

Soil mycoflora studies of some locations in Lagos State, Nigeria.

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Abstract: Soil mycoflora studies were carried out on a non-industrial site and three industrial sites in Lagos State, Nigeria. Samples were collected from different spots in each location. The pH of the samples ranged from 6.31-9.78. The mycoflora of the samples was also determined and a total number of sixty-one(61) fungal species belonging twenty-one(21) genera molds and four(4) genera of yeasts were isolated. *Aspergillus* species (29.60%), *Penicillium* species (24.50%) and *Trichoderma* species (11.22%) were the most encountered in the study. Of all the locations sampled, the non-industrial site, Lagos State University (LASU), was the richest in terms of fungal species with twenty-six (26) species, this was followed by Polyethylene Nigeria Limited with twenty-three(23) species, Chemstar Nigeria Limited, Ikeja with (19) species, while Chemstar Nigeria Limited, Alakuko had the least with fourteen(14) species. These results were discussed in relation to effect of industrial effluent on the mycoflora of soil environment. [Ewekeye, Tolulope; Oke, Oyedamola; Li-Hammed, Morufat. **Soil mycoflora studies of some locations in Lagos State, Nigeria.** Rep Opinion. 2012;4(4):52-57]. (ISSN: 1553-9873). <http://www.sciencepub.net/report>. 10

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1. Introduction

Soil is a very species-rich habitat containing all major groups of micro-organisms like bacteria, algae, protists and fungi (Hagvar, 1998). Soil is also an oligotrophic medium for the growth of fungi (Dighton *et al.*, 2005). Fungi are very important component of the soil micro-biota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth and Bisby, 1995). Fungal species are especially important components of biodiversity in tropical forest as major contributors to the maintenance of the earth's ecosystem, biosphere and biogeochemical cycles. Fungi perform unique and indispensable activities on which larger organisms including humans depend (Nilima *et al.*, 2007). The habits adopted by fungi in the soil fall into three types; decomposers, mutualists and pathogens (Ingham, 1999; Abbott and Daniel, 2003; Christian and Irina, 2007). The great majority of fungal species have at least some part of their life cycle in soil (Bridge and Spooner, 2001). The saprobic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in the decomposition of plant structural polymers, such as cellulose, hemicelluloses and lignin, thus contributing to the maintenance of global carbon cycle (Mahmood *et al.*, 2006; Saravanakumar and Kaviyaran, 2010). Fungi play a fundamental role for the functioning of the ecosystem and due to their ability to decompose complex macromolecules like lignin or chitin, they are essential for making the nutrients like carbon, nitrogen, phosphorus, sulphur available (Wuczkowski *et al.*, 2003).

The presence of fungi in different environments

have been reported by Saksena, 1965; Maren, 2002; Wuczkowski *et al.*, 2003; Mahmood *et al.*, 2006; Al-Nur and Abdul, 2007; Nilima *et al.*, 2007; Suhail *et al.*, 2007; Aderiye *et al.*, 2008; Muthezhilan, 2008; Sameera *et al.*, 2008; Panda *et al.*, 2010 and Saravanakumar and Kaviyaran, 2010. Common soil fungi inhabitants such as, *Penicillium*, *Aspergillus*, *Cladosporium*, *Chaetomium* have been implicated to degrade paintings (Orio, 1999). According to Dale (2007), *Aspergillus*, *Penicillium*, *Mortierella*, *Gliocladium*, *Trichoderma*, *Mucor*, *Acremonium*, *Arthobotrys*, *Botryotrichum*, *Cladosporium*, *Chaetomium*, *Fusarium*, and *Paecilomyces* have also been isolated from waste paper gradual recycling materials. Furthermore, *Rhizopus*, *Mucor*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Trichoderma* have been isolated from dye degrading industrial effluents (Muthezhilan *et al.*, 2008).

