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Mycological Pollutants in Air and Phenol Utilization by *Aspergillus niger*

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Abstract

Ambient air at the University of Ilorin was sampled mycologically at four different locations, namely: student sitting area outside computer Based Test Centre, student sitting area outside the Department of Microbiology, walk way of block 1 and walkway of block 4. This was actualized using the settling plate method by exposing the plates of Potato Dextrose Agar (PDA) to air at the different points for 5 minutes. The fungal load ranged from 20,740 to 31,450 cfu/m^3. Six species of fungi were isolated and identified. They are *Aspergillus niger*, *Aspergillus fumigatus*, *Geotrichum candidum*, *Mucor circineloides*, *Trichosporon mucoides and Aspergillus terreus.* Degradation of different concentrations of phenol was studied using *Aspergillus niger* over a period of 12 days. Biodegradation rate was read using spectrophotometer at an absorbance of 400nm. Phenol utilization took place at the different concentrations and incubation periods while the maximum phenolic utlisation occurred at day 12 for the different concentrations. For the first concentration 0.090M, maximum phenolic degradation occurred at 0.045 absorbance at day 12, while for the last concentration (0.436M), maximum phenolic degradation occurred at 0.002 at day 12. The study showed that *Aspergillus niger* is promising in the biodegradation of phenol.

Key words: Air, Mold, Phenol, Biodegradation, Indoor, Outdoor.

1. Introduction

Microbes borne in air can result in serious health effects (Fracchia *et al*., 2006). Fungi are

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prevalent in outdoor and indoor air and about 12 % of the human poppulation are allergic to fungi (Pasanen *et al.,* 1996). In many places the existence of aerosols carrying microbes can adversely affect normal activities, making useful auditing crucial (Stetzenbach, 2007; Okafor & Opuene, 2007). Microbiological harm indoors and outdoors are linked mostly to bacteria and molds. These microbes have a very vital function in the biogeochemical cycle. They can move indoors by passive or active ventilation systems. Many fungal genera are released by indoor sources including wastebaskets, man, flowers and animals.

The common fungi isolated from hospitals are *Penicillium* spp., *Fusarium* spp., *Paecilomyces* spp and *Aspergillus* spp. *(*Abbasi & Samaei, 2018). *Alternaria* sp and *Penicillium* sp have been reported to have the capability to degrade phenol (Jacob & Sohail, 2010). Phenol degradation by filamentous fungi is influenced by incubation time, initial phenol concentration and strain (Santos & Linardi, 2004).

Outdoor air contamination is a principal environmental and public health challenge which affects everyone in developed and developing countries (Bascom *et al.,* 1996). Air indoor also contains fungal spores. Indoor air spores enter from outdoor whenever doors or windows are opened. If the indoor spores originate from outdoors, the spore composition in indoor and outdoor air will be closely related but the population indoors will be lower than that outdoors.

Normal air flora does not cause disease except for persons who are immunocompromised due to illness, disease, stress, old age and infancy. Fungal growth influencers such as excessive humidity and/or high water content of building materials are in most cases described as the limiting factor for microbial growth (Dhanasekaran *et al.,* 2009; Madukasi *et al.,* 2010). Air sampling of microorganisms is a popular method of conducting microbiological experiments as it allows a direct toxicological evaluation (Velmurugan *et al.,* 2008; Cuthbertson *et al.,* 2010).

Biodegradation involves the breakdown of organic substances into smaller compounds by living microbial organisms (Marinescu *et al.,* 2009). Majority of hydrocarbon chemical compounds and entities succumb to biodegradation. The relative rates of such processes however differ. A

number of factors determine the speed of biodegradation of organic compounds. Some of the factors are light, temperature, water and oxygen. The degradation rate of most organic compounds is determined by their bioavailability (Sims & Cupples, 1999; *Sims, 1991*).

