# Environmental Health Effects and Biosurfactant Production Potentials of Microorganisms Isolated from Five Petroleum Stations in Fate, Ilorin, Nigeria

**David Olugbenga Adetun* and Khadijat Yetunde Amidu**

Department of Microbiology,
Faculty of Life Sciences,
University of Ilorin, Nigeria

***Corresponding Author Email**: adetitun.do@gmail.com,
adetitun.do@unilorin.edu.ng

## ABSTRACT

Air harbors large quantity of bacteria and fungi which makes it imperative to carry out microbiological analysis of outdoor air. In this study the microbiological quality of air of five fuel stations at Fate, Ilorin were assessed. The isolated bacteria were tested for their ability to produce biosurfactant. Settling plate technique was employed for sample collection using nutrient agar plates and potato dextrose agar plates for bacterial and fungal isolation. The bacterial counts in the selected locations ranged from 3.72 $\times 10^2$ cfu/m$^3$ to 1.6592$\times 10^4$ cfu/m$^3$, while the fungal count ranged from 1.57 $\times$ $10^2$ cfu/m$^3$ to 1.18$\times 10^3$ cfu /m$^3$. The highest bacterial and fungal count was observed at station five which was the busiest during the sampling period. Seven bacterial isolates and five fungal isolates were identified. The microorganisms identified include *Bacillus* sp, *Lactobacillus* sp, *Micrococcus* sp and *Corynebacterium* sp as the bacteria isolates while *Aspergillus* sp, *Alternaria alternata, Fusarium oxysporum* and *Rhizopus oryzae* as the fungi isolates. *Bacillus* sp and *Aspergillus niger* had the highest microbial occurrence at 100% all through the sampling period. The *Corynebacterium* sp *and Bacillus coagulans* isolated exhibited biosurfactant producing potential that can be further tested for bioremediation purposes. The opportunistic pathogenicity of the bacterial and fungal species isolated in this research has vividly demonstrated that the microflora of the fuel stations at Fate, Ilorin, has public health implications. It is therefore recommended that proper sanitation and hygiene should be observed by everyone who associates with the petroleum stations environment.

**Keywords**: *Bacteria, Biosurfactant Production, Environmental Health, Fungi, Petroleum Stations, Ilorin.*

## 1.0 INTRODUCTION

The air is a mixture of gases which component is variably important to all forms of living things, its oxygen component is required by aerobic living things while its

nitrogen component can be utilized by anaerobic living things. While air is mostly gas, particles referred to as aerosols and microorganisms which are regarded as bioaerosols are also present in the air and can travel long distances through the air (Grennfelt *et al.,* 2019). Jasim *et al.* (2024) reported that dust particles in air contribute to health problems.

There are no microbes that are native to the air, rather they are allochthonous to the atmosphere their presence in the air must have emanated from living or nonliving source (Yaghoub and Elagbash, 2010). The air is not protected from desiccation, so most of the microbial forms in the air will die thus, the air is believed not to support microbes but serve as dispersal medium for microorganisms. However, the air inhaled can be loaded with microorganisms, so the air quality is one of the factors affecting health and productivity of people. Microbial contamination of air is mostly by bacteria and fungi, they can be dangerous as pathogenic living cells and can also secrete substances harmful for health (Sheik *et al.,* 2015).

Understanding the composition and diversity of microorganisms in the air of fuel stations is essential as the presence of certain microorganisms of health implication can lead to significant impact on the personnel and customers in the fuel stations, as poor air quality can affect or harm human health and the environment (Wemedo *et al.*, 2012) also the presence of beneficial microorganisms could offer opportunities for biodegradation purpose, as microorganisms are expected to reside in favorable conditions, thus can be employed for bioremediation purposes.

The air microflora of petrol stations represents a dynamic and complex ecosystem influenced by various factors, including vehicle emissions, fuel handling activities, and environmental conditions (Adebiyi, 2022). The reasons behind targeting petrol stations is because we desire to test the isolated bacteria for biosurfactant production. In a way we are trying to see if the bacteria isolated have affinity with petroleum products that are being stored and sold at the stations. This is because bacteria that bind easily to hydrocarbons are known to be high in biosurfactant production. This biosurfactant production ability usually translate to biodegradability (Soni *et al*., 2024).

