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Bacterial Density in Soil Samples Collected from Hospital Wastes Dumping Site at Chukwuemeka Odumegwu Ojukwu Medical Centre, Uli, Anambra State, Nigeria.

Nwakoby, N.E[1] and Ejimofor, C.F[2]

[1]Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.
[2]Department of Biological Science, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.
Corresponding author email: cf.anyaegbu@coou.edu.ng

ABSTRACT

The soil quality is characterized by adequate nutrients as a result of abundant nutrients cycling bacteria. Most soil in Nigeria has been subjected to different kinds of pollutants resulting from anthropogenic activities which have become a major threatening factor to the quality of soil. This study was carried out to evaluate bacterial density in soil samples collected from hospital wastes dumping site at Chukwuemeka Odumegwu Ojukwu Medical Centre, Uli, Anambra State. A total of 30 composite soil samples were aseptically collected from the site using a sterile soil auger. The samples were analyzed for total heterotrophic bacterial count (THBC) and phosphate solubilizing bacterial count (PSBC) using standard plate technique. The predominant bacterial isolates that aided nutrients cycling were appropriately characterized, and their densities in both impacted and non-impacted soil samples were enumerated. Also, the bacterial isolates were characterized based on morphology, microscopy, and biochemical characteristics. There was an increase in the THBC and PSBC in the impacted soil, and Gram negative rods such as *Pseudomonas*, *Klebsiella,* and *Micrococcus* species (Gram positive) were the bacterial isolates. The study has revealed that wastes generated from hospital enhances microbial proliferation in the soil, which invariably increases soil fertility.
Keywords: Bacterial Density, Soil Samples, Hospital Wastes and Dumping Site

INTRODUCTION

Soil minerals are the remnants and newly formed minerals of bedrock weathering and parent materials through soil formation [1-3]. Soil minerals are the main components of solid soil particles, including various primary minerals, layered silicate clay minerals and oxides of iron, aluminum and manganese. Approximately 80–90% of the microorganisms living in soil adhere to the surface of minerals or mineral–organic complexes to form a single microbial colony or biofilm [4-5]. There are many factors affecting the diversity of the soil microbial community structure, including the soil physical and chemical properties and the vegetation community types, such as soil particle size, water content, pH, enzyme activity and organic matter content, which directly affect the quantity and activity of soil microorganisms. In summary, previous studies on soil microbial diversity under different conditions have mainly focused on soil properties and human factors. Few studies on the impact of soil CO_2 changes on microbial diversity have been conducted [6-8].

To analyze the influence of soil CO_2 and mineral composition on the microbial community, soil microorganisms in different environments were selected for

analysis in this study. The relationship between the microbial community and environmental factors was illustrated through analysis of carbon dioxide and mineral composition. Minerals provide nutrients for the life activities of bacteria, and the metabolic activities of bacteria can also change the solubility of minerals. Early bacterial mineral mechanisms were widely used in heavy metal pollution control; for example, clay minerals can react with surrounding redox bacteria, resulting in significant changes in soil physical and chemical properties, and there was a significant positive correlation between clay minerals and soil bacterial biomass [8-9]. This research work is aimed at evaluating bacterial density in soil samples collected from hospital wastes dumping site at Chukwuemeka Odumegwu Ojukwu Medical Centre, Uli, Anambra State.

MATERIALS AND METHODS

Study Area

The study was conducted at Umuchima, Uli, Ihiala Local Government Area, Anambra State. Uli is a village located between latitudes 5.47°N and 5.783°N and longitude 6.52°E and 6.87°E on the South eastern part of Nigeria. Uli extends westward to the confluence of the rivers of Atammiri and Eyinja, and across Usham lake down to the lower Niger region. Uli has rainforest vegetation with two seasonal climatic conditions: rainy season and dry season, which is characterized by the harmattan between December and February. Uli is characterized by double maxima of rainfall with a light drop in either July or August known as dry spell or August break. The annual total rainfall is about 1,600 mm with a relative humidity of 80 % atdawn.

Sample Collection

The soil surface was carefully scrapped out using sterile spoon. The soil auger was derived to a plough depth of 15 cm in the sampling site, and soil sample was drawn up to 10 samples from each sampling unit into a sterile tray. The samples were thoroughly mixed and foreign materials such as roots, stones, pebbles and gravels were carefully removed. The soil sample was then reduced to half by quartering the sample. Quartering was carried out by dividing the soil sample into four equal parts and the two opposite quarters were discarded and the remaining two quarters were mixed. The process was repeated for the rest of soil samples used for this study. The samples were carefully labeled and then kept in a disinfected cooler, to maintain its temperature and stability of the number of the isolates. The samples were transported to the laboratory for analysis.

Sample Preparation

This was carried out using the modified method of [4]. One gram of the soil sample was weighed into a 50 mL beaker (Pyrex) using analytical weighing balance (JJJ430BC), little normal saline (0.85% NaCl) was added; this was shake thoroughly and made up to 10 mL using the normal saline. Then ten-fold serial dilution was carried out by transferring one milliliter of the prepared sample into nine milliliters of the diluent (normal saline), and this was serially carried out to form dilution 10^{-6}.

