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Dynamics in Physicochemical and Bacteriological Properties of Simulated Leachate from Dump Site Soil in Ikhueniro, Benin City, Edo State, Nigeria

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Abstract

Municipal solid waste (MSW) management in Nigeria faces significant challenges due to rapid urbanization and poor waste management practices. Leachate, the liquid generated from MSW, poses a major pollution threat to natural resources, including surface and groundwater, and adversely affects human health and hygiene. This study investigated the dynamics in physicochemical and microbiological properties of simulated leachate from dump site soil in Ikhueniro, Edo State, Nigeria using standard Microbiological testing techniques. Samples were collected from a non-sanitary open dumpsite in Ikhueniro, Edo State, Nigeria, and leachate was obtained by filtering 150 g of waste-impacted soil mixed with sterile distilled water (1000 mL). Over 28 days, microbial detection, enumeration and physicochemical characterization were conducted on the filtrate leachate sample maintained in a rotary shaker at 28 ˚C and 150 rpm. Results indicated high densities of microbial contamination with total heterotrophic bacteria (1.5±0.5 to 6.9±1.0 × 10^4 CFUmL^{-1}) and coliform (2.7±0.8 to 7.8±1.0 × 10^3 CFUmL^{-1}) counts. These included potential pathogens like Klebsiella spp., Salmonella spp., Bacillus spp., Pseudomonas spp., and Yersinia spp. Physicochemical analyses revealed significant ($p<0.05$) variations in parameters such as electrical conductivity (EC) with values ranging from 9183.3 - 9758.7µscm^{-1}, total dissolved solids (TDS) with values ranging from 7004.0 - 8210.3 ppm, with changes in pH ranging from 6.48-7.62, biochemical oxygen demand (BOD) with values ranging from 561.7 - 651.3 mgL^{-1}, and chemical oxygen demand (COD) with values ranging from 1981.7-2058.7 mgL^{-1}. The pH dropped from 7.62 to 6.48, and the levels of BOD and COD indicated a decreasing trend that was strongly and positively correlated ("r" ranging from 0.949 to 0.968) with bacterial counts, indicating organic molecule degradation. This study underscores the environmental and public health risks posed by improperly managed waste and its resultant leachates while highlighting the potential for bioremediation strategies using the isolated bacterial species. Furthermore, transitioning to sanitary landfills and improving waste management practices are crucial for mitigating these risks.

Keywords: Municipal solid waste, leachate, microbial occurrence, physicochemical changes, open-dumpsite

INTRODUCTION

Human and municipal solid waste are two parallel and yet opposing entities existing in the global space. The generation of solid waste is an inevitable consequence of human activity, and its management directly impacts the health of the people and the environment surrounding it (Khan *et al.*, 2022). The high population growth rate, urbanization, increase in socioeconomic activities, and inadequate knowledge in modern solid waste management that involves collection, processing, disposal, and reuse efficiently in developing countries, including Nigeria, is limited compared to the advanced economies (Ahsan *et al.*, 2014).

Municipal solid waste (MSW) is refuse from households, non-hazardous solid waste from industrial, commercial, and institutional establishments, including hospitals, market waste, and yard and street sweepings (Singh, 2021). It can be regarded as garbage, rubbish, discards, or junk, depending on who defines it. Nigerians are permanently challenged with waste management issues, evidence of which is seen by the day-to-day indiscriminate discharge of solid waste into drains and, at times, on the highway (Adi, 2023).

Generally, municipal solid waste gets disposed of in dumps as the simplest, convenient, inexpensive, and technologically less advanced method. Waste management is a serious issue due to its implication on human health and environmental sustainability as it remains a pressing issue Nigeria is facing today since a higher percentage of waste is currently discarded by open dumping (Purity *et al.*, 2016).

Following the dumping of waste on open dumpsites accompanied by landfilling, solid waste undergoes biological and physicochemical alterations through biodegradation in the presence of oxygen. Aerobic biodegradation of waste involves microbial oxidation of wastes by modifying and consuming waste as a source of food (energy), leading to changes in the physicochemical properties of the waste (Gopikumar *et al.*, 2020). Besides aerobic biodegradation, organic fractions, as a major component of municipal solid waste, undergo biodegradation (under the anaerobic conditions prevailing in landfills) in combination with percolating rainfall, which consequently releases greenhouse gases and a darkly coloured, highly polluted liquid called leachates (Mojiri *et al.*, 2016).