The subject of airborne microorganisms in both indoor and outdoor settings has received extensive research attention in public health (Ruiz-Gil *et al*., 2020), hence the composition and diversity of microorganisms in the air around petrol stations have implications for both environmental and public health.

The primary objectives of this study are twofold: to assess the environmental health effects stemming from air microflora of petrol stations in Fate, Ilorin, and to explore the biosurfactant production potentials of bacteria isolated from the stations.

## 2.0 MATERIALS AND METHODS

### 2.1 Sampling Area

This study was carried out at five selected fuel stations along Fate Road, Ilorin. The five selected fuel stations (Figure 1) had varying human activities during the periods of sample collection. Figure 2 shows process of sample collection in one of the sampling points.

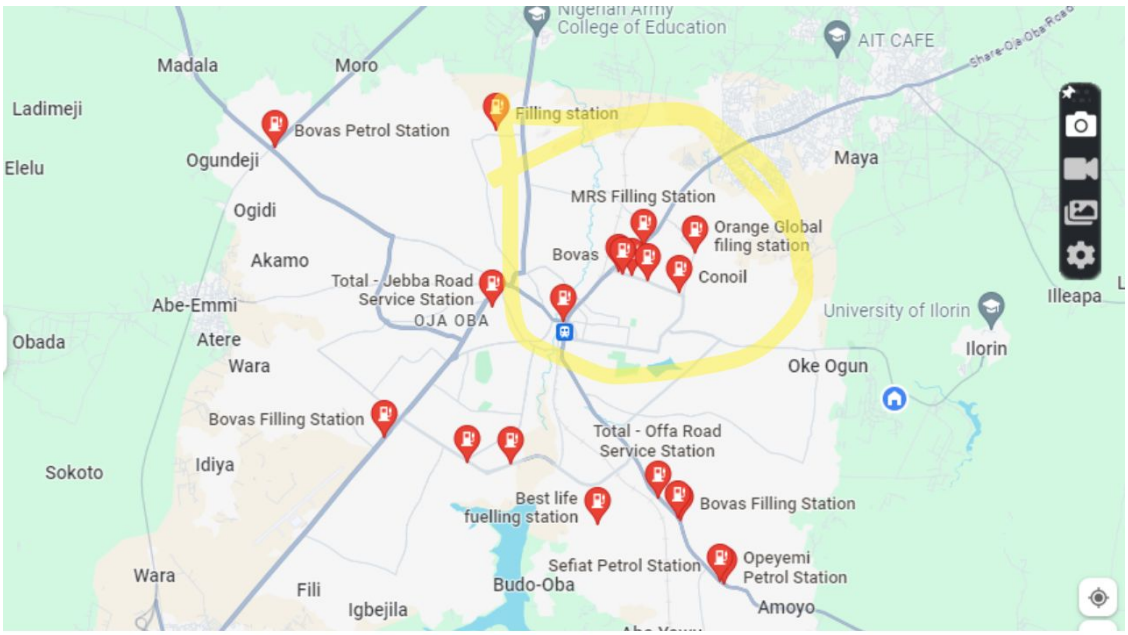


***Figure 1:*** *Location of the five petroleum stations sampled on Fate Road, Ilorin (yellow circle)*

### 2.2 Sterilization of materials

The materials used were sterilised before and after use to avoid contamination. The autoclave was used to sterilise the materials for 15 minutes at 121°C. Every piece of glassware was washed with soap, rinsed with distilled water, and then sterilised in a hot air oven at 170 °C for 60 minutes. Before and after usage, the inoculating loops and the straight wire loops were both sterilised by heating them until they turned red over a Bunsen flame. A 70% ethanol solution was used to wipe the laboratory bench with cotton wool. Sterile pipette tips and sterile disposable Petri dishes were utilised (Fawole and Oso, 2007).

## 2.3 Air Sampling Procedure

The air sampling was done using the settling plate technique as employed by Adetitun and Oladele (2016), this was done by exposing sterile plates of Nutrient Agar and Potato Dextrose Agar to the air of the environment for 15 minutes at two different spots in duplicates at the selected fuel stations (Figure 2). One spot was the air around the top of the fuel dispenser while the other spot was the air around the pavement of the fuel dispenser, the exposure was done five times at one-week intervals. Nutrient agar plates were incubated at 37°c for 24 hours for bacterial growth, while potato dextrose agar plates were incubated at 25°c for 48 hours for fungal growth. The mixed culture obtained were characterized and counted. The CFU range was calculated using the formula:

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

Where **N** is microbial load in $cfu/m^3$,

**a** is the total number of colonies,

**b** is the diameter of the Petri dish, and

**t** is the exposure time in minutes.

The mixed colonies obtained were sub-cultured until pure cultures were obtained for the bacterial and fungal isolates.