Effects of Hospital Waste on Bacterial Load in Soil Samples

Estimation of Total Heterotrophic Bacterial Counts (THBC)

The prepared samples were aseptically introduced (1.0 mL) into Petri dishes (90 mmX 15 mm) containing sterile prepared nutrient agar (BIOTECH) as described by [6,8]. These were placed in electric incubator in vertical positions at 35±°C for 24 h. THBC were enumerated by counting the number of colonies in each plate after 24 h, and the mean counts were calculated and presented in form of mean ± standard deviation [7].

Estimation of Phosphate Solubilizing Bacterial Count (PSBC)

The prepared samples were aseptically cultured on sterile poured plates (90 mm X 15 mm) containing National Botanical Research Institute's Phosphate Growth Medium (NBRIP) which comprises 10 g glucose, 5 g $Ca(PO_4)2$, 5 g $MgCl_2.6H2O$, 0.25 g $MgSO_4.7H_20$, 0.2 g KCl and 0.1 g $(NH_4)_2SO_4$ in 1000 mL of distilled water. These were placed in electric incubator (STXB128) in vertical positions at 30±2°C for 24–48 h. PSBC were enumerated by counting the number of colonies in each plate after 24–48 h, and the mean counts were calculated and presented in form of mean ± standard deviation.

Characterization of Predominant Bacterial Isolates that Aided Nutrients Cycling from the Studied Samples

Purification of the Isolates

The plates that showed discrete colonies were selected after 24 h, and aseptically streaked each colony on sterile plates (90mm×15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturer's description. The streaked plates were placed in a bacteriological incubator in inverted positions and incubated at 35±2°C for 24h as described in [4].

Characterization of the Pure Isolates

The pure isolates were characterized using the morphological, biochemical and molecular characteristics.

Morphological characterization of the pure isolates

The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out. The presence or absence of capsule was also carried out. The presence or absence of flagellum was determined by carrying out motility test as described by [4].

Gram staining technique

A thin smear was made in a cleaned grease free microscopic slide (75mmX25mm X 1mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95%w/v ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens.

Biochemical characterization of the pure isolates

The capability of the pure isolates to produce catalase, indole, oxidase, acetoin, grow in 6.55 % NaCl and to utilize sugars, sugar alcohols and other substances (ribose, sorbitol, arabinose, sacharose, glucose trehalose, lactose, starch, inulin, salicin, hiparate) and also the haemolytic activity of the isolates were done using the methods described by [4].

Indole test

Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 ml of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the pure isolates and kept in an incubator at 37°C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test

The capability of the pure isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham's tubes. The medium was then sterilized by autoclaving. The sugar solution were prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate pure isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production

This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The pure isolates were aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for hydrogen sulphide production.

Citrate utilization test

The Simmon's Citrate Agar was prepare according to the manufacturer's direction and the pure isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test

The test was carried out as described by [4]. A smear of the pure isolates was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production

Statistical Analysis

The densities of the bacterial group in the impacted and non-impacted soil were compared using students' T test, and P values greater than 0.05 were considered non-significant ($P > 0.05$).

RESULTS

Effects of Hospital Wastes on Bacterial Density and Quality of the Impacted Soil Samples

The study revealed the enumeration of total heterotrophic bacterial counts (THBC) and phosphate solubilizing bacterial counts (PSBC) as presented in Table 1. From the result, the impacted soil recorded higher THBC (16.80±0.07) and PSBC (0.98±0.01) when compared to the normal soil (control) which had 14.80±0.21 and 0.50±0.17, respectively.

Table 1: Enumeration of Phosphate solubilizing bacterial count from hospital wastes sites

Bacterial Group	Impacted site	Control site
THBC ($X10^6$CFU/g)	16.80±0.07	14.10±0.10
PSBC($X10^4$CFU/g)	0.98±0.01	0.50±0.17

Characteristics and Identities of the most Predominant Bacterial Isolates in the population that Aid Nutrient Cycling

The cultural and morphological characteristics of the implicated bacterial isolates are shown in Table 2. The isolates; M, N, and O exhibited varying characteristics culturally and microscopically. Isolates N and O were Gram negative rods, circular colonies with varied appearance on nutrient agar plates. Isolate M was colorless, while isolate N had yellow coloration, with entire margin. Isolate O had pale yellow color with entire margin, and convex elevation. The isolates were catalase positive and utilized glucose. They exhibited varied degree of utilizing sugar molecules as shown in Table 3. All the isolates utilized glucose as their carbon source while other sugars and sugar alcohols such as sucrose, maltose, mannose, lactose, mannitol, and sorbitol were rarely utilized (Table 3). Similarly, all the bacterial species were catalase and citrate positive while hydrogen sulphide and indole were not produced by all the isolates.