Leachate is the viscous liquid material found in open dump or landfill sites. It is generated when water from rainfall infiltrates into the solid waste and reacts physically, chemically, and biologically with waste to produce leachate. Recent studies and research reveal that leachate generation occurs when the rate of precipitation exceeds the evapotranspiration in the (sanitary) landfill region (Maqhuzu *et al.*, 2018). Mainly, municipal waste and industrial waste placed on or beneath the ground surface are the two most significant sources of the formation of leachate (Pming *et al.*, 2016).

The quality of leachate produced is variable in both volumetric flow and chemical composition. The variation of leachate composition depends on various factors, including waste composition, age of dumpsite and/or landfill, site hydrology, specific climatic conditions, moisture routing through the landfill, and landfill design and operation (Roy *et al.*, 2018). Leachate contains harmful and complex compounds that include organic matter, leading to high chemical oxygen demand (COD) and biological oxygen demand (BOD), as well as heavy metals like iron, copper, etc. (Naveen *et al.*, 2017).

Leachate, when not properly collected and managed can lead to potential contamination of nearby surface and groundwater, tainting its quality, and with the potential to compromise human health. In addition to this contamination, soil quality alterations and ecosystem imbalances occur (Cortés-Lorenzo *et al.*, 2014), thus making the removal of persistent leachate pollutants a significant challenge. Moreover, leachate is one of the most difficult problems to handle, as it could be released from the start of the dumpsite and landfill operation until potentially many decades after closure. Consequently, it should be treated until it fulfills environmental safety criteria. However, as long as landfills and open dumps remain a disposal option, the generation of leachate and gas remains a potential environmental risk to the public. Microbial communities in contaminated soils play a vital role in the degradation and transformation of pollutants. Analyzing the microbial occurrence in leachate provides insights into the biodegradation processes and potential bioremediation strategies (Sharma *et al.*, 2022). Microbial identification in leachate is essential for public health, as these pathogens can pose serious risks if they contaminate drinking water sources. (Xiang *et al.*, 2019).

In addition to organic and inorganic compounds, pollution parameters such as pH, conductivity, COD (chemical oxygen demand), BOD (biological oxygen demand), and the presence of heavy metals serve as indicators of pollution levels. The present study aimed to determine the dynamics in bacterial composition and physicochemical properties of simulated leachate from soil heavily contaminated with MSW. Characterizing these properties would help reveal the magnitude of contamination, which is critical for developing suitable remediation and treatment protocols.

MATERIALS AND METHODS

Study Area

This study was carried out in an open dump site (non-sanitary) for over five (5) years at Ikhueniro along the Benin- Lagos bypass road in Benin City, Edo State, Nigeria (located at approximately 6.32668°N latitude and 5.74605°E longitude). The site is unsightly and receives all forms of hazardous organic and inorganic fractions of municipal solid waste, including food, garbage, metal scraps, paper, polythene, plastics, and electronic waste.

Sample Collection and Preparation for Analysis

Raw landfill soil samples were collected from landfill sites (open dumpsites) having low-lying land areas located at Ikhueniro in Benin City, Edo State. The field-moist surface soil samples were collected using a sterile soil auger at a depth of between 0-15cm and delivered into a sealed plastic bag to the laboratory immediately for analysis.

Samples were collected during the dry season in the month of February. Leachate simulation was done by introducing two hundred and fifty grams (250g) of MSW-impacted surface soil samples collected at a depth of 0-15cm into 2L flat bottom conical flasks. Concisely, a litre (1000 mL) of distilled sterile water was added to the polluted-soil for extraction. The waste mixture was thoroughly mixed and allowed to stand for 4 h at room temperature (28±2 °C) with continuous manual stirring. This stirring was done manually at regular intervals of 30 min to dislodge all attached microbes from the waste-soil mixture. After 4 hours of periodic mixing to homogeneity, the solid and liquid portions were thereafter filtered to remove debris. The concentrated resultant filtrate was designated for use as simulated leachate. The simulated leachate was kept in a rotary tank for 28 days at room temperature (28±2 °C) and monitored for microbiological and physicochemical changes over the retention period. A speed of 150 rpm was employed to allow for adequate aeration and uniform homogeneity while also preventing excessive scum formation and foaming, as well as limiting shear stress on the microbial populations.

