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Physicochemical Composition and Heavy Metals Tolerance of Bacterial Isolates in Leachate from Solid Waste Dumpsites in Delta North Senatorial District, Delta State

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Abstract

Leachates from solid waste dumpsites are a source of environmental contaminants which negatively affect the ecosystem and subsequently human health. The continuous bio magnification of heavy metals in the environment has remained as source of worry to public health experts. The aim of this study was to assess the physicochemical and heavy metals concentrations as well as heavy metals tolerance profile of bacteria isolated from leachate collected some towns; Isele-Uku (6^0 25ꞌ 0ꞌN and 6^0 42ꞌ 0ꞌE), Umunede (6^0 16ꞌ 0ꞌN and 6^0 18ꞌ 0ꞌE) and Agbor (6^0 7ꞌ 20ꞌN and 6^0 5ꞌ 20ꞌE) in Delta State, Nigeria. Standard laboratory procedures were used to analyse the samples. The mean concentrations of the physicochemical results ranged from 1060.00±3.15 to 2070.00±4.11µS/cm, 29.50±1.11 to 62.40±0.40mg/l, 78.9078.90±1.12 to 120.5078.90±0.22mg/l, 18.69±0.01 to 31.26±0.23mg/l, 0.05±0.11 to 0.90±0.01mg/l and 21.60 ± 0.49 to 32.80 ± 0.12 electrical conductivity, biological oxygen demand, chemical oxygen demand, Iron, Lead, nitrate respectively. Overall, Umunede had the highest bacterial load of 95%. Also, samples from Umunede had the most prevalent isolate, *Corynebacterium* sp. (26.87%) followed by *Pseudomonas aeruginosa* (25.76%) and least in *Bacillus cereus* (5.97%) at Isielu – uku. *Pseudomonas aeruginosa* displayed the highest resistance (70.59%) against chromium, *Bacillus subtilis* had the highest (83.33%) against cobalt. *Arthrobact*er sp. had the highest of 75.00% against lead and mercury. *Corynebacterium* sp. had the highest of 86.67% against and lead, *Alcaligenes* sp. displayed the highest resistance 81.82% against cobalt while *Acinetobacter* sp. had the highest (66.67%) against lead and mercury. The leachate contained electrical conductivity, chemical oxygen demand, biological oxygen demand, nitrate and iron and lead at concentrations beyond the acceptable limit for effluent discharge which makes it unfit for discharge without prior treatment.

Keywords: Heavy Metals, Leachate, Permissible Limit, *Pseudomonas Aeruginosa*, Public Health

1. Introduction

Leachates are solutions that results from leaching of soluble constituents in landfills which among other issues; disrupt environmental quality and by downward movement percolate into surrounding ground water (Enerijiofi and Ajuzie 2012; Bhalla et al., 2012;). Despite much stricter statutory controls, leachates from modern sites are known to contain range of contaminants; pathogenic microorganisms, antibiotics, heavy metals and lots more (Enerijiofi and Ekhaise, 2019a). These contaminants particularly heavy metals highly affect microorganisms in soil leading to alteration in diversity, population size and activity of the soil microbial community (Mgbemena et al., 2012). When plants grown on heavy metals laden soils, are consumed by man either as food for energy or medicine for treatment, the heavy metals gets into man's system which causes serious lethal effects including health and reproductive challenges (Oluseyi et al., 2014; Enerijiofi, 2021). In animals, induced micronuclei in mouse bone marrow cells in vivo by municipal landfill leachate was used to describe the biotoxicity of leachate, with the mouse been damaged when fed with leachate at low concentration of 5mg/L COD.

Biological and physicochemical techniques remain the most common methods of treating leachates because they possess the different removal potentials for varieties of pollutants. These discharged effluents must satisfy the discharge criteria (Bhalla et al., 2012). However, the characteristics of leachate depends on several factors such as waste composition and the age of waste, degree of decomposition, moisture content of waste, rate of water movement as well as temperature (Aluko et al., 2002, Fatubarin and Olojugba, 2014). The natural attenuation of assessing environmental risks of leachates that flow into the underground water determines the suitable corrective methods as well as monitoring regular mitigations (Yusof et al., 2009; Jelic et al., 2011).

Groundwater is a major source of potable water within the vicinity of this study area as well as Nigeria in general and its contamination leads to series of public health issues. The aim of this study was to assess the physicochemical composition and heavy metals tolerance of the isolated bacteria species from of leachate located within solid waste dumpsites in Delta North Senatorial District of Delta State, Nigeria.

