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A COMPARATIVE STUDY OF THE OUTDOOR AIR QUALITY OF A SECTION OF EBONYI STATE UNIVERSITY OLD LABORATORY COMPLEX AND ANTIBIOGRAM OF CYMBOPOGON CITRATUS ON THE AIR CONTAMINANTS

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ABSTRACT

The Bacteriological determination of outdoor air quality of a section of Ebonyi state university old laboratory complex was examined. The microbial counts in the outdoor air environment of the sample site ranged from 10cfu/m3 to 80cfu/m3. The highest mean bacterial count (68.7cfu/m^3) was recorded at noon and the lowest mean bacterial count (15cfu/m3) was recorded in the morning. However, there was no significant difference ($p>0.05$) between the counts obtained in the morning and those recorded in the afternoon. The isolated organism were tested against aqueous extract of lemon grass plant grown in the region, it was observed that the inhibition zone diameter of aqueous extract of lemon grass plant against the isolates ranged from 17 to 48 mm showing better antimicrobial activity against common pathogenic bacteria species. Based on the obtained results, lemongrass has demonstrated varying degree of antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus sp.*, *Pseudomons* spp, Therefore, this signifies that some bacteria that have not been tested with lemongrass extract in this research may also be susceptible to the antibacterial effect of lemongrass.

KEYWORDS: However, there was no significant difference ($p>0.05$) between the counts obtained in the morning and those recorded in the afternoon.

INTRODUCTION

Microorganisms are found everyway in our environment and impact the whole ecosphere in one way or the other. They influence man in different ways. The diversity of microbial activities varies from causing diseases in human, other animals and plants. Microorganisms are sensitive indicators of environmental quality. Air serves as a very good dispersal medium for microbiota. The type of species and amount of organisms present in the air depends on physicochemical factors like the temperature, viscosity, lighting, suspension of organic and inorganic material and food availability. Human activities are also an important determining factor for the diversity of microbes in an area (Kumari et al., 2011). The air inhaled by people is abundantly loaded with microorganisms in form of bioaerosols (Wojtatowicz et al., 2008). Karwowska (2005) defined bioaerosols as a colloidal suspension, formed by liquid droplets and particles of solid matter in the air, whose components contain or have attached to them viruses, fungal spores and conidia, bacterial endospores, plant pollen and fragments of plant tissues. Basically, bioaerosols are airborne particles that are living or originate from living organisms. Bioaerosols are ubiquitous, highly variable, complex, natural or man-made in origin. Aerosol particles of biological origin (cells, cell fractions or organic matter of animal, plant and microbial origin) form a significant portion of atmospheric aerosols, sometimes reaching close to 50% numerically of all aerosol particles (Jarnicke, 2005). Pillai and Ricke (2002) opines that composition and size of bioaerosol varies from 20 nm to >100 µm depending on the source,

aerosolization mechanisms, and environmental conditions prevailing at the site. The inhalable fraction (PM 2.5) is of primary concern because it is the most susceptible portion of the bioaerosols to reach the deeper parts of the respiratory system. Indoor air quality is one of the most important factors that influence our general life quality. An average human breathe 10 m3 air every day, and spend 80–95% of his or her live indoors (Dacarro et al., 2003). Indoor air pollution can result in health problems and even an increase in human mortality (Cabral et al., 2003). 2 Most people in urban areas spend about 90% of their time outdoors (WHO, 2000). It has been also reported that many people spend most of their lives in different outdoor environments of homes, care facilities, schools and workplaces (Srinivasan et al., 2003). Health care facilities or other private and public buildings where people spend a large part of their life is an essential determinant of healthy life and people's well-being (WHO, 2010). Globally, an estimate of 3.8 million deaths was attributed to outdoor air pollution in 2016 (WHO, 2018; Andualem et al., 2019) and the level of indoor pollutants is said to be 2–5 times more than that of outdoor pollutants (EPA, 2017). It has also been reported that poor air quality is associated with lungs and heart diseases (Shinn et al., 2000).

