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BACTERIAL ISOLATES OF SOILS OF DIFFERENT WASTE TYPES IN YENAGOA CENTRAL SOLID WASTE DUMPSITE, NIGERIA

Juliana Okwena Pondei[1*] and Cynthia Chidimma Okeke[2]

[1]Department of Microbiology, University of Abuja, PMB 117, FCT, Nigeria.

[2]Department of Microbiology, Federal University Otuoke, PMB 126, Yenagoa, Nigeria.

[*]Corresponding Author Email: julipondei@gmail.com

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ABSTRACT: *Managing wastes has remained a continuous issue for developing countries like Nigeria. Microorganisms can be utilized to augment the efficiency of degradation of undesired wastes. The microorganisms associated with soils of solid wastes in Yenagoa Central Waste Dumpsite of Bayelsa State, Nigeria were investigated. Soil samples were collected from four locations having sorted wastes of plastics, metals, glass, general wastes and a control area devoid of any waste within the dumpsite. The soil samples were examined for some physicochemical parameters and culturable bacteria using standard procedures. Soil temperature across the locations was 29°C, pH values were between 7.3 and 7.7, moisture contents ranged from 1% – 9%, soil particle sizes were 63.3g/cm – 91.4g/cm, electrical conductivity 62 mS/m – 200 mS/m, and the total organic carbon ranged from 0.88% to 5.64%. The Total Heterotrophic Bacterial (THB) counts were between 1.50 ± 0.00 x 10^5 cfu/g and 7.96 ± 3.21 x 10^5 cfu/g, with general wastes having the highest counts and the control soil with the least counts. The frequencies of occurrence of the isolates were Bacillus spp. (21.88%), E. coli (18.75%), Salmonella spp. and Vibrio spp. (15.62%), filamentous bacteria Streptomyces spp. (12.50%) and Eikelboom Type 0092 (9.38%), and Streptococcus spp. (6.25%). All bacterial species were present in the general wastes. Salmonella and Streptococcus species were absent in plastic wastes, Eikelboom type 0092, Vibrio and E. coli were absent in metal wastes while for glass, Eikelboom type 0092, Vibrio and Streptococcus species were not isolated. The control soil was devoid of Streptomyces and Salmonella species. The presence of specific bacterial species with location could be indicative of their association with the biodegradation of the specific waste type. On further research, these microorganisms could be explored for their potential in waste management.*

KEYWORDS: Bacteria, Biodegradation, Nigeria, Solid waste management.

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INTRODUCTION

Waste collection, disposal and management remains a major and complex environmental problem existing in low-income countries such as Nigeria (Aliu *et al*., 2014; Amasuomo & Baird, 2016; Nwosu & Chukwueloka, 2020). The open and indiscriminate dumping of human, animal and other wastes is a main issue and considered a gross irresolvable nuisance with urban centers, (Agamuthu & Fauziah, 2010). In the majority of developing countries, municipal solid wastes are usually disposed of without sorting, reducing, reusing or recycling (Angaye & Abowei, 2017). These wastes are composed of household wastes, electrical appliances, plastic packaging, wood, glass/bottles, food, paints, metals, and clothing, among others; it is therefore not uncommon to find other industrial, agricultural, medical, electrical, commercial and hazardous wastes in municipal wastes.

Improper disposal of waste poses serious public health and environmental consequences where piles of decomposing garbage and litter in strategic locations deface the aesthetics of a city. Major Nigerian cities are littered with solid wastes; along the roads, on drainage lines, and on most vacant lands (Pukkalanun *et al*., 2013; Ike *et al*., 2018). Numerous microorganisms are involved in the biodegradation of solid wastes and improper management of these wastes can lead to the introduction of pathogenic microorganisms and harmful chemicals into the environment. Furthermore, issues associated with improper waste disposal have resulted in contamination of rivers and also drinking water in residential areas (Hamatschek *et al*., 2010).

Due to the threat posed by improper waste management, studies on waste reduction and potency are highly encouraged. Biodegradation describes the enzymatic breakdown of organic material by microorganisms and plants to nutrients useful to other organisms. The degradation of environmental pollutants, including municipal solid wastes, and other types of solid wastes are carried out by various classes of microorganisms. These microorganisms, most of which are pathogenic, are domicile in refuse dumps (Odeyemi *et al*., 2011). Microorganisms found to be associated with degradation of municipal wastes at dump sites include *Bacillus, Escherichia coli, Klebsiella, Proteus, Pseudomonas, Staphylococcus and Streptococcus, Aspergillus, Fusarium, Mucor, Penicillium* and *Saccharomyces* (Williams & Hakam, 2016).