***Figure 2:*** *Sample collection from the air surrounding of a fuel dispenser at a fuel station in Fate, Ilorin.*

## 2.4 Characterization and Identification of bacterial Isolates

Pure cultures of the bacterial colonies were characterized based on colonial and cellular morphology. Their cellular morphology was determined according to their reaction to Gram staining, endospore staining, motility test and capsule staining. The bacterial isolates were further characterized by routine biochemical tests. Identification of bacterial isolates was done using the standard procedures according to Cheesbrough (2005). Reference was made to Fawole and Oso (2007), for there colonial characteristics.

## 2.5 Characterization and Identification of fungal Isolates

Macroscopic and microscopic examination was carried out on the pure cultures of the fungal isolates for their colonial and cellular characterization respectively as described by Adetitun and Oladele (2016), Reference was made to Campbell and Stewart (1980) for identification of the fungal isolates.

## 2.6 Biosurfactant Analysis

The bacterial isolates were tested for their biosurfactant activity. They were tested against three different test oils; crude oil, engine oil and kerosene. Hemolytic assay and oil displacement assay were carried out on the bacterial isolates according to Thavasi *et al.* (2011), with slight modifications. The bacterial cultures were grown on nutrient agar medium and human blood agar was used for the hemolytic assay. Drop collapse assay and Emulsification test was done according to Charan and Patel (2017), with slight modification to the emulsification test, 1ml of culture supernatant and 1ml of the test oils were added in a sample bottle and vortexed at high speed for 2 minutes. The mixture was left to stand for 24 hrs. The emulsification index is the height of the emulsion layer divided by the total height and expressed as a percentage. The drop collapse experiment involved extracting the supernatant from a bacterial culture suspension. When drops of the polar supernatant were placed on an oil-coated surface without surfactant production, the drops remained stable as they were repelled from the surface. However, in the presence of surfactant production, the drops exhibited changes – they either spread out or collapsed. This behavior was attributed to the reduction in surface tension between the liquid supernatant drop and the oil-coated surface caused by the surfactant.

## 3.0 RESULTS

The bacterial isolates obtained from the sampling site were identified as *Bacillus circulans, Bacillus coagulans, Micrococcus varians, Micrococcus roseus, Lactobacillus* sp *and Corynebacterium* sp. The cellular characteristics of the six bacterial isolates are presented in table 1.

Table 2 shows the bacterial load obtained at the sampling sites over the five-week sampling period which ranged from 3.72 $\times 10^2$ cfu/m³ to 1.6592$\times 10^4$ cfu/m³. The highest bacterial loads were observed at station 5 all through the five weeks sampling period with the highest count on week 1 to be 16,592 CFU/m³ while the least bacterial loads were observed at station three all through the five weeks sampling period with the least count on week 3 to be 3,722 CFU/m³. The result of the one-way ANOVA showed no statistically significant difference (F= 0.8319, P= 0.5207, (a=0,05)).

The occurrence of the bacterial isolates across the sampling period showed that *Bacillus* sp. had the highest frequency of occurrence (100 %) while *Micrococcus roseus* had the least frequency of occurrence (40%) (figure 3). The result of the biosurfactant activity of the bacterial isolates is presented on table 3, *Corynebacterium* sp and *Bacillus coagulans* both exhibited significant biosurfactant producing potential.

### Fungal isolates

Seven fungal isolates in total were isolated and 5 different fungus was identified which include; *Aspergillus niger, Aspergillus fumigatus, Alternaria alternata, Rhizopus oryzae* and *Fusarium oxysporum*. The fungi load recorded during the sampling period as shown on table 4 ranged from 1.57 $\times$ $10^2$ cfu/m³ to 1.18$\times 10^3$ cfu /m³, having station five as the highest count all through the five weeks sampling period with 1,180 cfu/m³ count and fuel station 4 as the least count all through the five weeks sampling period with 157 cfu/m³ count.