Little information is available on mycoflora of soil around industries in Nigeria. However, soil mycoflora of some commercial ventures in South West Nigeria was carried out by Aderiye *et al.*, (2008) and the following fungi were isolated: *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Absidia* sp., *Curvularia* sp., *Neurospora* sp., and *Penicillium* sp. Also, the mycoflora of drinking water from Omuihuechi stream near Port Harcourt, Nigeria has been investigated and fungal genera; *Aspergillus*, *Byssoschlamys*, *Candida*, *Cephalosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Saccharomyces*, *Sporobolomyces* and *Trichoderma* were isolated (Obire *et al.*, 2008).

Therefore, this study was aimed at investigating the diversity and frequency of fungi in different locations and to compare the fungal flora of a

non-industrial environment with industrial sites.

2. Materials and methods

2.1 Sampling Sites Samples were collected from four different locations in Lagos State, Nigeria. The sites included: Lagos State University, Ojo (LASU); Chemstar Industry Nigeria Limited, Alakuko, Chemstar Nigeria Industry Limited, Ikeja and Polyethylene Nigeria Limited, Ikeja.

a) Lagos state University (LASU), Ojo

Samples were collected from three locations within Lagos State University:

Site 1 - Faculty of Science shopping complex

Site 2 -Central Generator area

Site 3 -Abandoned dump site

b) Chemstar Industry Nigeria Limited, Alakuko and Ikeja

The industry is a paint manufacturing company.

c) Polyethylene Nigeria Limited, Ikeja

The industry is involved in making cartons and paper millings for flexible packages.

2.2 Collection of Samples

Total numbers of 15 samples (12 soil samples and 3 water samples) were collected altogether from the sampling sites. Samples were collected from three different sites in LASU and four different sites on each industrial location. Sampling was carried out twice during the raining season between May and July, 2010. At each sites, samples were collected at a depth of between 1-5cm beneath the soil surface using clean sterile spatula and universal specimen bottles. The samples from the industrial sites were collected at various depths such as effluent samples, sediment samples at the point of effluent, 100metres away from the point of effluent and soil samples from the surroundings. The samples were brought into the laboratory and kept in the refrigerator till when needed.

2.3 pH Determination

The pH of the samples was determined using an electronic pH (PerpHect logR meter, model 310). The pH metre was first standardized using pH 4 and 7 buffer solutions. The pH knob was switched off and the glass electrode was removed from the buffer solution. It was rinsed with distilled water and the tip of the electrode was dried with tissue paper. The clean and dried electrode was inserted into the water samples respectively and the pH knob was switched on. The pH value for each water sample was read directly from the scale. In case of the soil samples, 10% each of the soil samples was made into distilled water and the same procedure as done to the water samples was repeated.

2.4 Media

Potato Dextrose Agar (PDA), Malt Extract agar

(MEA) and Czapek-Dox Agar (CZEP) were used. Each medium was prepared following the manufacturer's instruction.

2.5 Isolation Technique

The mycoflora of the samples were isolated using the soil dilution-plating technique (Johnson *et al.*, 1960; Warcup, 1960). 1g (or 1ml) of each sample was dissolved into 9ml of sterile distilled water and from each dilution, series of dilutions up to 10^6 were prepared. Pour plate method was used for inoculation. Two dilutions (10^2 and 10^4) were used for analysis of total complexes of fungi present in the samples. 1ml of each of the desired solution (10^2 and 10^4) was pipetted and transferred aseptically into sterile disposable Petri dishes. The inoculation was done in triplicates for all the media used. PDA, MEA and CZEP were poured aseptically onto the inoculums contained in three Petri dishes respectively. The plates were rotated in a slow swirling motion to dispense the soil suspension with the medium. Plates were turned upside down after the media solution had solidified and incubated at $28 \pm 2^\circ\text{C}$ at ambient room temperature. Fungal growth was observed for 5days. After 5days of incubation, a small portion of mycelium from each fungal colony was transferred aseptically onto the different media. Fungi from the media were purified by repeated sub-culturing.

2.6 Determination of number of fungal colonies

The number of fungal colonies isolated on each medium was determined using the formula:

$$\frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of inoculums}}$$

2.7 Identification of Isolates

From the growth of the pure isolates, an accurate description of each fungus as grown on the medium was observed and examined at frequent intervals for colonial or cultural characteristics.