Components of dumpsites like plastics (e.g. polyvinyl chloride, PVCs), are resistant to microbial attack. However, studies revealed that bacteria, namely: *Bacillus subtilis, Pseudomonas* spp., *Staphylococcus aureus, Streptococcus lactis, Proteus vulgaris, Micrococcus* spp., *Clostridium* spp., *Streptomyces* spp. and *Alcaligenes faecalis* are able to degrade synthetic or biodegradable plastics (Kathiresan, 2003; Shah *et al*., 2008; Ahmed *et al*., 2018). The presence of metals in wastes may inhibit organic pollutant biodegradation in addition to affecting degradation rates. Some heavy metals are required for microbial processes while others induce oxidative damages. Heavy metals affect the physiological and biochemical properties of microorganisms (Liu *et al*., 2016). Other hazardous products and hospital wastes found in municipal wastes, especially in developing countries, are detrimental to the environment.

In Yenagoa, Nigeria, there are few sanitary landfills because of urban residential problems and the waterlogged nature of the environment. Waste materials are deposited in certain “open” dumpsites, allowed to accumulate before they are taken to the central waste dump site. The wastes are left for a considerable period of time at the site before incineration. Wastes from these dumpsites contaminate underground water and soil as is the case with wetlands such as in Bayelsa State, Nigeria. There is an increased need for environmentally friendly studies on

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waste management options essentially, biodegradation, to reduce harmful microbial levels in soil and the general impact on the environment.

MATERIALS AND METHODS

The Study Area

The study was carried out at Yenagoa LGA of Bayelsa State, Nigeria; Latitude 4º55'29" N, Longitude 6º15'51" E. Yenagoa is made up of 19 communities, namely: Swali, Yenagoa, Ovom, Onopa, Amarata, Ekeki, Okaka, Yenezue-epie, Kpansia, Yenezue-gene, Opolo, Okutukutu, Etegwe, Edepie, Akenpia, Agudama-epie, Akenfa, Yenegue, Igbogene. These communities take their wastes to the central waste dumpsite at Tombia, Latitude 5º0'0" N, Longitude 6º15'54" E.

Sample Collection

Soil samples were collected from five locations (L1, L2, L3, L4 and L5) in the waste dumpsite. The surface debris was removed and the subsurface soil dug to a depth of about 15 cm using soil auger. Soil samples (30 kg) were scooped using a trowel from depths of 0 – 15 cm into clean ziplock polythene bags within the perimeter of each refuse collection point. Samples of soils were collected at six different points per location. Soil temperature was determined on site using a thermometer. The control soils were collected from areas devoid of wastes. All soil samples were well labeled and transported to the laboratory for further analysis. Samples from each location were mixed to give a composite sample before analysis. Soil samples were sorted to remove debri within two (2) hours after collection.

Physicochemical Analysis of the Soil Samples

Physicochemical properties of the various soil samples determined were the moisture content, pH, electrical conductivity, temperature and organic carbon (Kalra & Maynar, 2010).

Moisture Content

The moisture content was determined by taking the initial weight of the sample before drying it in the oven at 105ºC for 24 hours. The dried sample was also weighed and was subtracted from the initial weight. Percentage Moisture Content = difference in weight/initial weight of soil sample X 100.

Electrical Conductivity

The electrical conductivity (EC) of the soil samples was measured with a conductivity meter, Model H1 8033. The standard reference solution of electrical conductivity, 1413 mhos/cm which relates to the concentration of dissolved mineral salts was made by dissolving 0.7546 g of dry KCl in water (0.01 N) and making up the volume to 1000 ml at 25º C. A suspension of ten (10) grams of soil sample was made in 200 ml of distilled water. The conductivity meter was dipped into the solution and allowed to stand for about 10 minutes to obtain a stable reading.