Bacteriological Examination of Leachate

Bacterial enumeration was carried out using the standard viable plate count (VPC) method, as described by Egong *et al*. (2016). Briefly, by pipetting 1ml of aliquot sample into 9ml of sterile water at a dilution factor (1: 100000) and allowed to stand for 5 min, serial dilution (10-fold) of the properly mixed sample was carried out. From the dilution, 1ml was then plated out by pour plate method on nutrient agar and MacConkey agar to determine the densities of total heterotrophic bacteria (THB) and total coliform loads, respectively. The Nutrient agar and MacConkey agar plates were incubated at 37 °C for 24 h. After incubation, discrete bacterial colonies on nutrient agar and MacConkey agar plates were totaled, and the count was expressed in $CFUmL^{-1}$. By way of subculture, bacterial colonies were re-cultivated on fresh nutrient medium plates, and isolates were stocked in McCartney bottles containing freshly prepared nutrient agar slants for further biochemical characterization. These characterizations included oxidase, urease, citrate utilization, catalase, coagulase, hydrogen sulphide, indole, methyl red, Voges Proskauer, and sugar fermentation assay (Cheesbrough, 2006). Isolate identification was performed using the keys provided in the determinative scheme of Holt *et al*. (1994).

Physicochemical Examination of the Leachate

The respective leachate samples were placed on a shaker for 28 days. Parameters, which included soil pH, were analysed using a portable pH probe carefully immersed in the simulated leachate and the reading taken by the meter. Other properties like electrical conductivity, were ascertained using techniques described by Kalra and Maynard (1991). The particle size distribution of the total dissolved solid was determined according to the methods enumerated by Mohammed *et al*. (2024). Also, biochemical oxygen demand and chemical oxygen demand were determined in accordance with standard procedures of the *Association of Official Analytical Chemists* (AOAC, 2016).

Data Analysis

All experiments were performed in duplicates and reported as mean values with standard deviation. Correlation coefficients of determination (r) was established between bacteriological and physicochemical variables. All data were curated and analyzed using Microsoft Excel, 2016.

RESULTS

The total heterotrophic bacterial and total coliform counts for the leachate across the 28-day retention period are shown in Table 1. Day 1 had the highest heterotrophic bacterial count ($6.9 \pm 1.0 \times 10^4$ $CFUmL^{-1}$), while the least ($1.5 \pm 0.5 \times 10^4$ $CFUmL^{-1}$) was from Day 28. The coliform bacterial counts showed the maximum count on Day 1 ($7.8 \pm 1.0 \times 10^3 CFUmL^{-1}$) and were undetected on Day 28.

Table 1 Total Heterotrophic Bacteria and Coliform Loads of Municipal Solid Waste Leachate

Days	THBC ($\times 10^4$ $CFUmL^{-1}$)	TCC ($\times 10^3$ $CFUmL^{-1}$)
Day 1	6.9±1.0	7.8±1.0
Day 8	4.5±0.8	2.7±0.8
Day 21	3.1±1.0	ND
Day 28	1.5±0.5	ND

Data are mean values ± standard deviation; THBC = total heterotrophic bacterial count; total coliform count (TCC); ND = not detected

The cultural, morphological, and biochemical characteristics of bacteria isolates revealed the presence of eleven (11) species of bacteria, namely *Yersinia* sp, *Yersinia enterolitica*, *Pseudomonas* spp, *Shigella* sp, *Bacillus amyloliquifaciens*, *Bacillus* spp, *Salmonella* sp, *Bacillus subtilis*, *Bacillus* spp, *Salmonella typhi*, *Enterobacter aerogenes*, *Klebsiella pneumonia* was observed. Of the bacteria isolated, *Pseudomonas* spp, *Bacillus* spp, *Yersinia* spp, *and Salmonella* spp. were the predominant species encountered.

