17.60	<i>A. niger</i> Yeast cells
	5.2	28.9	27.8	220.0	5x10 ⁶	100	<i>Aspergillus spp</i>	4.6	31.5	28.6	200.0	106 x10 ⁶	100	Yeast cells

Key: AT = Ambient Temperature, ST = Soil Temperature, Rad = Radiation, Cfu = Colony forming unit, PO = Percentage occurrence

Table 3: Fungal enumeration and identification in soils of 'O' Flow Station at various distances from Flare in the Dry and Wet Seasons

Distance from Flare	Dry season					Wet season								
	pH	AT (°C)	ST (°C)	Rad (μw/cm ²)	Cfu/g	PO (%)	Fungi	pH	AT (°C)	ST (°C)	Rad (μw/cm ²)	Cfu/g	PO (%)	Fungi
50 m	5.9	28.3	35.3	300.0	54x10 ⁶	100	<i>Aspergillus spp.</i>	5.6	33.8	37.8	346.7	54x10 ⁶	100	<i>Yeast Cells</i>
100 m	5.7	26.1	25.8	240.0	5x10 ⁶	50	<i>Rhizopus spp</i> <i>Aspergillus spp.</i>	5.3	32.4	37.0	333.3	80x10 ⁶	100	<i>Rhizopus spp</i>
150 m	6.0	26.7	25.6	233.3	300x10 ⁶	100	Yeast cells	5.6	31.5	35.8	326.7	300x10 ⁶	100	Yeast cells
Control	4.9	34.1	26.8	206.7	2x10 ⁶	50	<i>Rhizopus spp</i>	4.6	29.4	30.6	213.3	300x10 ⁶	50	<i>A. niger</i>
						25	<i>Botryodiplodia theobromae</i>						50	Yeast cells
						25	<i>Aspergillus spp.</i>							

Key: AT = Ambient Temperature, ST = Soil Temperature, Rad = Radiation, Cfu = Colony forming unit, PO = Percentage occurrence

same season. *Aspergillus* spp(100%) was isolated at 50 m; in the dry season .Yeast cells (100%) only were isolated at same distance. At the distance of 100 meters, *Rhizopus* spp (50%) and *Aspergillus* spp (50%) were isolated in the dry season and at same distance in the wet season, *Rhizopus* spp(100%) was isolated .At 150 meters distance away from the flare, yeast cells (100%) only were isolated in the dry season and in the wet season at the same distance, yeast cells(100%) only were isolated. At the control, *Rhizopus* spp (50%), *Botryodiplodia theobromae* (25%) and *Aspergillus* spp (25%) were isolated in the dry season, whereas at the same distance in the wet season, *Aspergillus niger* aggregate (50%) and Yeast cells (50%) were the isolated fungi. The least Cfu was revealed at the control in the dry season and the highest at 150 m in the dry and wet season and at the control in the wet season. At the 'O' flow station *Aspergillus* spp was the most abundant fungi isolated and identified in all the distances away from the flare except at the 150 meters away which had yeast cells completely dominating at that distance. The control area also had *B. theobromae*, which also occurred at 25% at the 150 meters away from the flare and in the wet season, *Rhizopus* spp (100%) was observed at 100 meters away from the flare. Among the diversity of fungi isolated, the *Aspergillus*

group and yeast cells were found to be more dominant when compared to the other fungal isolates in all the stations.

DISCUSSION

This study has shown that wet season had higher values of isolated fungi than the dry season in all three flow stations studied. This has suggested clearly that the season affected the fungal population in the three flow stations and does not agree with the study of Obire and Womodo (2002) who found that seasons did not clearly affect microbial population. There was no particular fungus peculiar to any distance away from the flare, some had higher frequency of occurrence than others at any given distance away from flare. Akpoturi *et al.*, (2017)observed in their study that there was no fungus isolated or peculiar to any distance and any location which is similar to the findings of this study. This study revealed the presence of the same fungi in all three flow stations except in the control 'O' where *Botryodiplodia theobromae* spp was isolated which was a forest reserve. The presence of this fungus could have been as a result of the mango trees and banana found at this distance which agrees with the findings of Meah *et al.*, (1991) that *Botryodiplodia theobromae* thrived best at 28°C on ripe mango extract and dextrose or sucrose media. The presence

of the mango trees would have aided the growth.