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2 Materials and Method

2.1 characteristics of study area and collection of samples

The study area is along Agbor - Asaba Express way, a very busy road with different communities. It leads to the entire South East Geopolitical zones of Nigeria. The surface debris were removed and 200mls of leachate samples were aseptically collected from dumpsites located at Isele-Uku ($6^0\ 25^{\prime}\ 0^{\prime\prime}$ N and $6^0\ 42^{\prime}\ 0^{\prime\prime}$ E), Umunede ($6^0\ 16^{\prime}\ 0^{\prime\prime}$ N and $6^0\ 18^{\prime}\ 0^{\prime\prime}$E) and Agbor ($6^0\ 7^{\prime}\ 20^{\prime\prime}$ N and $6^0\ 5^{\prime}\ 20^{\prime\prime}$ E) into sterile screw capped bottles. The samples were immediately transported to the microbiology laboratory for analyses.

2.2 Determination of physicochemical parameters

The method of APHA, (2011) was used to determine the physicochemical parameters which included pH, temperature, electrical conductivity, chloride, total dissolved solids, total soluble solid, chemical oxygen demand, biological oxygen demand, dissolved oxygen, turbidity, sulphate, nitrate and phosphate. The cations (sodium, potassium, calcium and magnesium) were determined with a flame photometer, model Jenway model PFP7 while the heavy metals (iron, copper, zinc, Aluminium, Manganese, Lead, Nickel and Cobalt) concentrations were analysed with the aid of atomic absorption spectrophotometer, model PG 550 (Enerijiofi et al., 2017a).

2.3 Inoculation and enumeration

The method of Holt et al. (1994) was used for isolation and enumeration of bacterial isolates. One mililitre of the leachate sample was homogenized in ten mililitres of distilled water to form the stock, followed by serial dilution of the leachate samples in ten -fold. Thereafter, 0.1ml aliquots from the third, sixth and ninth dilutions were pour - plated with freshly prepared nutrient agar plates in triplicate containing fushin, which prevents fungal growth The nutrient agar plates were incubated at 37^oC for 48h and were observed for growth. The discrete colonies of bacteria were counted, calculated and expressed as colony-forming units per millilitre (CFU/ml)

2.4 Isolation, identification and characterization

The colonies were sub-cultured into Nutrient agar plates that were freshly prepared to obtain a pure colony. Isolation and Characterization of pure cultures of the various isolates were made and stored in the refrigerator at 4^oC. They were identified by culturally and morphologically (Cheesbrough, 2005). The sub - cultured bacterial isolates were subjected to an array of relevant physiological and biochemical tests which included; catalase production, oxidase production, spore staining, sugar fermentation tests, Gram staining. The results were put together and the bacterial isolates were identified by comparison with the protocol of (Holt et al., 1994 and Cullimore, 2000).

2.5 Preparation of selected heavy metals solution

The stock solutions of the tested heavy metals were prepared separately by dissolving 500mg the respective metal salts; $HgCl_2$, $CdCl_2$, $CoCl_2$, $Pb(NO_3)_2$ and CrO_3 in 100ml of de ionized water which was followed by filtration. The mixture was thereafter filtered using 0.22µm membrane filters (Nucleopore Corp., Pleasanton, CA, USA). This was stored in sterilized flasks at $4^{\circ}C$ in dark room for 24hrs before use for further studies (Enerijiofi and Ajuzie, 2012).

2.6 Heavy metals tolerance testing

Heavy metals resistance testing was carried out according to the method of (Mgbemena et al., 2012). Muller hinton agar was prepared with varying concentrations 0.00mg/ml, 0.50mg/ml, 1.00mg/ml, 1.50mg/ml, 2.00mg/ml, 2.50mg/ml, 3.00mg/ml, 3.50mg/ml, 4.00mg/ml, 4.50mg/ml and 5.00mg/ml respectively for the different heavy metals compounds of $HgCl_2$, $CdCl_2$, $CoCl_2$, $Pb(NO_3)_2$ and CrO_3. Zero point one mililitre of pure bacterial isolate was poured on the surface of heavy metal incorporated medium and spread evenly on the surface of the medium to allow for even distribution of the isolates. The medium without heavy metal served as control. The cultured plates were incubated at 37^oC for 24hrs and thereafter observed for bacteria growth.

2.7 Statistical analysis of data

The means and standard errors were computed using conventional statistical method. The statistical package for social sciences (SPSS version 20) was used to generate analysis of variance (ANOVA), thereafter, Duncan's multiple range test was used to compare the means (Ogbeibu, 2005).

3 Results

The results of the concentrations of the physicochemical parameters of leachate samples are presented in Table 1.