The occurrence of the fungal isolates at the sampling sites shows *Aspergillus niger* had the highest occurrence with 100% presence all through the sampling period while *Alternaria alternata* had the least occurrence with 60% presence all through the sampling period, (figure 4). Figure 5 depicts the microscopic view of *Aspergillus fumigatus,* while in figure 6 is shown the colonial morphology of *Aspergillus fumigatus* on PDA.

***Table 1**: cellular characteristics of the bacteria isolated from the five fuel stations at Fate, Ilorin*

| S/N | I | GS | S | SS | CS | OR | MT | OT | CT | CaT | CoT | IT | MR | VP | TSI | SF | MF | StF | Probable identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | + | R | + | _ | FA | + | _ | _ | _ | _ | _ | + | _ | G | + | +, g | +, g | *Bacillus circulans* |
| 2 | B | + | R | _ | _ | FA | + | _ | _ | + | _ | _ | + | _ | G | + | + | +, g | *Micrococcus varians* |
| 3 | C | + | C | _ | _ | FA | + | + | + | + | _ | _ | + | _ | G, g | + | +, g | +, g | *Corynebacterium* sp |
| 4 | D | + | R | + | _ | FA | + | _ | + | + | _ | _ | + | _ | G | + | + | +, g | *Lactobacillus* sp |
| 5 | E | + | C | + | _ | FA | + | _ | + | + | _ | + | + | _ | G, $H_2S$ | + | +, g | + | *Micrococcus roseus* |
| 6 | F | + | R | _ | _ | FA | + | _ | _ | + | _ | _ | + | _ | G, g | + | +, g | +, g | *Bacillus coagulans* |

**Keys**: i = bacterial isolates, + = positive, - = negative, R= rod, C= cocci, S= shape, GS= Gram stain, SS= spore stain, CS= capsule stain, OR= oxygen relationship, MT= motility test, OT= oxidase test, CT= citrate test, CaT= catalase test, CoT= coagulase test, IT= indole test, MR= methyl red test, VP= Voges Proskauer test, TSI= triple sugar ion test, SF= sucrose fermentation, MF= maltose fermentation, StF= starch fermentation, FA= facultative anaerobes, $H_2S$= H2S production, g= gas production, G= positive for glucose fermentation.

***Table 2:*** *Bacterial load of the five sampling points at Fate, Ilorin*

| S/N | Sampling points | Week 1 CFU/m³ | Week 2 CFU/m³ | Week 3 CFU/m³ | Week 4 CFU/m³ | Week 5 CFU/m³ | Mean CFU/m³ |
|---|---|---|---|---|---|---|---|
| 1 | Station 1 | 14,705 | 6,265 | 7,737 | 5,793 | 6,182 | 8,136 |
| 2 | Station 2 | 14,233 | 6,213 | 7,172 | 5,374 | 5,662 | 7,731 |
| 3 | Station 3 | 9,614 | 5,505 | 4,901 | 3,722 | 4,561 | 5,661 |
| 4 | Station 4 | 10,826 | 5,662 | 5,924 | 4,640 | 4,587 | 6,328 |
| 5 | Station 5 | 16,592 | 7,759 | 8,834 | 6,107 | 6,291 | 9,117 |
| | Mean | 13,194 | 6,281 | 6,910 | 5,127 | 5,457 | |