Microscopy morphology was determined using simple staining method called 'wet mount' with lactophenol cotton blue stain. This was done by using a sterile inoculating needle to pick mycelial growth from culture plates onto a clean grease free glass slide on which a drop of saline water had been added. The fungal mycelia were teased out properly. One drop of lactophenol cotton blue stain was added and the preparation was covered with clean cover slips. The preparation was subsequently viewed under $\times 10$, $\times 20$ and $\times 40$ microscope objectives. Reference was made to Onions *et al.*, 1981; Collins *et al.*, 1989; William and Dennis, 1990 for identification.

3. Result

The pH value of the samples ranges from

6.31-9.78 (Table 1). The water samples from the industrial locations had lower pH value compared to the soil samples. In LASU, the generator site has the lowest pH value (6.86) while the cooking area has the highest pH value (8.15). In the industrial locations, the effluent samples of Chemstar Limited, Alakuko and Polyethylene Limited is slightly acidic while that of Chemstar Limited, Ikeja tends towards neutral. The sediment samples were also alkaline with the pH of soil sample from Polyethylene surroundings being the highest (9.78).

Table1: pH of samples at different sampling sites

Locations	Sampling points	pH
Lagos State University, Ojo	CA	8.15
	GA	6.86
	DS	7.65
Chemstar Nigeria Limited, Alakuko	W	6.31
	P	8.01
	100	8.60
Chemstar Nigeria Limited, Ikeja	W	7.37
	P	7.95
	100	8.23
Polyethylene Nigeria Limited, Ikeja	W	6.89
	P	7.80
	100	8.81

Key

CA - cooking area

GA - generator area

DS - dump site

W - water/effluent sample

P - soil sample at point of effluent

100 - soil sample at 100metres away from the point of effluent

S - soil sample from the surroundings

The number of viable fungi count also varies according to the dilution factor and medium used (Table 2). The number of fungi growing on PDA varied from 0-1178 thousands CFUg⁻¹/ml⁻¹, the number of fungi growing on MEA and CZEP agar varied from 0-1200 thousands CFUg⁻¹/ml⁻¹ and from 0-708 thousands CFUg⁻¹/ml⁻¹ respectively. The highest number of fungi growth was on dilution factor 10² as compared to 10⁴. The highest number of viable fungi count was from the water samples of Chemstar Limited, Ikeja and Polyethylene Limited as a result of yeast

colonies present in the water samples. The highest number of fungal colonies was isolated from LASU; this was followed by Polyethylene Limited and Chemstar Limited, Alakuko with Chemstar Limited, Ikeja having the least number of viable fungal counts.

A total number of 141 isolates were obtained from the analysis of the 15 samples. The identification of these isolates resulted into 61 species belonging to 21 genera of molds and 4 genera of yeasts (Table 3). Among the identified species, 11 belonged to *Aspergillus*, 19 to *Penicillium*, 5 to *Trichoderma*, 2 to each genus of *Fusarium*, *Gliocladium*, *Mucor*, *Ulocladium* and 1 each to the genus of *Aureobasidium*, *Cladosporium*, *Coccidioides*, *Byssoschlamys*, *Mortierella*, *Moniliella*, *Oidiodendron*, *Paecilomyces*, *Rhizopus*, *Scopulariopsis*, *Syncephalastrum*, *Neurospora*, *Zygorhynchus*, *Rhodotorula*, *Saccharomyces*, *Sporobolomyces* and *Candida*.

Of all the locations studied, LASU has the highest population of fungi recorded with 13 genera and 26 species. *Aspergillus* sp. and *Penicillium* sp. are the most abundant and the only species that is common to the three sampling points in LASU is *Penicillium frequentans*. The highest number of organisms was recorded in the cooking area, followed by the generator area with the dump site having the least number of organisms.

In the three other (industrial) locations, the mycoflora also varies and each site has organisms peculiar to it. Polyethylene Nigeria Limited is the next to LASU in terms of species richness. The presence of 8 genera and 23 species of fungi make up the mycoflora of Polyethylene Limited with *Aspergillus* sp., *Penicillium* sp. and *Trichoderma* sp. being the most abundant. Chemstar Paint Limited, Ikeja is the next in abundance of species after Polyethylene Limited with 9 genera and 19 species of fungi, Chemstar Paint Limited, Alakuko has the least population of fungi with 9 genera and 14 species.