Collection and Preparation of Samples

The settle plate method was used to collect outdoor air samples from the laboratory unit gy. Sampling was done at two regular intervals of a day. The first set of petri dishes were exposed before the arrival of staff and students in the morning by 9.00a.m. When the number of staff and students were minimal while the second sets of petri-dishes were exposed to air in the afternoon by 4.00pm when the day's work was done and staff and students were about leaving the laboratory (Kabir et al., 2016). 90mm diameter petri dishes containing 20 ml of sterile nutrient agar prepared in triplicates were exposed to outdoor air in the different corner of the laboratory unit for thirty minutes after which the plates were covered and transported to the Department of Microbiology laboratory and incubated at 37^0C for 24 - 48 h.

Plant Materials used

Lemongrass leaves were collected from a nearby farm in Ebonyi State University, Abakaliki and identified by a botanist. The leaves were washed first under running tap water, followed by sterilized distilled water and dried at room temperature in dark then grinded to powder using an electrical blender.

Preparation of Samples (Dilution Method)

A colony of bacteria cells were collected from each of the culture media with a sterile wire loop and placed into four sterile beakers and 10ml diluent (sterile water) added to it. They were shaken for 5mins and allowed to soak for 30mins. 9ml sterile water was poured into thirty-six (36) test tubes for a ten (10) fold dilution process. 1ml each of the diluted samples were then poured into each of the test tubes carrying out a serial dilution of 10-1 to 10-10 and labeled.

Preparation of Plant Extracts

The leaves of the plants were air dried at room temperature for 3 weeks and grounded to coarse powder. 15g of the powder was placed in 100ml of distilled water (cold water extract), methanol, and hexane in conical flask and the crude preparations were left overnight in the shaker at 35°C and then centrifuged at 2500rpm for 10mins. The supernatants containing the plant extracts was then transferred to a pre-weighed beakers and the extracts were concentrated by evaporating the solvents at 60°C. The crude extracts were weighed and dissolved in a known volume of dimethyl sulphoxide, to obtain a final concentration of 200mg/ml and sterilized by filtration through (0.45µm) millipore filters. The aqueous extracts were stored in sample bottles at 4°C prior to use (De and Ifeoma, 2002). The percentage (%) yield of the sample before and after extraction was calculated as follows: % $Yield = mass\ of\ sample\ after\ extraction\ (g)\ mass\ of\ sample\ before\ extraction\ (g)\ x\ 100$.

Bacteriological Analysis

1ml each of the 10-9 and 10-10 dilution of each samples were poured into eight sterile petri dishes and the nutrient agar was poured into the dishes containing the samples. It was swirled to ensure even distribution of the samples and the agar was allowed to gel and incubated at 37^OC for 24hrs by inversion. Sub-culturing was carried out inside the laminar airflow. The isolated colonies of bacteria grown on MacConkey, Meuller Hinton, and Mannitol Salt agars were picked and transferred to the 36 Nutrient agar and Nutrient broth by streaking in a zig-zag method and mixing respectively using a sterile wire loop. To avoid contamination, the lid of the plates were lifted to minimum. It was then closed, inverted and incubated for 24 – 48 hours. This subculture was done in order to obtain a pure culture of the isolated bacteria from air. 3.6.2 Standardization of Inoculum Exactly 0.2ml of 24/hours old culture of each microorganism was dispensed into 20ml of sterile nutrient broth and was incubated for 3-5/hours to standardize the culture to 106cfu/ml (Collins et al., 1995).

Antibacterial Testing

This was done using the agar wells diffusion method of (Odeyemi and Fagbohun, 2005). 0.5ml of overnight broth culture of each clinical isolates containing 106cfu/ml was gently transferred to the solidified nutrient agar and spread uniformly on the agar surface using a sterile glass spreader. Four 6mm wells were bored unto the agar and filled with the aqueous extracts (distill water, methanol, and hexane) while the distill water serves as the control. The Petri dishes were incubated at 37°C for 18-24/hr and the inhibition zones were measured (mm).