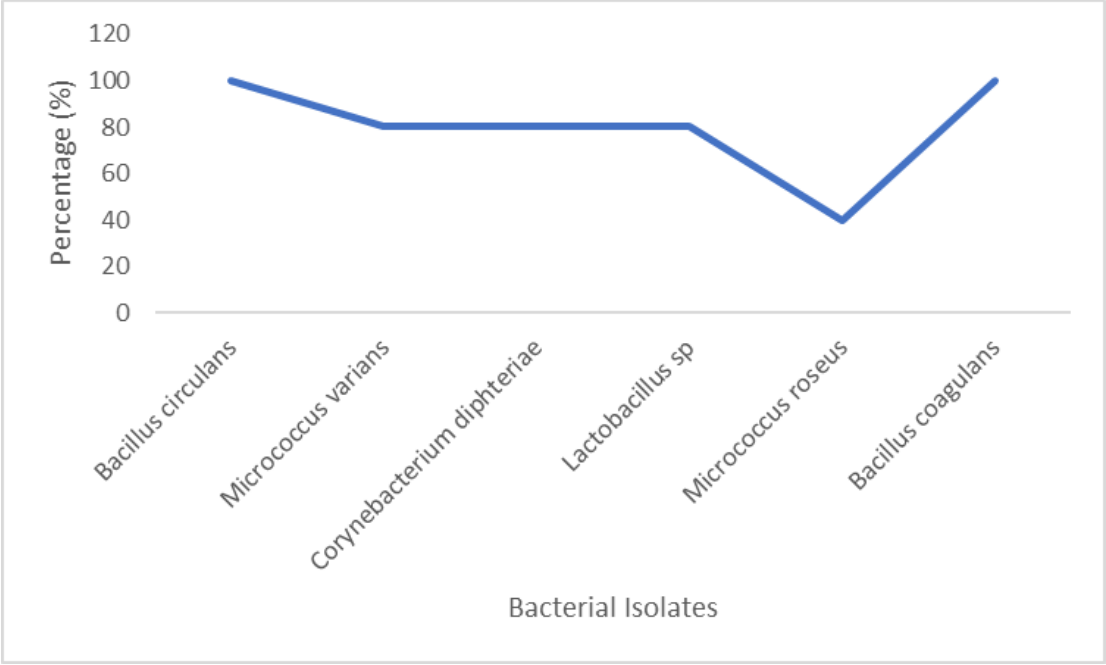


***Figure 3:*** *Occurrence of the bacterial isolates at the five selected fuel stations in Fate, Ilorin*

***Table 3:*** *Biosurfactant activity test of the bacteria isolates obtained from five selected fuel stations at Fate, Ilorin.*

| **Isolate** | **Oil Displacement Test** | | | **Emulsification Test** | | | **Drop Collapse Assay** | | | **Hemolysis** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Kero** | **Engine oil** | **Crude oil** | **Kero (%)** | **Engine (%)** | **Crude (%)** | **Kero** | **Engine oil** | **Crude oil** | |
| *Bacillus circulans* | 0 | 0 | 0.7 | 20 | 0 | 3 | _ | _ | + | Beta |
| *Micrococcus varians* | 0 | 0.6 | 0 | 7 | 0 | 0 | _ | + | _ | Beta |
| *Corynebacterium* sp | 1.6 | 0 | 2.6 | 47 | 0 | 8 | + | _ | + | Gamma |
| *Micrococcus roseus* | 0 | 0 | 0 | 38 | 0 | 7 | _ | _ | _ | Gamma |
| *Lactobacillus* sp | 0 | 0 | 0 | 23 | 0 | 7 | _ | _ | _ | Gamma |
| *Bacillus coagulans* | 1.4 | 0.35 | 1.3 | 46 | 0 | 7 | + | + | + | Beta |

**Legend**: + = positive, - = negative, Kero = kerosene, % = percentage

***Table 4:*** *Fungal load of the five sampling sites selected at Fate, Ilorin*

| S/N | Sampling sites | Week 1 CFU/m³ | Week 2 CFU/m³ | Week 3 CFU/m³ | Week 4 CFU/m³ | Week 5 CFU/m³ | Mean CFU/m³ |
|---|---|---|---|---|---|---|---|
| 1 | Station 1 | 567 | 341 | 367 | 263 | 1048 | 517.2 |
| 2 | Station 2 | 393 | 341 | 236 | 315 | 1,154 | 487.8 |
| 3 | Station 3 | 498 | 263 | 289 | 315 | 865 | 446 |
| 4 | Station 4 | 367 | 157 | 184 | 184 | 629 | 304.2 |
| 5 | Station 5 | 603 | 366 | 446 | 367 | 1,180 | 592.4 |
| | Mean | 485.6 | 293.6 | 304.4 | 288.8 | 975.2 | |