Table 2: Morphological and Biochemical Characteristics of Bacterial Isolates

G.R	Shape	Spore Staining	Motility	Catalase	Starch Hydrolysis	Indole	MR	VP	Citrate	H_2S	Glucose	Lactose	Dextrose	Mannitol	Probable Organism
-	R	-	+	+	-	+	-	+	-	-	AG	A	A	AG	*Yersinia* sp
-	R	-	+	+	-	+	-	+	-	-	A	A	A	A	*Yersinia enterocolitica*
-	R	-	+	+	-	-	-	+	-	+	-	-	-	A	*Pseudomonas* sp
-	R	-	-	+	+	-	+	-	-	+	A	-	A	A	*Shigella* sp
+	R	+	+	+	+	-	-	+	+	-	A	_	AG	A	*Bacillus amyloliquifaciens*
+	R	+	+	+	+	-	-	+	+	-	AG	-	A	A	*Bacillus* spp
-	R	-	+	+	+	-	+	-	+	-	A	-	A	A	*Salmonella* sp
+	R	+	+	-	-	+	-	+	-	+	AG	AG	-	A	*Bacillus subtilis*
+	R	+	+	+	+	-	-	+	+	-	AG	-	A	AG	*Bacillus* sp
-	R	-	+	+	+	-	+	-	+	-	AG	A	A	A	*Salmonella typhi*
-	R	-	+	-	-	-	-	+	+	-	A	AG	A	A	*Enterobacter aerogenes*
-	R	-	+	-	+	+	-	+	+	+	A	A	A	AG	*Klebsiella pneumonia*

Key: G.R= Gram Reaction, MR= Methyl Red, VP= Voges Proskauer, + = Positive, - = Negative, R = Rod, A = Acid only, AG = Acid and Gas produced

Table 3: Occurrence of Bacteria in the Leachate

Bacteria Isolates	No. of Occurrence	Percentage of Occurrence (%)
Yersinia sp	1	5.88
Yersinia enterica	1	5.88
Pseudomonas sp	5	29.41
Shigella sp	1	4.88
Bacillus amyloquifaciens	2	11.76
Bacillus sp	2	11.76
Salmonella sp	1	5.88
Baillus subtilis	1	5.88
Salmonella typhii	1	5.88
Enterobacter aerogenes	1	5.88
Klebsiella pneumoniae	1	5.88
Total	**17**	**100**

The physicochemical parameters of the leachate samples are shown in Table 4. The degree of chemical contamination and biodegradability of the leachate was determined by studying the physicochemical parameters (EC, TDS, pH, BOD, and COD) of the leachate sample within 28 days under the studied condition. Notable correlation coefficients of determination (r) were observed between all the analyzed properties (Table 5). For instance, the number of days was negatively correlated with all other parameters, indicating that as time progressed, the values of these parameters declined.

Table 4: Physicochemical Changes in Leachate Over a 28-day Retention Time

Days	EC (μscm^{-1})	TDS (ppm)	pH	BOD (mgL^{-1})	COD (mgL^{-1})
Day 1	9758.7±0.07	8210.3±1.00	7.62 ±0.01	651.3±0.01	2058.7±0.04
Day 8	9469.3±1.00	8198.7±0.06	7.56 ±0.02	623.3±0.05	2044.3±0.01
Day 21	9647.0±0.09	8104.7±0.07	7.51 ±0.01	611.0±0.04	2006.0±0.03
Day 28	9183.3±0.05	7004.0±1.00	6.48 ±0.01	567.7±1.00	1981.7±0.04

Keys: EC = electrical conductivity; TDS = total dissolved solids; BOD = biochemical oxygen demand; COD = chemical oxygen demand. The values presented are the mean of the designated sampling days and the standard deviation.