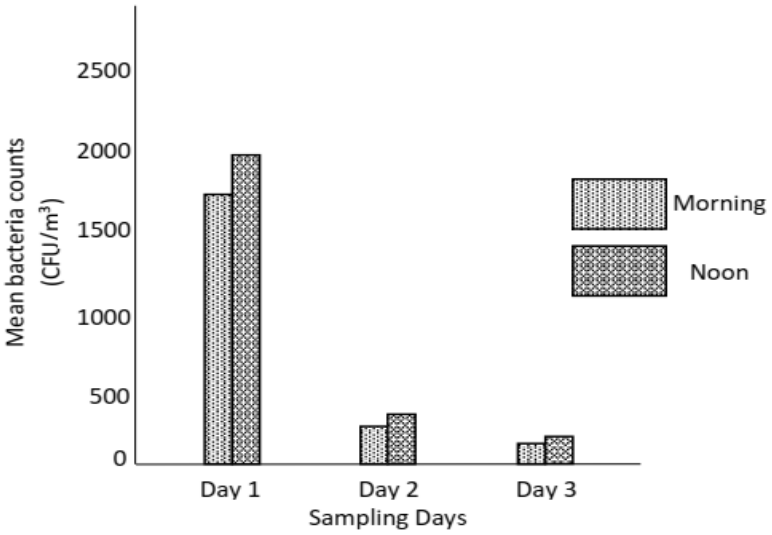
Enumeration of Bacteria

The colonies formed on petri dishes were enumerated and converted to colony-forming units per cubic meter of air (CFU/m3) using the equation described by Omeliansky (Hayleeyesus and Manaye, 2014). N = 5a × 104 (bt) - 1 Where: N = CFU/m3 of air a = number of colonies on the petri dish b =Surface area of the petri dish (cm2) t = Exposure time (mins).

3.6.5 Identification of Bacterial isolates Bacteria isolates were characterized to genus level based on morphological, cellular and biochemical characteristics as described by Bergey's manual of determinative bacteriology (Holt et al., 1994).

Statistical analysis

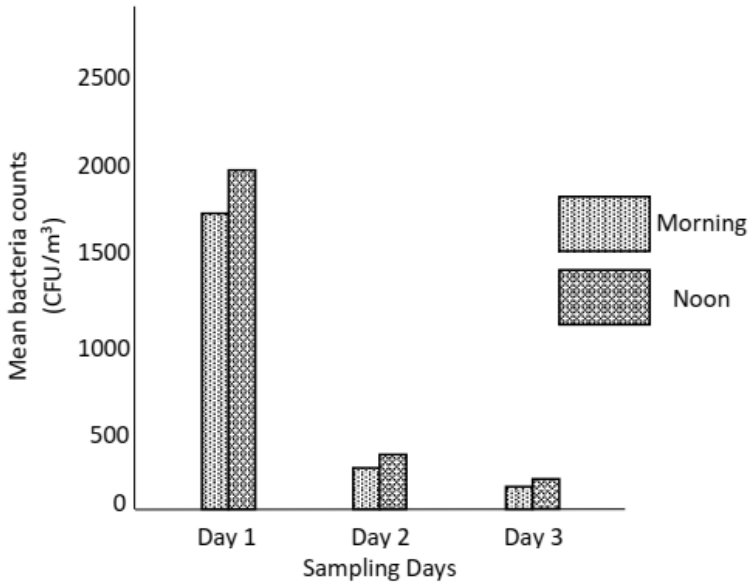
The data recorded during the course of investigation were statistically analyzed by three way classifications and conclusion was drawn on the basis of analysis of variance technique. The calculated value of F was compared with the tabulated value at 5% and 1% level of significance for appropriate degrees of freedom.304 38.