In recent years, interest in the microbial biodegradation of pollutants has increased (Eduardo, 2008). Biological reactions play a vital part in the removal of contaminating substances and take advantage of the catabolic versatility of microorganisms to degrade or convert such compounds. Phenol is a monosubstituted aromatic compound with the molecular formula C_6H_5OH and is apprehended as major environmental pollutant. It is a white crystalline solid and it is volatile. Phenol is a vital industrial chemical as a raw material to many useful compounds (Weber *et al*., 2004). When compared to aliphatic NaOH completely to lose H^+ whereas most alcohols react only partially and the increased acidity over alcohol can be explained through resonance stabilization of the phenoxide anion by the aromatic ring.

Despite phenol toxicity, a number of microorganisms utilize it as carbon and energy source. The commonly isolated fungal genera from phenol include *Fusarium*, *Aspergillus, Alternaria* and *Penicillium* (Leitao *et al*., 2007). Pollution with phenol is linked with wood preservation plants, pulp mills, coal mines, refineries and various chemical industries as well as their waste water. Phenol is a very important pollutant and is added in the list of EPA (2002).

The aim of this study is to identify the fungi present outdoors in some areas within the University of Ilorin and test for phenol utilization of one of the identified isolates.

2. Materials and Methods

All materials used for this work were properly sterilized before and after use to prevent contamination. The work bench was properly disinfected using cotton wool soaked in 70% ethanol. Every work carried out was done near flame to avoid contamination.

Samples were taken in some areas within University of Ilorin where human activities were predominant. The areas sampled included Walkway of Block 1, Walkway of Block 4, Student sitting area outside the Department Microbiology and Student sitting area outside Computer Based Test Centre (CBT).

The manufacturer's instruction was strictly followed in the preparation of 1 litre of potato dextrose agar (PDA). The medium was allowed to cool to about 45 to 50^{o} C and streptomycin was added to inhibit bacteria growth before pouring into sterile Petri dishes. The settling plate technique was adopted in obtaining air samples (Plate 1). The samples were collected daily for 7 days. The sterile media was poured into sterile plates and allowed to set. The plates were placed on the floor in triplicates placing each plate few distances away from the other. The plates were exposed for five minutes and closed. The PDA plates were incubated at room temperature (25±2^{o}C) for 72hours.

Plate 1: Settling plate air sampling at Block 4 walkway

The mixed culture obtained from the incubated PDA plates were subcultured into fresh sterile PDA plates which was done after counting the total number of colonies. Also slants were also prepared in bottles as stock using this same method. Once colony forming units (CFU) were

calculated, CFU/m^3 were computed using the following formula reported by Omeliansky (Borrego *et al.,* 2010; Gutarowska, 2010; Adetitun & Oladele, 2016).

$$N= 5a\times10^4(bt)^{-1},$$

where t=exposure time in minutes, b=dish surface (cm^2), a=number of colonies per Petri dish and N=CFU/m^3 of air.

Isolates were characterized macroscopically and microscopically. The macroscopic aspect of characterization of the isolates was examined with the use of the naked eye noting the color of mycelia, spores, sizes and shape. The colonies were also observed microscopically in which their vegetative structure and spores were noted (Aneja *et al*., 2005; Onions *et al.,* 1981).

Mineral salts medium composition used include; 0.125g/ml of KH_2PO_4, 0.35g/ml of Na_2HPO_4, 0.05g/ml of $MgSO_4.7H_20$, 0.075g/ml of KNO_3 and 0.025g/ml of $(NH_4)_2\ SO_4$ (Vecchioli *et al*., 1990).

PDA broth was prepared by measuring 9.7 grams of PDA into conical flask with 250ml distilled water, stirred and heated for 3minutes to dissolution and allowed to cool. The suspended media was carefully poured into MacCartney bottle used as slants and organism to be used (*Aspergillus niger*) was inoculated using inoculating needle which was done aseptically. This was incubated for 37^0C for 72hours.