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Soil pH

Twenty (20) grams of air dried soil samples were weighed and transferred into a 100ml beaker. Forty (40) ml of distilled water was added and stirred at intervals with a glass rod. This was allowed to stand for about 30 minutes. The electrode of a calibrated pH meter was submerged into the soil suspension and stirred gradually until a steady reading on the meter was obtained.

Soil Organic Carbon Content

One (1) gram of soil sample was added to 10 ml of 1N K_2CrO_7. This was mixed gently and 20 ml of concentrated H_2SO_4 added. This was allowed to stand for 30 minutes after which 100 ml of distilled water was introduced, 3 – 4 drops of 0.025m ferroin added and titrated with 0.5 normal $FeSO_4$.

Microbiological Analyses

Total Heterotrophic Count of the Soil Samples

Normal saline was autoclaved and 9 ml was dispensed into test tubes. One gram of the respective fresh soil sample was weighed using a weighing balance and dissolved in 9 ml of sterile normal saline under aseptic conditions. Ten (10) fold serial dilutions were then conducted in series up to 10^{-6} and one (1) ml of dilution 10^{-4} plated in triplicate using the pour plate method. Twenty (20) ml of molten nutrient agar, was poured into Petri dishes and swirled gently to disperse the inoculum evenly in the medium. The plates were allowed to solidify for 30 minutes, inverted and then incubated at $30^oC \pm 2^oC$ for 24 – 48 hours. After incubation, the plates were observed for the occurrence of growth, the resulting colonies were counted using the colony counter and expressed as cfu/g. Dilution 10^{-4} of soil samples were also plated on Luria Bertani (LB) and MacConkey agar and observed for growth.

Isolation of Pure Cultures

Bacterial colonies were picked and sub-cultured on freshly prepared nutrient agar, Luria Bertani (LB) agar and MacConkey agar. This was repeated to obtain a pure culture. Pure cultures of the organisms were inoculated on agar slants and left to grow at 24 – 48 hours after which the stock cultures were refrigerated at 4^oC and used within a period of two weeks.

Characterization and Identification of Isolates

The isolated bacteria were identified by morphological, cultural and biochemical characterization methods. The morphology of the isolates on gram's reaction and some cultural characteristics on nutrient agar were observed and documented. Standard biochemical tests were utilized for identification (Cheesbrough, 2006).

Identification of Filamentous Bacteria

Filamentous bacteria were identified using Gram reaction and morphologies on Gram staining (filament shape and size), cell shape and size and the presence of visible cell "septa". Other characteristics noted were the presence or absence of a sheath, epiphyte (attached growth) and branching (Spiers *et al*., 2009).

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Data Analysis

The mean and standard deviation of values were calculated using Microsoft Excel (2016). All analyses were done using Microsoft Excel (2016).

RESULTS AND DISCUSSION

The physicochemical properties of the soils are laid out in Table 1. Soil temperature at the various locations ranged from 29.52^0C to 29.60^0C with no statistical significant differences ($P>0.05$) among the mean values. The average values of pH ranged from 7.4 ± 0.09 – 7.75 ± 0.10 with the control soil and sections with bottles having the least values. In a similar study in Port Harcourt, which is also in the South-South zone of Nigeria, Obire *et al*. (2002) reported dumpsite soil temperatures of 27ºC – 28°C and pH range of 5.4 – 7.9 while Obianefo *et al.* (2017) reported pH values of 4.86 – 7.66. Osim *et al*. (2020) recorded soil pH values of 5.32, 7.43 and 7.69 at three dumpsites in Yenagoa, Nigeria with the control pH of 5.29. However, all pH values in this study were alkaline. The pH differences in dumpsites could be due to the presence of liming materials and microbial activities (Ayade *et al*., 2003; Ideriah *et al.*, 2006; Obianefo *et al*., 2017). Moreso, the pH of soils of municipal solid wastes was documented to increase due to the decomposition of organic matter (Essien and Hanson, 2013).

The electrical conductivity was highest in the soil with plastic wastes (203.17 ± 0.14 mS/m), followed by metal (185.67 ± 163 mS/m), bottles (167.33 ± 1.03 mS/m) and general wastes (152.17 ± 2.14 mS/m). The control soil had the least electrical conductivity of 63.50 ± 1.05 mS/m. Post-hoc analysis of variance (ANOVA) shows statistically significant differences ($P<0.05$) in the average values of electrical conductivity from these different sections of the dumpsite. Normal soil electrical conductivity values are 110 – 570 mS/m which shows available nutrients to enhance microbial growth in all the waste types except in the control soil devoid of wastes and subsequently, nutrients.