RESULTS

Table 1: Total Microbial Count of the Sampling Site during Day and Noon. Microbial Count (cfu/m3) Sampling Time Morning Noon.

The microbial counts in the outdoor air environment of the sample site as presented in Table 1 ranged from 10cfu/m3 to 80cfu/m3. The highest mean bacterial count (68.7cfu/m^3) was recorded at noon and the lowest mean bacterial count (15cfu/m3) was recorded in the morning. The highest mean bacterial count in the afternoon is due to the fact that the sampling site is always overcrowded at this time with staff and students, which increases the rate of air pollution than in the morning. The highest total microbial count is recorded in Day 3 (100cfu/m^3), while the lowest total microbial count is recorded in Day 2 (75cfu/m3). This is as a result of high rate of activities around the sampling site in Day 3 than in Day 1 and Day 2.



NB: < 500 cfu/m3 is permissible standard

Table 2 below shows the organism isolated from each of the days after exposure for some minutes. It was observed that only *Bacillus* spp was isolated in day 1, E. coli in d S. *aureus* on the second day whereas *Peudomonas* spp and E. coli was isolated on the third day.

Table 2: Representation of isolated organism from day 1 to day 3

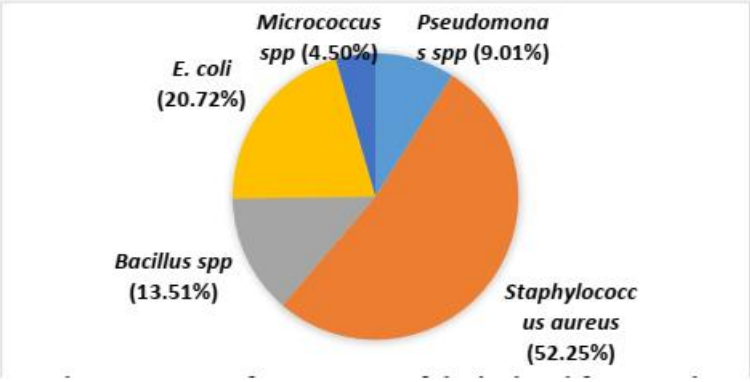
Sampling Days	*Pseudomonas spp*	*Staphylococcus aureus*	*Bacillus spp*	*E. coli*	*Micrococcus spp*
Day 1	-	+	+	-	-
Day 2	-	+	-	+	-
Day 3	+	+	-	+	-

Key :
+=present
-=absent

The distribution of the isolated microorganism in the outdoor air environment of the sample site as presented in Table 3 ranged from 10cfu/m3 to 80cfu/m3. The distribution of the isolated microorganism highest mean bacterial count (68.7cfu/m^3) was recorded at noon and the lowest mean bacterial count (15cfu/m3) was recorded in the morning. The highest distribution of the isolated microorganism was S. *aureus* followed by E. coli the least was *micrococcus* spp

Table 3: distribution of the isolated microorganism.

Sampling Day	Sampling Time	*Bacillus spp*	*E. coli*	*Pseudomonas spp*	*Staph. aureus*	*Micrococcus spp*
Day 1	Morning	0	2	3	10	0
	Noon	1	10	5	5	1
Day 2	Morning	3	0	0	20	3
	Noon	6	1	1	7	0
Day 3	Morning	5	0	0	9	1
	Noon	0	10	1	7	0
Total		**15**	**23**	**10**	**58**	**5**



In this study, it was found that the inhibition zone diameter of aqueous extract of lemon grass plant concentration around 5 to 48 mm showing better antimicrobial activity against common pathogenic bacteria species. Based on the obtained results, lemongrass has demonstrated varying degree of antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus sp.*, *Pseudomons* spp,

Therefore, this signifies that some bacteria that have not been tested with lemongrass extract in this research may also be susceptible to the antibacterial effect of lemongrass.

Table 4: Antimicrobial activity of lemons grass against isolated bacteria species.