Figure 1 records the frequency of occurrence of bacterial isolates. Overall, Umunede had the highest bacterial load of 95%. Also, samples from Umunede had the most prevalent isolate, *Corynebacterium* sp. (26.87%) followed by *Pseudomonas aeruginosa* (25.76%) and least with *Bacillus cereus* (5.97%) at Isielu – uku. In the overall, Umunede had the highest of 95% while Agbor had the least, 66%.Table 2 records the percentage of heavy metals tolerance in bacterial isolates. *Pseudomonas aeruginosa* displayed the highest resistance (70.59%) against chromium, *Bacillus subtilis* had the highest (83.33%) against cobalt. *Arthrobact*er sp. had the highest of 75.00% against lead and mercury. *Corynebacterium* sp. had the highest of 86.67% against lead, *Alcaligenes* sp. displayed the highest resistance (81.82%) against cobalt while *Acinetobacter* sp. had the highest (66.67%) against lead and mercury.

Table 1. physicochemical characteristics of leachates

PARAMETERS	A	B	C	FEPA (1991) limit
pH	7.80±0.21^{a}	6.90±0.01^{a}	7.10±2.01^{a}	6 – 9
Temp. ($0^{o}C$)	29.60±0.01^{a}	29.50±0.20^{a}	29.60±1.03^{a}	30
EC (us/cm)	1060.00±3.15^{a}	1780.00±3.01^{a}	2070.00±4.11^{b}	1000
Cl^{-}(mg/l)	142.00±0.46^{a}	319.50±0.42^{c}	284.00±0.23^{b}	600
TSS (mg/l)	6.00±0.27^{a}	4.00±0.21^{a}	4.00±0.16^{a}	30
TDS (mg/l)	51.00±0.31^{a}	92.00±0.18^{a}	1030.00±1.34^{b}	2000
Turbidity (NTU)	740.00±2.45^{b}	480.00±2.13^{a}	420.00±1.99^{a}	300
COD (mg/l)	120.50±0.22^{b}	86.20±0.34^{a}	78.90±1.12^{a}	40
DO (mg/l)	4.00±0.18^{a}	6.00±0.51^{a}	5.00±1.01^{a}	40
BOD (mg/l)	62.40±0.40^{b}	30.80±0.11^{a}	29.50±1.11^{a}	10
Sulphate (mg/l)	5.24±1.11^{a}	8.41±0.14^{a}	6.20±0.23^{a}	50
Nitrate (mg/l)	32.80±0.12^{b}	24.00±0.19^{a}	21.60±0.49^{a}	1
Phosphate (mg/l)	13.51±0.44^{a}	13.80±0.31^{a}	12.42±0.02^{a}	5
Calcium (mg/l)	42.40±0.23^{a}	40.20±0.19^{a}	38.80±0.61^{a}	100
Potassium (mg/l)	16.80±0.12^{a}	18.20±0.42^{a}	16.40±0.42^{a}	N/A
Magnesium (mg/l)	26.40±0.11^{a}	25.80±0.16^{a}	26.00±0.17^{a}	100
Sodium (mg/l)	14.60±0.42^{a}	12.80±0.27^{a}	12.10±1.16^{a}	200
Fe^{2+} (mg/l)	2.61±0.23^{b}	1.69±0.22^{a}	1.04±0.01^{a}	20
Cu^{2+} (mg/l)	0.05±0.01^{a}	0.06±0.14^{a}	0.03±0.01^{a}	1.5
Zn^{2+} (mg/l)	0.48±0.10^{a}	0.61±0.11^{a}	0.50±0.01^{a}	1
Al^{3+} (mg/l)	0.68±0.10^{a}	0.74±1.17^{a}	0.72±0.12^{a}	N/A
Mn^{2+} (mg/l)	0.35±0.10^{a}	0.41±0.10^{a}	0.48±0.01^{a}	N/A
Pb^{2+} (mg/l)	0.90±0.11^{b}	0.02±0.01^{a}	0.03±0.01^{b}	0.5
Ni^{2+} (mg/l)	0.09±0.32^{a}	0.03±0.01^{a}	0.05±0.01^{a}	1
Co^{2+} (mg/l)	0.01±0.01^{a}	0.01±0.01^{a}	0.02±0.01^{a}	0.5