The strongest negative correlation was observed with COD (-0.996), followed by THBC (-0.976) and TCC (-0.910). Total heterotrophic bacterial count (THBC) correlated positively with total coliform count (TCC) (r = 0.949), BOD (r = 0.968), and COD (r = 0.960). A strong positive correlation of total coliform count (TCC) was observed with BOD (0.842) and COD (0.870) as well as a moderate positive correlation with EC (0.644). A moderate to strong positive correlation of electrical conductivity (EC) with TDS (0.876), pH (0.894), BOD (0.902), and COD (0.722) was seen. Total dissolved solids (TDS) showed a very strong positive correlation with pH (0.999) and a strong positive correlation with EC (0.876), BOD (0.903), and COD (0.825). A very strong positive correlation between pH and TDS (0.999) and a strong positive correlation with BOD (0.911) was also witnessed. The biochemical oxygen demand (BOD) showed a strong positive correlation with COD (0.949), EC (0.902), and pH (0.911). Chemical oxygen demand (COD) exhibited a strong positive correlation with BOD (0.949), THBC (0.960), and TCC (0.870).

Table 5: Correlation Matrix of Microbiological and Physicochemical Changes

	Days	*THBC*	*TCC*	*EC*	*TDS*	*pH*	*BOD*	*COD*
Days	1							
THBC	-0.97603	1						
TCC	-0.91025	0.949189	1					
EC	-0.71799	0.809083	0.643659	1				
TDS	-0.78624	0.772191	0.532994	0.876012	1			
pH	-0.78778	0.78203	0.546475	0.893958	0.999256	1		
BOD	-0.94682	0.968324	0.842221	0.902326	0.903025	0.911129	1	
COD	-0.99603	0.960169	0.869893	0.721726	0.825052	0.824167	0.949316	1

DISCUSSION

From the study, varying microbial load was recorded from the leachate. This was a reflection that the waste materials on open dumpsites impacted microbial growth and influenced the proliferation of the leachate microflora (Sekhohola-Dlamini., *et al*., 2021). The bacterial load recovered from day 28 was comparatively lesser than all the days before. This trend may be due to the depletion of the available nutrients that can result in microbial community competition for these nutrients: organic compounds and nitrates (Rashid *et al*., 2016). The detection of coliform bacteria is of public health concern and indicates the potential for ground- and surface-water contamination by leachate from garbage dumps. Microorganisms, which include bacteria, archaea, and fungi, are observed in abundance within landfill and dump sites (Sekhohola-Dlamini and Tekere, 2020). This can be attributed to the presence of rich natural organic compounds and substrate complexity within those areas since dumpsites and/or landfills are considered as microbial pools (Song *et al*., 2015). Generally, studies have indicated that the microorganisms present within waste-dumpsite-impacted ecosystems are strongly selected by the predominant environmental conditions present and their substrate-specificity (Song *et al*., 2015). In this study, quite a number of Gram-positive bacteria consisting of Bacillus and Gram-negative bacteria belonging to the genera *Pseudomonas*, *Yersinia*, and *Enterobacter* were isolated during the 28 days. These organisms were similar to those reported by Kalfizadeh *et al*. (2011).

The bacterial isolates identified from the leachate samples have been reported to be associated with wastes and are adept in waste biodegradation (Obire *et al*., 2002).

Specifically, *Pseudomonas* spp. and *Bacillus* spp. Possess higher degrading potential than the others (Devi *et al.,* 2019). Besides waste biodegradation, some of them, like species of Salmonella, Yersinia, and Klebsiella, are putative pathogens implicated with disease causation (Ilieva et al., 2023). The microorganisms found in these waste dumpsites derive their nutritional requirement from the waste, hence the high bacterial growth profile (Sekhohola-Dlamini., *et al.* 2021). One of the major concerns for the indiscriminate dumping of waste in open dumps is their proximity to residential buildings, potentially leading to environmental risk (Dladla *et al.,* 2021).

The pH value of the leachate declined from 7.62±0.01 to 6.48±0.01 due to the degradation of organic fractions and corresponding production of intermediate acids (bicarbonates) with buffering capacity occurring at the maturity phase of the leachate (Hussein *et al.,* 2019). The decreased pH to 6.48±0.01 on day 28 was a result of the decrease in methanogenesis. This is in accordance with the normal pH changes occurring at the different phases of landfill stabilization (Gotvajn and Pavko, 2015).