This study revealed the presence of yeast cells, *Rhizopus spp*, and *Mucor spp* occurring more and in both seasons. In a similar study by Obire *and* Wemedo (2002), eighteen different microorganisms were identified across different seasons. The groups of fungi found in all the seasons were *Penicillium and Rhizopus spp*. which is in contrast to this study, as *Rhizopus spp*. was found in 'U' and 'I' flow stations and in both seasons. This probably indicates that season did not have so much impact in the distribution and diversity of the fungi found around the studied flow stations.

The impacts of both seasons were considered on the diversity of fungi in the soils of the three study flow stations. There was no significant impact of seasons on the diversity of the isolated fungi as almost all the fungi isolated were in all season but some had higher colony forming units than others as well as the frequency of occurrence. However the wet season had more fungal load than the dry season. This conforms to the findings of Johannes and Erland (2011) that found that high moisture in the soil encourages rapid growth. There was an increase in the fungal load in respect to increase in distances away from the flare in 'U' flow station. The flow stations 'I' and 'O' did not follow any particular trend. This might be as a

result of the designs of the flare stacks in these flow stations. The finding conforms to that of Johannes and Erland (2011) that high temperature reduces microbial growth. The findings of Abdulkareem (2005) revealed that the distribution of pollutants can be attributed to flare stack type which can lead to high or low deposition in atmosphere and eventually cause acid rain and consequently on soil resulting in acidic soil environment. The entire study area and their control sites had pH ranging from moderately acidic to strongly acidic in both seasons. Ambo *et al* (2013) also reported that soils from this region are acidic and this is a characteristic of this region. This may also be a favorable condition for presence of the isolated fungi.

However, other characteristics such as soil pH, temperature may also play a role on the type and fungal functional diversity associated with the soil samples. Yeast cells and the *Aspergillus* group had higher frequency of occurrence in all three studied flow stations. Most yeast according to Bahafid *et al.*, (2017) exhibit the ability to adapt to extreme conditions of pH and high levels of organic and inorganic contaminants. Kutty and Philip (2008) in their report mentioned that yeasts are ubiquitous in distribution and population and mainly depends on the type and concentration of organic materials, further more they

are also more in silt mud than in sandy sediments and as such also abundant in marine environments. Zhang *et al.*, (2015) reported that the *Aspergillus* group grows optimally at 37°C and at pH range of 2 - 8.8 and are also among the fungi that grow within the marine sediments. Most filamentous fungi and yeast are known to grow abundantly in soils contaminated by petroleum residues (Ekundayo and Obuekwe, 2000). The 'U' and 'O' flow stations are marine environments and according to Arora (2003), fungi also degrade hydrocarbons in streams and lakes. *Aspergillus*, *Penicillium* and yeast amongst others are some of the fungi used for bioremediation. In 'U' flow station, soil temperature increased with increase in distance in the wet season, and decreased with increase in distance in the dry season, while radiation decreased with increase in distance in both seasons. The presence of stream around the flow station and the increase in water volume of the stream during the wet season might be responsible for the lowered temperature across the distances.

CONCLUSION

The study showed that fungal population increased as distance increased in all three flow stations. This showed that heat radiation affected population. Fungal load was also higher in the wet season, as the study also revealed that

wet season also favoured the presence of fungi. Frequency of occurrence and diversity of fungi isolated did not follow any particular trend and may not be influenced by season or distance but may be influenced by heat emitted from flares which may have reduced the diversity of fungi as most fungi isolated may be present as a result the ability to adapt to such environment created by the flare and heat radiation.

RECOMMENDATION

Fungal analysis would evaluate prominent members of the fungal communities in agricultural soils to better catalogue and understand fungal biodiversity for possible bioremediation measures. The knowledge of fungal community structure and composition will allow for proper agricultural management practices.

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