This was carried out by dispensing 20ml of MSM into 5 different dispenser bottles. Phenol was added thus; for the first bottle of 100mM concentration, 0.2ml of phenol was measured into the bottles containing distilled water using pipette and this was done for all the samples at different concentrations. 0.5ml of the *Aspergillus niger* (broth) was also introduced into the sample with phenol. The absorbance was read at day 0, 3, 6, 9 and 12 at wavelength 400nm. Distilled water was used as blank (Garcia *et al*., 2000).

3. Result and Discussion

In Table 1, occurrence of the isolates on PDA plates and the percentage frequency were illustrated. It was seen that *Aspergillus niger* had the highest number of occurrence per day. This was followed by *Aspergillus terreus.* The least fungal occurrence was exhibited by *Geotrichum candidum.*

In Table 2 is depicted the average number of the fungal isolates per day expressed in cfu/m^3. For each day at the different sampling sites and from the average calculated, CBT area had the highest number of air fungi while the least was from BLOCK 4 area. Fungal levels in air exceeded AIJ standard levels (1000 CFU/m^3). This correlates the report of Shinohara (2018).

Fungi were isolated from all the sampled areas daily. The ubiquitous nature of fungi is further confirmed even in air. Various human activities increase the diversity of microorganisms in air (Gordon, 2004). The fungi isolated had high number of occurrence and distribution on the plates each day. This may be due to high and vast human activities as the sampling sites are known for large concentration of students.

In the report of Wamedo *et al.* (2012) it was opined that indoor domain are prime environmental factors having the ability of influencing health. Particulate activities like sneezing, talking, coughing, can generate biological particulate matters that are carried by air. The fungi isolated in this work are similar to that obtained in the work of Adetitiun and Oladele (2016) where they isolated fungi from some offices (indoor air) in the University of Ilorin. Some of the fungi they isolated were *Aspergillus niger and Geotrichum candidum*. Occurrence of this same fungi especially *Aspergillus* species elucidates and agrees with Flannigan *et al.* (1991) who stated that the ratio of the sum of species of *Aspergillus* outdoors to that indoors may either be more or reversed.

The isolated fungi have been implicated as the causal agent of diseases in man such as the case of primary cutaneous zygomycosis caused by *Mucor circineloides* (Chandra and Woodgyer,

2002), Aspergillosis caused by *Aspergillus* species and causal agent of food spoilage such as *Aspergillus niger* spores and vegetative forms on foodstuffs. Aspergillosis is increasingly common ubiquitous fungal infection of birds and occasionally other animals including man. About twenty species have been documented as causal factor of opportunistic infections in man. Among these, *Aspergillus fumigatus* is the most rampant (Denning *et al.,* 1997). This is followed by other species of *Aspergillus* (Arikan *et al.*, 1998).

The ability of *Aspergillus niger* isolated from air to degrade phenol was investigated and the result showed that *Aspergillus niger* can grow using phenol as sole carbon and energy source. There was a sharp drop in phenol concentration from day 1 to day 6 (0.09M to 0.05M). From day 7 the drop in phenol concentration was minimal and only ranged from 0.05 to 0.04M (Figure 1). In Figure 2, there was a sort of lag phase up to day 6 after which an exponential drop was observed. In Figure 3, the exponential phenol reduction was found between day 3 and 7. There was a combination of lag-log phase in Figure 4. Exponential drop was observed from day 3 to day 7 in Figure 5.