The following isolated organisms from Polyethylene Nig. Limited; *Aspergillus flavus*, *A. japonicus*, *Cladosporium cladosporioides*, *Penicillium restrictum* and *Trichoderma harzianum* were found to be common with the mycoflora of LASU while *Coccidioides immitis*, *Neurospora sitophili* and *Aureobasidium pullulans* were peculiar only to this site. Chemstar Nig. Limited, Ikeja has *Aspergillus flavus*, *A. niger*, *A. terreus*, *Gliocladium deliquescens*, *Penicillium restrictum* in common with the mycoflora of LASU while *Mucor racemosus*, *Oidiodendron tenuissimum*, *Penicillium oxalicum*, *P. corylophilum*, *P. piceum*, *P. rugulosum*, *P. expansum*, *P. fellutanum*, *Zygorhynchus moelleri* and *Candida* sp. were only peculiar to this site. Chemstar Nig. Limited, Alakuko with the least fungi population has organisms such as *Aspergillus flavus*, *A. niger*, *Cladosporium cladosporioides*, *Trichoderma*

harzianum and *T. reesii* in common with the mycoflora of LASU.

Of all the 15 sampling points, cooking area in LASU is the highest in terms of species richness and is followed by the generator site in LASU and sample from effluent point in Polyethylene industry (P) with the same number of species. This is succeeded by samples from 100metres away from point of effluent and surroundings of Chemstar Industry, Ikeja with the same number of species and dump site in LASU while sample from point of effluent (P) in Chemstar Industry, Ikeja is the least in terms of species richness. Overall in all the sampling points, members of the *Aspergillus* (29.60%), *Penicillium* (24.50%) and *Trichoderma* (11.22%) were the most frequently isolated organisms and were common to all the sites. However, no particular species of organism was common to all the sampling points but *Aspergillus niger* and *A. flavus* were the most frequently isolated species. Also, *Aspergillus flavus*, *A. niger*, *Mucor* sp. and *Saccharomyces* were common to both factories of Chemstar Nig. Limited. The species richness at the point of effluent collection of both factories was also low compared to other sampling points.

4. Discussion

The soil pH, organic content and water are the main factors affecting the fungal population and diversity (Saravanakumar and Kaviyaran, 2010). However, soil moisture has been said to be more important for microorganisms than temperature and pH value (Donnelly *et al.*, 1990). pH values from this study range from slightly alkaline and fungi are known to dominate acid soils but can also tolerate pH of beyond 9.0. However, it is noteworthy that all fungi encountered during this investigation were able to tolerate pH range from 6.31-9.78.

The richest location in terms of species was Lagos State University. The cooking area sampled in the University had the highest organisms recorded and this may be due to availability of different organic food substrates present in the soil and have accumulated over time. Similarly, in the generator area of LASU, fungal species of *Aspergillus*, *Penicillium* and *Paecilomyces* isolated were found to be similar to previous observation (Judith *et al.*, 2002). The fungal species found in the generator site may have the

potential of degrading petroleum products. Fungal species found on the dump site were not as much as the other two sampling sites in the University and this may be due to the fact that the dump site had been abandoned for a very long time and the site might have depleted in nutrient availability.

Organisms found in the other three locations (industrial sites) have also been previously documented (Orio, 1999, Dale, 2007, Francesca and Claudia, 2008, Muthezhilan *et al.*, 2008). Of the industrial locations, Polyethylene Industry had the highest number of fungal species and this may be due to the presence of cellulose from paper waste. It is noteworthy that the number of fungal species from the location decrease with respect to the collection spots, i.e. from the point of effluent to 100 metres from the point of effluent. However, a previous study by Clausen (1999) observed that *Aureobasidium pullulans* which is one of the organisms isolated from this site is the most common mold fungus associated with wood. Some of the organisms isolated from the paint industry have also been reported to degrade paintings (Orio, 1999, Francesca and Claudia, 2008). Low concentration of fungal species at the point of effluent in both factories of Chemstar Industry may be due to the toxicity of chemicals that have accumulated at the spot. Also, the Ikeja factory of Chemstar paint industry had more organisms than that of its factory at Alakuko and this may be due to the fact that Ikeja is an industrial site while Alakuko is a residential site and human activities could also have affected the fungal flora. Fungal species isolated from the paint industry may also have the ability to degrade paints. Further investigation may be useful in this regard.