Table 2: Cultural and morphological characteristics of some selected bacterial isolates

Parameter	M	N	O
Appearance on NA	Colorless	Yellow	Pale yellow, later white
Shape of colony	Circular	Circular	Circular
Elevation	Raised	Raised	Convex
Margin	Smooth	Smooth	Entire
Gram Reaction	Negative	Positive	Negative
Cell Morphology	Rods	Cocci	Rods
Possible Bacterium	*Klebsiella*	*Micrococcus*	*Pseudomonas*

Table 3: Biochemical characteristics of the selected bacterial isolates

Parameter	X	Y	Z
Catalase	+	+	+
Citrate	+	_	+/_
Indole	_	_	_
Hydrogen sulphide	_	_	_
Glucose	+	+	+
Maltose	+	_	_
Lactose	+	_	_
Mannitol	+	_	_
Mannose	+	_	_
Sorbitol	+	_	_
Bacterium	*Klebsiella*	*Micrococcus*	*Pseudomonas*

DISCUSSION

A hospital produces waste by giving their services to the patients. This waste can be produced directly in combination with the service (e.g. injection) or in the upstream (e.g. blood or urine cultures in the laboratory) or downstream (vaccine) process. The slight increase in the THBC and PSBC observed in this study could be attributed to high organic matter of the wastes. Research had shown that both solid and liquid waste generated in hospitals are biodegradable, which enhances microbial proliferation, especially fungi and bacteria. This observation is in agreement with the finding of several researchers [5-8]. The predominance of Gram negative bacteria in the sampled dumping sites could be ascribed to environmental selection and the ability of the isolates to degrade high organic substances. The Gram negative bacteria such as *Pseudomonas*, *Bacillus, Micrococcus*, and *Enterobacter* had been reported to be involved in nutrient cycling and in decomposition of complex organic substances in the environment. Nutrient cycling such as nitrogen fixation, nitrification, phosphate solubilizing etc. are vital in the survival of living organisms in the soil and also determine the extent of crop yield. Similar bacteria were isolated by [6] who investigated microbial distribution in biomedical wastes. For instance, phosphates are needed for optimum growth of crops but can only be utilized in soluble form. These bacterial isolates solubilize phosphates in the form that would be absorbed by plants. The ability of the bacterial isolates to utilize glucose and sugar alcohols could be attributed to high metabolic activity. This observation corroborates to the study documented by [3] who evaluated microbial biodegradation in solid wastes.

CONCLUSION

The study has revealed that wastes generated from hospital enhance proliferation of soil microorganisms, especially heterotrophic bacteria and phosphate solubilizing bacteria. Therefore, the proliferation of these bacterial species that are highly beneficial to farmers can be enhanced by introducing wastes generated in hospitals.

REFERENCES

1. Anitha, J. and Jayraaj, I.A. (2012). Isolation and identification of bacteria in biomedical wastes (BMW).International journal of pharmacy and pharmaceutical sciences4(5):386–388.

2. Bisht, D., Yadav S.K., Darmwal, N.S. (2012). Enhanced production of extracellular alkaline lipase by an improved strain of *Pseudomonas aeruginosa* MTCC 10,055. American Journal of Applied Science 9:158 –167.
3. Chaudhuri, G., Dey, P., Dalal, D., Venu-Babu,P., Thilagaraj, W.R. (2013). A novelapproach to precipitation of heavy metalsfrom industrial effluents and single-ionsolutions using bacterial alkalinephosphatase. Water, Air, and Soil Pollution 224: 16 – 25.
4. Cheesbrough, M. (2010). District Laboratory Practice in Tropical Countries (2nd ed.). Cambridge University Press: Cambridge, England.pp.45–70.
5. Cui, H.B., Zhou, J., Zhao, Q.G., Si, Y.B., Mao, J.D., Fang, G.D. and Liang, J.N. (2013). Fractions of Cu, Cd, and enzyme activities in a contaminated soil as affected by application of micro- and nanohydroxyapatite. *Journal of Soils and Sediments* 13(4): 742 – 752.
6. Sharma, S. B., Sayyed, R. Z., Trivedi, M. H. and Gobi,T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils, *Springer Plus* 2: 587 - 593.
7. Soleimani, N., Fazli, M.M., Mehrasbi, M. and Darabian, S, M. (2015). Highly cadmium tolerant fungi: Their tolerance and removal potential. Journalof Environmental Health Science and Engineering 13: 19
8. Udofia, E.A., Fobil, J. and Gulis, G. (2018). Stakeholders' Practices and Perspectives on Solid Medical Waste Management: A Community Based Study in Accra, Ghana. AJEST*Journal* 534–54.
9. Wu, Y.H., Zhou, P., Cheng, H., Wang, C.S., Wu, M. (2015). Draft genome sequence of Microbacterium profundi Shh49T, an Actinobacterium isolated from deep-seasediment of a polymetallic nodule environment. Genome Announcments 3: 1 – 2.

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