Table 1: Occurrence and distributions of the fungal isolates on plates

Isolated Fungi	Sample sites	Days							Percentage Frequency
		1	2	3	4	5	6	7	
Aspergillus niger	CBT	+	+	+	+	+	+	+	100
	MCB	+	+	+	+	+	+	+	100
	BLK 1	+	+	+	+	+	+	+	100
	BLK 4	+	+	+	+	-	+	+	86
Geotrichum candidum	CBT	-	+	+	+	-	+	+	71
	MCB	-	+	-	-	+	-	+	43
	BLK 1	-	+	+	-	+	+	+	71
	BLK 4	-	+	-	+	-	+	+	57
Aspergillus terreus	CBT	+	+	+	+	-	+	-	71
	MCB	+	+	+	+	+	+	+	100
	BLK 1	+	+	+	+	+	+	+	100
	BLK 4	+	+	+	+	+	+	-	86
Aspergillus fumigatus	CBT	-	+	-	-	+	+	-	43
	MCB	+	-	+	+	+	+	+	86
	BLK 1	-	-	+	+	-	-	+	43
	BLK 4	-	+	+	+	-	+	+	71
Trichosporon mucoides	CBT	+	+	+	+	+	+	+	100
	MCB	+	+	+	+	+	-	+	86
	BLK 1	+	+	+	-	+	-	+	71
	BLK 4	+	+	+	+	-	+	+	86
Mucor circineloides	CBT	+	+	-	+	+	+	+	86
	MCB	-	-	-	+	+	-	+	43
	BLK 1	+	-	+	+	-	+	+	71
	BLK 4	-	-	-	+	-	+	+	43

Key: - = Absent; + = Present

Table 2: Number of fungal in air at different sampling weeks

Sampling time	Sampling sites and fungal isolates cfu/m^3			
Days	MCB	CBT	BLOCK 1	BLOCK 4
1	925	1,410	661	255
2	308	749	617	441
3	793	1,278	1,190	573
4	1,454	1,498	1,322	1,190
5	837	529	705	352
6	308	1,454	837	661
7	2,423	1,234	925	1,498
Total	7,048	8,152	6,257	4,970

Key: MCB=Microbiology CBT=Computer Based Test Centre

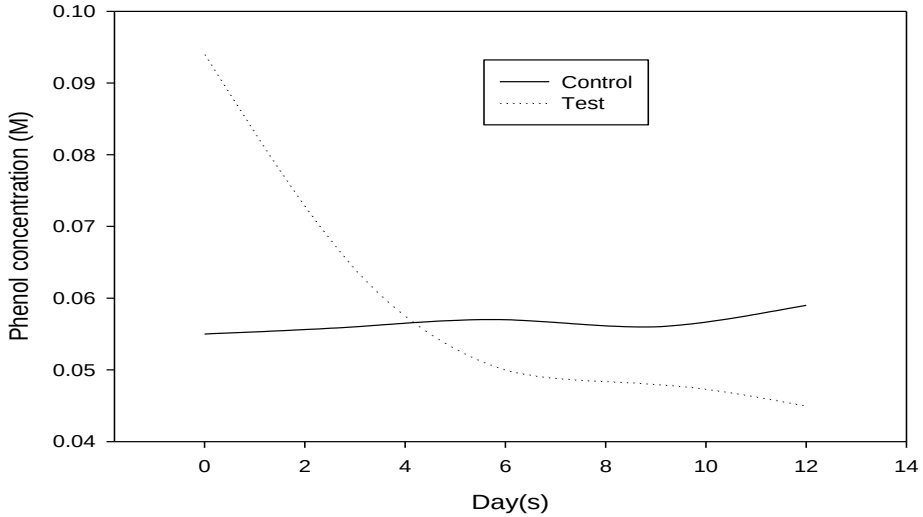

Figure 1: Utilisation of phenol by *Aspergillus niger* at 0.090M concentration of phenol