Values represent the Means± Standard Deviation of twelve samples collected over a period of six months
Legend: Legend: A: Agbor (6^0 7' 20'N and 6^0 5' 20'E), **B: Umunede** (6^0 16' 0'N and 6^0 18' 0'E), **C: Isielu –Uku** (6^0 25' 0'N and 6^0 42' 0'E), **NI= Not indicated, TDS= total dissolved solid, TSS= total soluble solid, EC= electrical conductivity, COD= chemical oxygen demand, DO= dissolved oxygen, BOD= biological oxygen demand Fe = Iron, Cu = Copper, Zn = Zinc, Al = Aluminum, Mn = Manganese, Pb = Lead Ni = Nickel, Co = Cobalt, N/A= not available**

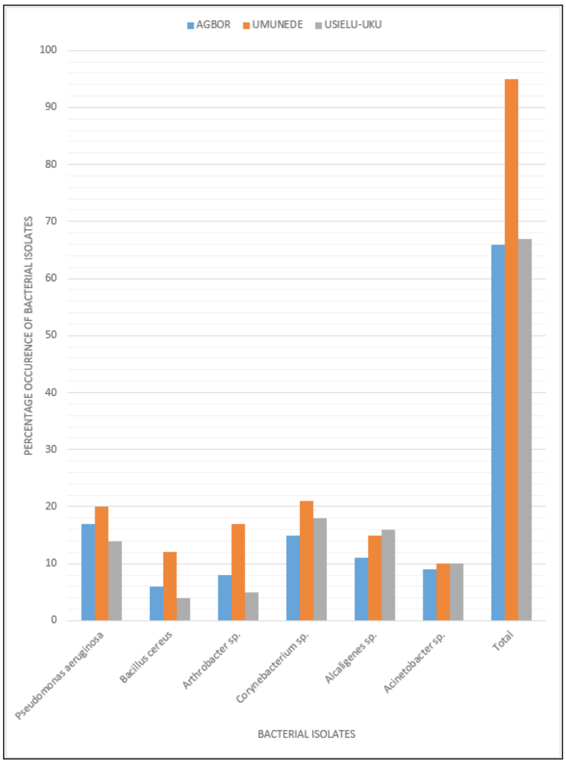

Figure 1. percentage frequency of occurrence of bacterial isolates

Table 2. percentage of heavy metals tolerance in bacterial isolates

Bacterial isolates	No. examined	Tolerance to Pb	Tolerance to Cd	Tolerance to Hg	Tolerance to Co	Tolerance to Cr
Pseudomonas aeruginosa	17	10 (58.82)	11 (64.71)	9 (52.94)	11 (64.71)	12 (70.59)
Bacillus subtilis	6	4 (66.67)	4 (66.67)	3 (50.00)	5 (83.33)	4 (66.67)
Athrobacter sp.	8	6 (75.00)	5 (62.50)	6 (75.00)	5 (62.50)	5 (62.50)
Corynebacterium sp.	15	13 (86.67)	10 (66.67)	12 (80.00)	9 (60.00)	11 (73.33)
Alcaligenes sp.	11	8 (72.72)	8 (72.72)	7 (63.64)	9 (81.82)	5 (45.45)
Acinetobacter sp.	9	6 (66.67)	5 (55.56)	6 (66.67)	5 (55.56)	5 (55.56)
Total	67					

4 Discussion

Leachates are liquid substances arising from municipal wastes. They are known to contain lots of biological and chemical contaminants which are hazardous to man and the environment. This study investigated the physicochemical composition and heavy metals tolerance of bacterial isolates in leachate from Delta State, Nigeria. The pH and temperature range reported support most biological functions in bacterial cells which are important in the natural treatment of leachate thereby reducing environmental contamination. The moderate acidic pH values obtained in the sites under study could be attributed to the removal of basic cations or nutrients from the surface of the soils in the dumpsites as a result of the effect of anthropogenic activities such as burning which resulted in the loss of nutrients. The results point to the obvious that certain reactions could be hastened by release of leachates and also, reduction in the oxygen solubility and intense odour due to anaerobic reaction. The turbidity values were higher than standard for discharge of waste water into stream (WHO, 2006; Oluseyi et al., 2014). This high value could have resulted from solubilisation of the organic component of the municipal solid waste. The electrical conductivity values reported indicates that the leachate samples have high concentration of soluble salts which is a good indicator of plant growth. There were significant difference in the electrical conductivity concentrations across the sites. The dissolved oxygen concentrations of the samples was within the stipulated limit FEPA (1991), which means that there was pollution by organic matter. Also, the high concentration of chemical oxygen demand and biological oxygen demand recorded showed high presence of organic contaminants which were actively decomposing due to their dissolved oxygen concentration and solubility in water. It is worthy to note that the result of this study showed that the chemical oxygen demand and biological oxygen demand were inversely proportional to dissolved oxygen which corroborated with earlier reports of Aluko et al. (2002) and Anyakora et al. (2011) from wastes dumpsites in Lagos. There were significant differences in the chemical oxygen demand and the biological oxygen demand across the sites. Nitrate recorded was above the limit. The high concentration could be responsible for the high number of bacterial diversity recorded. The presence of nitrate in the soil may be attributed to the mineralization of nitrogen as a result of organic matter in the soil. The phosphate concentration could be attributed to the presence of a high amount of organic matter and plant decomposition at the dumpsites (Ideriah et al., 2006). The high presence of lead could have been from incomplete combustion of hydrocarbons from automobiles and generating sets. It could also be due to the depositions of wastes from automobile mechanic workshops in these dumpsites. However, Michaela et al. (2018) reported that lead poisoning could lead to kidney failure, loss of appetite and brain damage as a result of prolong exposure. There were significant differences in iron and lead concentrations across the sites. Also, when plants are grown to maturity on such contaminated soils, there are possibilities that the heavy metals could get to man when they are taken in as drug or food (Momodu and Anyakora, 2010).

