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Soil Bacterial Dynamics: Assessing the Effects of Urine on Lipolytic and Cellulytic Bacteria

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Abstract

A good quality soil is characterized by adequate nutrients as a result of abundant nutrients cycling bacteria. Most soil in Nigeria has been subjected to different kinds of anthropogenic activities which can impact either positively or negatively. This study investigated the impact of human urine on soil bacterial populations, specifically lipolytic and cellulolytic bacteria, in Uli community. Thirty composite soil samples were collected from urinating sites and analyzed using in situ and standard plate techniques. The bacterial counts were subjected to statistical analysis using one-way analysis of variance (ANOVA) to determine significant differences between impacted and non-impacted soil samples. The results revealed a statistically significant increase in lipolytic and cellulolytic bacterial counts in impacted soil samples ($p < 0.05$). The predominant bacterial isolates identified were *Pseudomonas*, *Klebsiella*, and *Micrococcus* species, which are known to play a crucial role in nutrient cycling. Characterization of bacterial isolates was based on morphology, biochemical and molecular characteristics. The findings suggest that human urine deposition enhances microbial activity, potentially increasing soil fertility. The study's outcome has implications for understanding the role of human waste in shaping soil microbial communities and fertility.

Keywords: *Impacted, Predominant, Soil, Isolates, Characteristics.*

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Introduction

Urine is a liquid waste product from the kidney of both animals and humans. It is collected in the bladder and excreted through the urethra. As a waste liquid product, it contains some dissolved substances such as ammonia, urea, uric acid, and creatinine. These constitute the organic solids in the urine. Urine also contains inorganic dissolved substances such as sodium chloride, calcium, potassium, phosphate, and sulfates (Dedeke *et al.*, 2011).

The dissolved substances in the urine can be utilized by microorganisms of various groups as nutrients whenever urine finds its way into the environment. This is evidenced by the fact that urine-polluted environments usually have very strong odours, signifying that the biological oxygen demand (BOD) is high. This phenomenon is observed in toilets, bathrooms, street corners, and follow grounds.

The different groups of microorganisms can represent different functions and activities. Some can be harmful relating to public health risk, or beneficial relating to positive economic value. Urine leach into ground and surface waters consume the nitrogen. It results into a great bloom of growth. When this dies and decomposes, it pulls oxygen from the water or euthrophies, which can suffocate fish and other aquatic life. Underground nitrogen can seep into drinking water, posing a potential health hazard (Dada and Aruwa, 2014). Urine contains micro pollutants such as synthetic hormones, pharmaceuticals, and their metabolites, that is mainly excreted via urine and may be harmful to the ecosystem and human health.Today, many micro pollutants reach the aquatic environments because their degradation in waste water treatment plant is poor. More than just dirt hanging around the environment especially urine polluted is unhealthy. This research work is aimed at evaluating the effects of urine on nitrogen fixing bacteria and phosphate solubilizing bacteria in soil sample.

Materials and Methods

Study Area

The study was conducted at Umuaku, 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 % at dawn.

Sample Collection

The soil surface was carefully scrapped out using sterile spoon. 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 Cheesbrough (2010). 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}.

Estimation of Lipolytic Bacterial Counts (LBC)

The prepared samples will be aseptically cultured on sterile poured plates (90 mm x 15 mm) containing Tributyrin agar (TA) as described by Ibe *et al*. (2014) Abiodum *et al*. (2024b). The plates will be incubated in inverted position in electric incubator (STXB128) at 30±2°C for 24 – 48 h. LBC will be enumerated by counting the number of colonies surrounded by the clear zones.

Estimation of Cellulolytic Bacterial Counts (CEBC)

The prepared samples will be aseptically cultured on sterile poured plates (90 mm X 15 mm) containing cellulose agar (carboxy methyl cellulose) as described by Ibe *et al*. (2014) and Ekesiobi *et al*. (2017). The plates will be incubated in inverted position in electric incubator (STXB128) at 30±2°C for 24 – 48 h. CEBC will be enumerated by counting the number colonies with hydrolyzed zones.

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 ± 2^0C for 24h as described in Cheesbrough (2010).

Characterization of the pure isolates

The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al*. (2018), Iheukwumere *et al*. (2025a) and Iheukwumere *et al*. (2025b).

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 ($P > 0.05$) (Ekesiobi, 2025; Abiodum *et al*., 2024a; Iheukwumere *et al*., 2025c; Abiodum *et al*., 2024c; Iheukwumere *et al*., 2025e).

Results

The study revealed the enumeration of total heterotrophic bacterial counts (THBC) and phosphate solubilizing bacterial count (PSBC) as presented in Table 1. From the result, the impacted soil recorded higher THBC (23.80±0.07) when compared to the normal soil (control) which had 19.80±0.21

and the PSBC increased in the impacted soil compared to the control.

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 1: Enumeration of bacterial count from urinating sites

Bacterial Group	Impacted site	Control site
TLBC ($X10^5$ CFU/g)	53.00±0.07	19.80±0.10
TCC($X10^3$ CFU/g)	9.80±0.01	1.50±0.17

TLBC= Total Lipolytic bacterial count; TCC= Total Cellulytic bacterial count

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	M	N	O
Catalase	+	+	+
Citrate	+	_	+/_
Indole	_	_	_
Hydrogen sulphide	_	_	_
Glucose	+	+	+
Maltose	+	_	_
Lactose	+	_	_
Mannitol	+	_	_
Mannose	+	_	_
Sorbitol	+	_	_
Bacterium	*Klebsiella* species	*Micrococcus* species	*Pseudomonas* species

Discussion

Maintaining microbial distribution in the soil is vital in ensuring adequate crop production as some microorganisms especially bacterial are useful in nutrient generation and cycling.. The presence of soil microorganisms isolated from soil samples in this study is expected. The study has revealed that soils from urinating site consist of opportunistic microbial species of importance to human and agricultural sector. Bacterial isolates obtained from the sampled soils in this study are in agreement with the work of Dada and Aruwa (2014).

The slight increase in the nitrogen fixing bacteria observed in the impacted soil could be attributed to the composition of the urine. Research had shown that urine contains nitrogen which is required by soil microorganism for optimum growth. This observation conforms to the study of Dada and Aruwa (2014) and Dedeke *et al.* (2011) who investigated the effects of urine on soil microorganisms. 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 Poririer *et al*. (2017) and Olaniran *et al*. (2013) who investigated the effects of heavy metals in microbial biodegradation. However, this observation disagrees with the study documented by Siddiquee *et al.* (2015) who isolated mostly fungal species in heavy metal contaminated soil, which they attributed to high saprophytic activity of fungi. 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 Olaniran *et al*. (2013) who evaluated the effects of heavy metals in microbial biodegradation.

Conclusion

The study has revealed that urine enhances proliferation of various groups of bacteria mainly lipolytic and nitrifying bacteria etc. The nitrogen and carbon found in urine are responsible for the microbial proliferation. This shows that urine increases soil fertility and food production.

Conflict of interests: The authors declare that they have no conflict of interests.

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