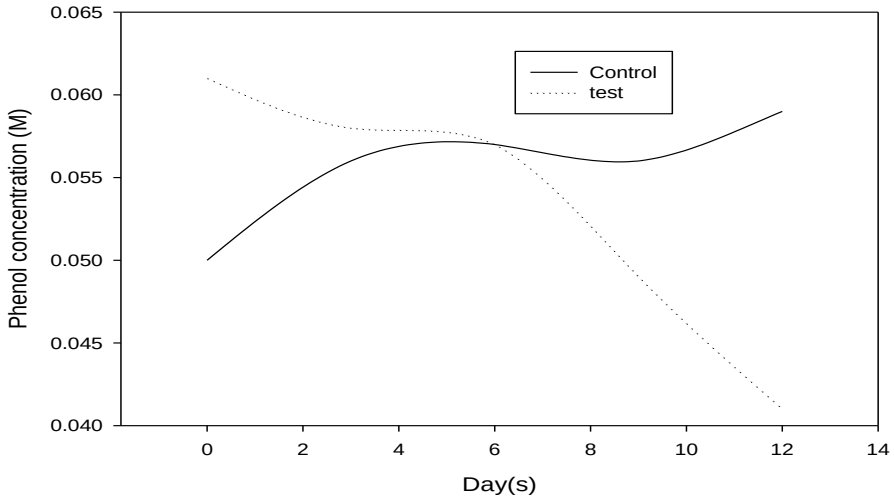

Figure 2: Utilization of phenol by *Aspergillus niger* at the rate of 0.179M concentration of phenol at 400nm.

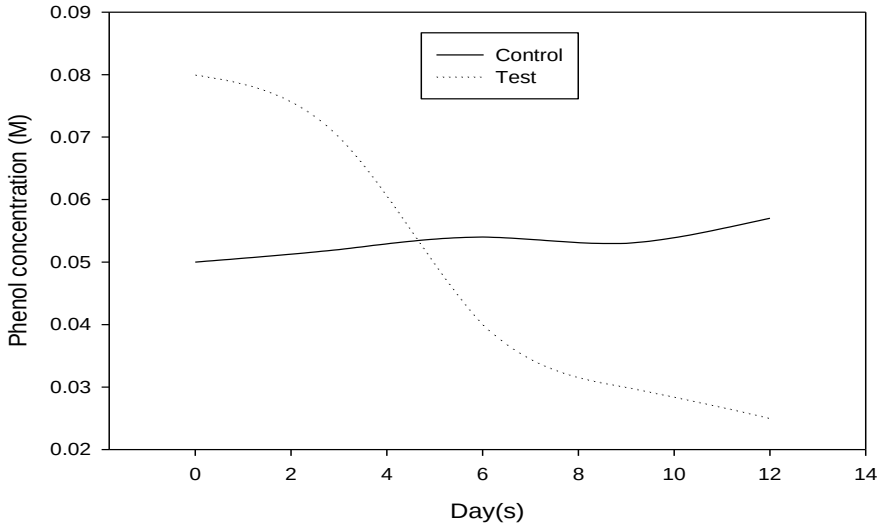

Figure 3: Utilization of phenol by *Aspergillus niger* at the rate of 0.265M concentration of phenol at 400nm