Organism tested	Concentration (mg/ml) /inhibition zone diameter(mm) 25	50	100
Staphylococcus aureus	-	10	48
Micrococcus spp	-	-	5
Eschericia coli	-	5	23
Pseudomonas spp	-	17	45
Bacillus spp	-	8	13

Key
- = no inhibition

DISCUSSION

The indoor air quality of the laboratory unit was determined in this research. Mean bacterial counts at different sampling times of Laboratory Unit in Ebonyi State University, Abakaliki, Nigeria shows that bacterial counts were higher at noon compared with counts obtained in the morning from the corners of the laboratory unit. This finding corroborates that of other researchers that there are higher concentrations of bacteria in the afternoon or evening when students are actively working or about to leave the laboratories compared to the morning period (Aniebo et al., 2016; Mohammed et al., 2016; Bukar et al., 2017; Enitan et al., 2017). However, there was no significant difference ($p>0.05$) between the counts obtained in the morning and those recorded in the afternoon. Aniebo et al., (2016) also had a similar report that the bacterial counts obtained in some major science laboratories in IBB University Lapai, Nigeria in the morning were insignificant.

The distribution of the bacteria isolated from the Laboratory Unit is presented in Table 3, *Staphylococcus aureus* was isolated the most, while E. coli was intermediate. These organisms are among the most commonly isolated bacteria in outdoor air environments (Hayleeyesus and Manaye, 2014; Luksamijarulkul et al., 2014; Aniebo et al., 2016; Mohammed et al., 2016; Bukar et al., 2017; Enitan et al., 2017). For the Measurement of Inhibition Zone Diameter (IZD) in millimeters of Tested Microbes, the highest inhibition zone (58 mm) was recorded for *Staphylococcus aureus* and the lowest inhibition zone (5 mm) was recorded for *Micrococcus* spp, while *E. coli* is 23 mm. 45 mm for *Pseudomona* spp, *Bacillus* spp 13mm. This result showed the dominance of Gram-positive bacteria in the outdoor air of the Laboratory Unit as reported in other related studies (Aniebo et al., 2016; Bukar et al., 2017; Bragosizewska et al., 2018). Also, as equally seen *E. coli* and *Staphylococcus aureus* were found to be highly dominant in the air environments of the location sampled. These organisms are commonly found in air, soil and water. Aniebo et al., (2016) also reported *Bacillus* species as the dominant bacteria in the outdoor air of the laboratory. *Bacillus* species are persistent and dominant in different environments because of their ability to produce spores which allow the organisms to withstand unfavorable conditions and partly also because they are present in high numbers in the air (Aniebo et al., 2016; Whyte et al., 2001). *Staphylococcus* is saprophytic in nature and generally associated with the human skin, which however gives favorable conditions and environment can become opportunistic pathogens to man. Staphylococcus aureus can cause skin infections, septicemia and some gastrointestinal infections. The presence of these organisms in the outdoor air also suggests contamination by human presence and activities.

This research has shown the presence of bacterial aerosols in the outdoor air quality of the Laboratory Unit sampled. The bacteria isolated from this laboratory unit are opportunistic pathogens which can cause adverse health challenges, especially in individuals with compromised immune systems. Bacteria in the air of this laboratory unit can also contaminate various research works carried out by students which can render the works invalid or give false results resulting in serious hazards to the public. The use of nose masks while working around laboratory units, adequate sanitary practices, as well as regular fumigation of science laboratories, can help to reduce the level of bioaerosols and the risks associated with these bioaerosols around the laboratory. Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development (Newman et al., 2007). In this study, it was found that the aqueous extract of lemon grass plant is concentration around 40 to 100 µg/ml showed better antimicrobial activity against common pathogenic bacteria species. Based on the obtained results, lemongrass has demonstrated varying degree of antibacterial activity against *Staphylococcus aureus, Escherichia coli, and Salmonella typhi*. Therefore, this signifies that some bacteria that have not been tested with lemongrass extract in this research may also be susceptible to the antibacterial effect of lemongrass.

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