The leachate samples had highly bacterial load than the control indicating contamination. This may be attributed to high quantity of organic matter present in leachate samples which favoured bacterial growth compared to the control. The bacteria reported in this study were similar to ones previously identified *Citrobacter* sp. (4.35%), *Bacillus* sp. (26.08%), *Enterobacter* sp. (4.35%) *E. coli* sp. (4.35%), *Klebsiella* sp. (4.35%), *Neisseria* sp. (4.35%), *Pseudomonas* sp. (4.35%), *Shigella* sp. (4.35%), *Staphylococcus* sp. (13.04%), *Streptococcus* sp. (26.08%), and *Vibrio* sp. (4.35%) (Obire et al. 2002; Michaela et al. 2018). The indiscriminate release of leachate from these municipal waste dumpsites could pose public health risks to the citizens whose food and water supplies may, perhaps, become contaminated as a result of leakage from dumpsites during rains (Eghomwanre et al. 2020). The bacteria identified could cause lots of diseases such as gastro-intestinal infections, shock, dysentery, typhoid fever and diarrhoea. *Citrobacter*, *Klebsiella*, *Enterobacter* and *Escherichia* are members of the coliforms group. They are gram negative rods that inhabit the human intestinal tract, with its main reserviour being the sewage system (Enerijiofi and Ajuzie, 2012). *Pseudomonas aeruginosa* is considered an opportunistic pathogen that can cause wound infections leading to sepsis and mortality from chronic septicaemia. The high organic matter recorded in leachate may have accounted for their high numbers in the samples studied. Other isolates; *Bacillus Arthrobacter*, *Alcaligenes, Corynebacterium* and *Acinetobacter* species have been described as human opportunistic pathogens which could lead to food poisoning upon ingesting contaminated food or water (Eja et al., 2010). All the bacterial and fungi isolates identified from leachate samples have been reported to be associated with wastes and waste biodegradation (Obire *et al*., 2002).

The heavy metals resistance was generally high for all metals especially lead and cobalt which were above 80%. This high values points to the obvious that the rate of discharge of heavy metals into the environment is on the increase which could lead to public health epidemic. The finding in this study in agreed with earlier reports Jelic et al. (2011) in Nigeria and Abdelately et al. (2011) in Egypt where they recorded a corresponding high bacterial resistance rate in effluent samples. These heavy metals have been reported to prevent ossification of bones in man by taking the place of Calcium in bones. They have also been implicated in reproductive disorders like inhibition of libido as well as prevent ovulation in males and females respectively (Enerijiofi and Ekhaise, 2019a). The implication is that resistance to heavy metals is a worldwide issue and worst off in developing countries where regulations are lacking and open disposal is fast becoming a culture.

5 Conclusion

This study revealed a significant difference in the concentrations recorded for electrical conductivity, chemical oxygen demand, biological oxygen demand, nitrate, iron and lead. The aforementioned physicochemical parameters were also beyond the acceptable limit and do not meet the standard for effluent discharge. The high microbial load reported could be linked to the increased nitrate and phosphate in the examined leachate. Also, the high heavy metals resistance recoded is worrisome. Education as well as the construction of well - lined landfills can reduce the level of contamination by leachate. However, the heavy metals resistance isolates could be used in remediating the soil of its heavy metals content.

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Authors' contributions

The first author conceived the research idea, designed the experiment and prepared the manuscript. The second and third authors did the literature search, generated results from the laboratory and performed data analysis. All authors did sampling, read and accepted the final version of the manuscript.

Competing interests

No competing interests exist between the authors weather financial or otherwise.

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