All fungi recorded from this study were known as inhabitants of different soils. *Aspergillus* and *Penicillium* were the most abundant and was followed by *Trichoderma*. In similar studies Saravanakumar and Kaviyaran (2010) also reported *Penicillium* and *Aspergillus* to be abundant. Al-Nur *et al.* (2007) also recorded *Aspergillus* and *Penicillium* as the most abundant taxa. Pandal *et al.* (2010) also recorded *Aspergillus* as the most abundant species with *Penicillium* and *Trichoderma* next in order to it. But of all the organisms, *Aspergillus* sp. were the most encountered. This is similar to the findings of Maren (2002), Suhail *et al.* (2007) and Sameera, (2008).

Table 2: Enumeration of fungi (CFUg⁻¹/ml⁻¹) according to the media and dilution factors at different sampling sites

Sampling Sites /Media and Dilution Factor		PDA		MEA		CZEP	
		X10 ²	X10 ⁴	X10 ²	X10 ⁴	X10 ²	X10 ⁴
LASU	CA	156	31	160	12	44	26
	GA	228	17	160	12	68	11
	DS	78	12	58	9	120	11
Chemstar, Alakuko	W	1162	720	400	468	708	160
	P	11	2	12	1	10	34
	100	5	1	7	0	6	54
	S	15	1	10	0	20	50
Chemstar, Ikeja	W	0	0	2	1	0	3
	P	15	1	13	3	10	1
	100	12	1	6	0	10	2
	S	32	4	16	2	30	45
Polyethylene, Ikeja	W	232	1178	240	1200	184	268
	P	22	2	11	0	13	6
	100	32	8	41	11	21	4
	S	5	3	13	1	10	22

Key

CA - cooking area

GA - generator area

DS - dump site

W - water/effluent sample

P - soil sample at point of effluent

100 - soil sample at 100metres away from the point of effluent

S - soil sample from the surroundings

Table 3. Isolated genera of fungi and their distribution

Sampling points	LASU			Chemstar, Alakuko				Chemstar, Ikeja				Polyethylene, Ikeja				Total	%
	CA	GA	DS	W	P	100	S	W	P	100	S	W	P	100	S		
Molds																	
<i>Aspergillus</i>	5	4	2	0	1	2	1	1	1	3	1	2	0	1	1	29	29.6
<i>Aureobasidium</i>	0	0	0	0	0	0	0	0	0	0	0	0	4	0	1	1	0
<i>Byssosclamyces</i>	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1.02
<i>Cladosporium</i>	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	4	1.02
<i>Coccidioides</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	4.08
<i>Fusarium</i>	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	1.02
<i>Gliocladium</i>	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	4	2.04
<i>Moniliella</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	4.08
<i>Mortierella</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1.02
<i>Mucor</i>	0	0	0	1	0	0	0	0	0	2	1	0	0	0	0	4	1.02
<i>Neurospora</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	4.08
<i>Oidiodendron</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1.02
<i>Paecilomyces</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.02
<i>Penicillium</i>	4	1	2	1	0	2	0	3	0	2	2	3	2	0	2	24	1.02
<i>Rhizopus</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	24.5
<i>Scopulariopsis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Syncephalastrum</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1.02
<i>Trichoderma</i>	1	0	1	0	0	1	2	0	0	0	0	0	3	0	1	11	1.02
<i>Trichophyton</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.02
<i>Ulocladium</i>	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	11.2
<i>Zygorhynchus</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	2
Yeasts																	
<i>Candida</i>	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2.04
<i>Rhodotorula</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1.02
<i>Saccharomyces</i>	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	2	
<i>Sporobolomyces</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1.02
														0			1.02
																	2.04
																	1.02
Total	14	11	7	4	3	5	5	5	2	8	8	6	11	3	6	98	100

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