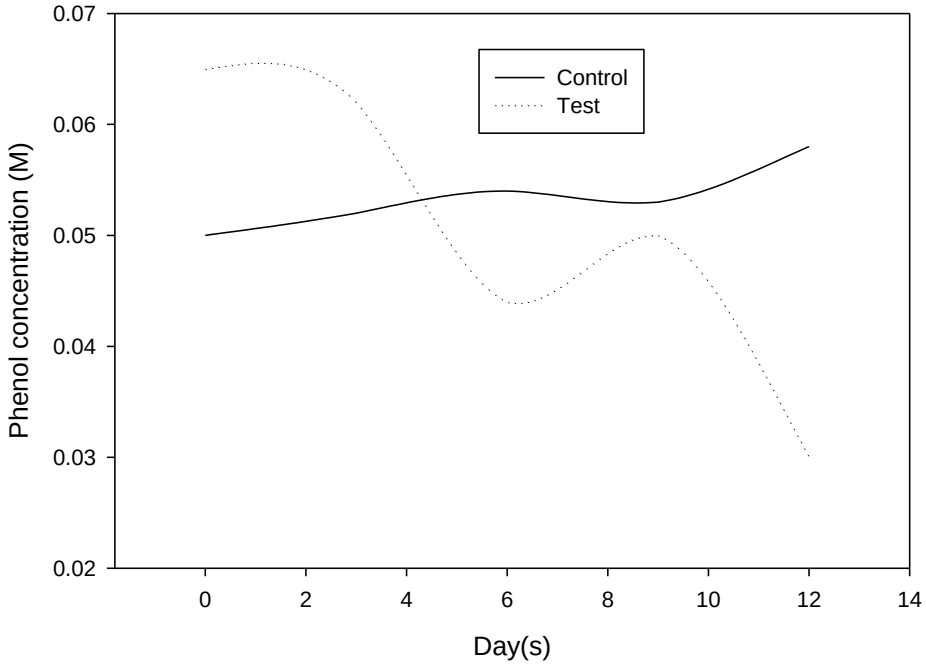

Figure 4: Utilization of phenol by *Aspergillus niger* at the rate of 0.350M concentration of phenol at 400nm

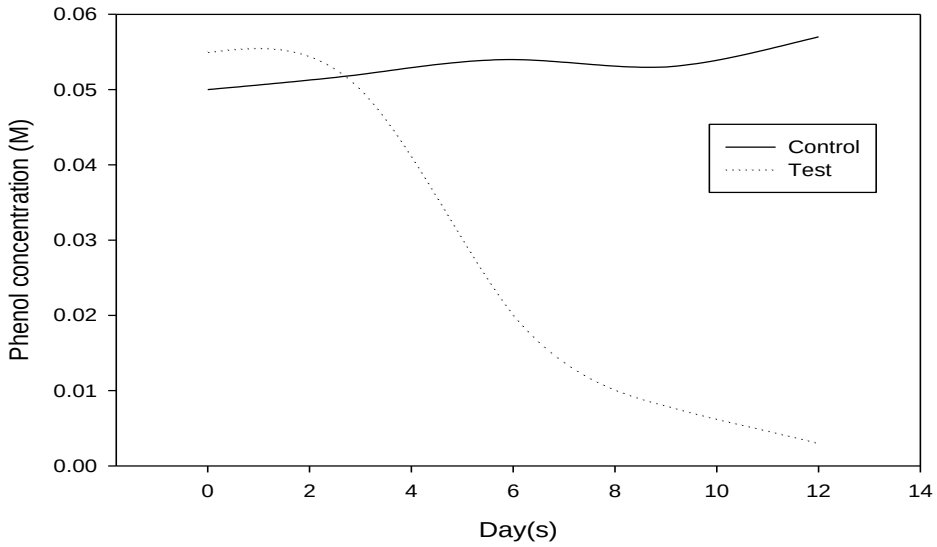

Figure 5: Utilization of phenol by *Aspergillus niger* at the rate of 0.436M concentration of phenol at 400nm

Supriya and Deva (2014) reported the effect of incubation period on phenol biodegradation. The result of these two workers showed that maximum phenol degradation occurred at 120 hours. In this work phenol degradation reached its maximum at 120 to 240 hours. Kumar *et al*. (2018) reported that immobilized cell of *Candida tropicalis utilized* 98% of phenol at a concentration of 1000 mg/l of phenol whereas free cells degraded up to 63% of the same concentration under 9 days of incubation.

In the aerobic degradation routes, the substrates are generally converted to a limited number of dihydroxylated intermediates such as catechol and protocatechate, followed by one of the meta or ortho ring-cleavage pathways. The enzyme systems resemble each other, although a good many different metabolic pathways have been identified to date (Vander meer *et al.*, 1992). The specific pathways of degradation will be explored in our subsequent work.

Maulin (2014) in his work on degradation of phenol by an administration of *Pseudomonas* species where the temperature effect (25 ^{o}C to 50 ^{o}C), difference in pH (5.5 to 10.5) and concentration of glucose (0, 0.25 and 0.5%) on the pace of degradation was investigated. This worker observed that the rate of phenol degradation was regulated by pH, temperature and glucose concentration.

4. Conclusion

This present work shows that *Aspergillus niger* is promising for application in biodegradation schemes in order to degrade phenol and possibly other related aromatic compounds. Different species of fungi are present in outdoor air and their use in biodegradation of hydrocarbons is possible.

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