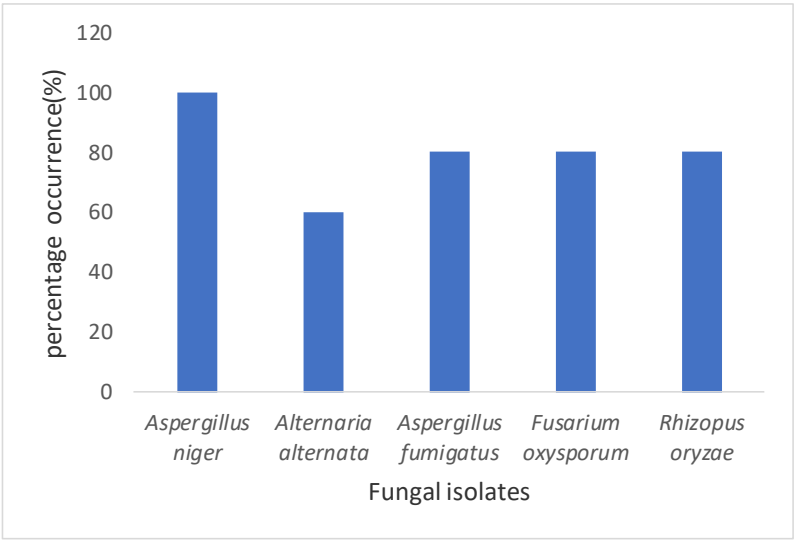


***Figure 4:*** *Occurrence of the fungal isolates at the five selected fuel stations in Fate, Ilorin*

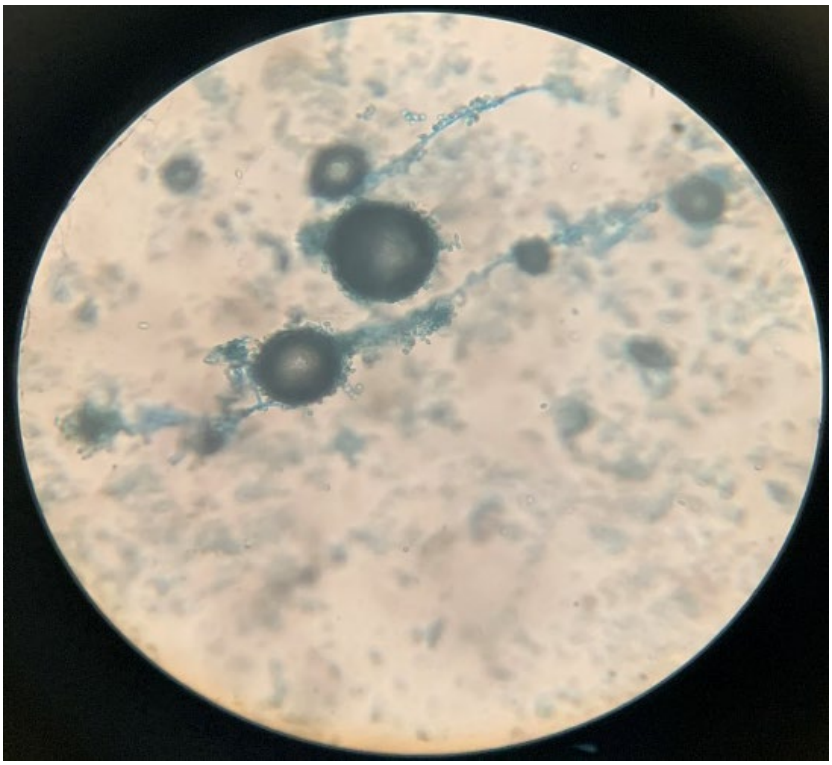

***Figure 5:*** *Microscopic view of Aspergillus fumigatus*

***Figure 6**: Apergillus fumigatus colonies on PDA*

## 4.0 DISCUSSION

The results of this study indicate clearly that bacteria and fungi were isolated from all the fuel stations all through the five weeks sampling period (table 2 and table 4). This is supported by the ubiquity of microorganisms, even in the air as reported by Adetitun and Oladele (2016). The result of the cellular characteristics of the bacterial isolates shown on table 1 shows that all the bacterial isolates stained positive to Gram reaction and this had been previously reported by Griffin *et al.* (2017). These workers reported that Gram positive bacteria are generally dominant in outdoor air. The possession of thicker peptidoglycan in Gram positive bacteria can be the reason why they are numerous in air. According to reports, Gram negative bacteria typically have a thinner peptidoglycan coating than Gram positive bacteria (Pismennõi *et al*., 2023).

Fuel station number five had the highest microbial load all through the sampling period. This is not surprising as the fuel station was the busiest all through the five-weeks of sampling. This is in terms of human and vehicular movement. and this agrees with the reports of several workers (Kalogeraskis *et al*., 2005; Chen and Hildermann, 2009), who observed higher microbial loads in residential and office areas that had higher human traffic. Makut *et.al*. (2014), also reported the highest

microbial load at the main market of Keffi metropolis in Nasarawa state due to human and vehicular movement.