Previous research showed that aged leachate contains lower levels of organic matter lower than 5,000 mg/l for COD and 3000 mg/l for BOD in immature landfills (Renou *et al.,* 2008; Sriram *et al.,* 2017) as a result of biotic stabilization processes in the body of the landfill dumps.

The BOD remains an indicator measurement for substances that can be degraded microbially, consuming dissolved oxygen in the degradation process. In this study, the reduction in the BOD level from 651.3±0.01 mgL^{-1} to 567.7±1.00 mgL^{-1} was an indication that the isolated bacteria from the simulated leachate sample consumed the available oxygen to degrade leachate by utilizing as their energy source the organic compounds - a major component of leachate. This observation was in consonance with the work of Dhall *et al.* (2012). The reduction may also attributed to the age of the landfill. This is consistent with other researchers (Sabour *et al.,* 2020). Greater BOD in leachate samples was reported by some other researchers (Javahershenas *et al.,* 2022).

The COD is a measure of the capacity of liquid to consume oxygen during the decomposition of the organic matter in the liquid. It increases as the organic compounds increase and thus decreases as decomposition occurs (Qi *et al.,* 2021). In this study, the COD level reduced from 2058.7 ±0.04 mgL^{-1} to 1981.7 ±0.04 mgL^{-1}, implying that the bacterial isolates were able to utilize the organic component of the leachate, decreasing the amount of organic compounds present in it, which in turn led to a decreased COD level. A similar event was also reported by Dhall *et al.* (2012), and the reduction was also attributed to the age of the landfill waste yard.

The total dissolved solids (TDS) are the dissolved organic matter, including inorganic salts (nutrients) in the liquid, while electrical conductivity (EC) is a measure of the ability of a substance to conduct or resist electric current. The TDS and EC of the leachate in this study reduced from day 7 to day 28 with mean values of 8210.3±1.0ppm to 7004±1.0ppm and 9758±0.07μscm^{-1} to 9183.3±0.05μscm^{-1}, respectively. This was suspected to be a result of the degradation, which tends to reduce the amount of the dissolved matter, ions, cations, and molecules present in the leachate, according to a previous study (Maiti *et al.,* 2016).

BOD and COD are both notable indicators of organic pollution and were strongly correlated with each other, and with the bacterial counts (THBC and TCC). These parameters, being strongly correlated with each other, highlight their common relationship with organic pollution and microbial activity. High THBC and TCC often indicate the presence of organic matter, which increases BOD and COD levels due to microbial decomposition. This outcome is consistent with previous literature (Verma and Singh, 2013) and suggests that as organic pollution increases, so do the bacterial counts. TDS and pH had an extremely high correlation, indicating they were closely related in the dataset. The strong correlation between pH and other parameters, such as TDS and BOD, is also supported by literature (Rahman *et al.,* 2021). The solubility and biological availability of chemical constituents in water and/or wastewater can be influenced by pH, affecting parameters like BOD and COD. The strong negative correlations observed with proceeding days suggest a general decline in these parameters over time, possibly due to the leachate treatment or natural attenuation processes. The correlation matrix revealed that EC and TDS are closely related because TDS measures dissolved solids, while EC measures the ability of these dissolved solids to conduct electricity. The high correlation (0.876) confirmed this association.

CONCLUSION

Based on the results obtained, the current study revealed the high-level contamination of simulated leachates from municipal waste-impacted soils with bacteria (*Yersinia* sp, *Yersinia enterolitica, Pseudomonas* spp, *Shigella* sp, *Bacillus amyloliquifaciens, Bacillus* spp, *Salmonella* sp, *Bacillus subtilis, Bacillus* spp, *Salmonella typhi, Enterobacter aerogenes, Klebsiella pneumonia*) as well as inorganic chemicals.

These findings emphasize the need to promote the construction and use of sanitary landfills as opposed to the practice of open waste dumping currently experienced in Nigeria. These leachates, when not properly channeled and managed, portend deleterious public and environmental health issues. However, the physical-chemical changes observed in the analysed leachate demonstrated potential for its decontamination/treatment through pollutant breakdown by these isolated bacteria, which may be adopted in the design of bioremediation strategies.

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