Fuel station number three had the least bacterial count all through the five-weeks sampling period. The fuel station was also observed to be the least busy in terms of human and vehicular movement. This agrees with the report of Makut *et al.* (2014), where Government Reserved Area (GRA) had the least movement and had the least bacterial load at 30 cfu/m³. However, the least fungal count observed at station four shows that population density may not correlate with fungal load. Makut *et al.* (2014) reported again that *Aspergillus niger w*as one of the most predominant fungal species isolated at Keffi metropolis which was also observed in this study.

The predominance of *Bacillus* sp. in this study (figure 3) is supported by the fact that they are relatively abundant in air and this could be attributed to their ability to form endospores which enable them to resist adverse conditions (Idris et al., 2024). Ulfat *et al.* (2022) reported that *Bacillus* and other bacteria are present in the water and soil of Lahore canal in Pakistan. Species of *Bacillus* have been considered to be soil organism and the soil to be the primary habitat of *Bacillus*. This assumption has been regarded to be an oversimplification of *Bacillus.* Their endospores have been found in diverse environment including rocks, dust, aquatic environments and the gut of various insects and animals (Nicholson *et. al.*, 2002).

*Corynebacterium* sp was observed to exhibit the highest biosurfactant activity and a correlation between its oil displacement, drop collapse and emulsification result was also observed which support the research work of Nayarisseri *et.al* (in 2018), where it was reported that the isolates (*Bacillus* sp*, Staphylococcus* sp*)* both had positive result for the drop collapse and oil displacement assay while *E. coli* had negative for both results. Nonpathogenic species of *Corynebacterium* are employed in significant industrial processes (Jiang *et al.,* 2024). Martins and Martins (2018) also reported that *Corynebacterium* is one of the microorganisms used to produce biosurfactant. *Corynebacterium* sp appear as non-hemolytic colonies on the blood agar, which is in conformity with the report of Ryan and Leulier (2014). These workers isolated non-hemolytic colonies of *Corynebacterium* sp on media containing blood.

Some of these microorganisms isolated have been reported for their economic importance in food, health, and industrial sector while some have been implicated to cause disease of human and animal and also food spoilage. *Bacillus coagulans* is regarded as 'the king of probiotics' because of its high stability in the gastrointestinal tract, non-toxic effect and also a high but not yet fully understood pharmacological activity (Bomko *et.al*., 2016). *Bacillus circulans* is an opportunistic pathogen that can cause sepsis, wound infection, mixed abscess infections and also meningitis especially in immunocompromised patients. Alebouyeh *et.al.* (2011),

reported a case of sepsis caused by *Bacillus circulans* in an immunocompromised patient which lead to the death of the patient.

*Aspergillus niger* is a serious plant pathogen and produces many mycotoxins that contaminate food (Tawfik *et al.,* 2022). Warris and Verweij in 2005 also reported *Aspergillus fumigatus* as the most frequent cause of invasive filamentous fungal infection in bone marrow transplant (BMT) patients. *Alternaria alternata* has also been identified as one of the most common fungi associated with asthma (de Souza et al., 2024).

The presence and type of microorganisms in the environment is affected by some factors which includes; the level of activities in the sampling area and its surrounding, the time of exposure, movement of individuals, the current content of the air at the time of sampling and the method employed in sampling (Omeokachie *et al.,* 2024).

## 5.0 CONCLUSION AND RECOMMENDATION

Finally, this study showed that bacteria that could be explored for biodegradation are present in air of filling stations and its immediate environment. It also shows that the examined petrol stations also had a high microbial load and might be sources of opportunistic and pathogenic bacteria. Individuals need to be careful when in such places. One way to be careful is not to stay too long in petrol stations. In order to guarantee that the air is free of harmful microorganisms, several preventive steps are recommended and they should be taken to lower the microbial load in the atmosphere. Some of these steps are availability of fuel in all fuel stations always in order to eliminate queues for fuel which exposes fuel buyers to fuel station air microbes. Another recommended method is fumigation, which can be used to spray the air and kill or impede air microorganisms. Some other recommended techniques include the use of dehumidifiers, to manage moisture as well as humidity, which may act as a promoter for the growth of microorganisms.

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