Soil moisture contents of the waste types were generally low (<10%), with general wastes recording the highest (9.22 ± 0.02%), plastics (7.53 ± 0.05%), metals (5.55 ± 0.02%), the control soil (4.53 ± 0.02%) and bottles with the lowest (1.67 ± 0.02%). The comparison of means using analysis of variance (ANOVA) shows statistically significant differences ($P<0.05$) in the average values of soil moisture contents in the various locations of the dumpsite. Available soil moisture determines microbial activity, and subsequently, the degree of utilization of available nutrients. Such low moisture contents have been documented in municipal solid wastes (Getahun *et al*., 2011).

The particle sizes of soils of the various wastes were between 63.22 ± 0.15 and 91.48 ± 0.15g/cm^3 with the control soil having the least value (63.22 ± 0.15g/cm^3). This is in contrast to the report of Ayade (2003) where soil particle sizes of 76g/cm^3 to 86g/cm^3 were recorded in some soils of the Niger Delta Region, Nigeria.

The mean value of total organic carbon content was lowest in the control soil (0.89 ± 0.04), moderate in the plastic (2.92 ± 0.05), metal (2.64 ± 0.02) and bottle (2.62 ± 0.02) soils and higher in the general wastes soil (5.52 ± 0.05). The low amount in the control could be due to the absence of a considerable amount of waste material in the soil. The total organic carbon content was highest in general waste which is expected as this consists of various types of organic wastes. There are statistical significant differences ($P<0.05$) in average values of total

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organic carbon content in all the locations with the exception of mean values of total organic carbon content of bottles and metals showing no statistical difference (P>0.05) in their average values.

The total heterotrophic bacterial count of the general wastes was highest ($7.91 \pm 0.05 \times 10^5$ cfu/g) while the control soil had the least ($1.60 \pm 0.11 \times 10^5$ cfu/g). This implies greater microbial activity in the soil with general wastes, due to accessibility of usable organic matter and least in the soil without waste materials.

Seven genera of bacteria, namely: *Bacillus*, *Escherichia*, *Streptomyces*, *Salmonella*, *Vibrio*, Eikelboom type 0092 and *Streptococcus* were isolated from the soils of the dumpsites (Table 2). Their cultural, morphological and biochemical characteristics are shown in Table 3. *Bacillus* spp. was most prevalent occurring in all the locations with a percentage frequency of 21.90% while *Streptococcus* spp. was the least isolated occurring only in two locations (6.30%). *Escherichia coli* was the second most isolated (18.75%), occurring in four locations, followed closely by *Vibrio* spp. (15.62%) in three locations and *Salmonella* spp. (15.62%) in four locations. *Streptomyces* spp. occurred in four locations with a frequency of 12.50%, and Eikelboom type 0092 (9.38%) at three locations.

All seven (7) bacterial genera occurred in soils of general wastes, which recorded the highest organic matter content. *Vibrio* spp. were the most isolated in this waste followed by *Escherichia coli*, *Salmonella* spp. and *Bacillus* spp. *Streptococcus* spp. and filamentous bacteria (*Streptomyces* and Eikelboom type 0092) were least isolated. Several authors have associated numerous bacteria as degraders of general wastes (Ekundayo, 2008; Hubbe, 2010; Cook *et al.*, 2013, Wemedo *et al*., 2020). The microorganisms of these dumpsites derive their nutritional requirement from the wastes and are associated with waste biodegradation because they produce enzymes that degrade the waste materials (Obire *et al.*, 2002; Chukwu *et al*., 2004; Osazee *et al.*, 2013; Dey *et al.*, 2023).

Soils of the plastic wastes had five (5) bacterial species (Eikelboon type 0092, *Vibrio*, *Escherichia coli*, *Streptomyces* and more of *Bacillus* spp.). Plastics are usually termed as being resistant to microbial attack but some bacteria such as *Streptomyces* spp., *Pseudomonas* spp., *Ochrobactrum* spp. *Alcaligenes* spp., and *Rhodococcus* spp. and a variety of fungi have been documented to degrade both natural and synthetic plastics (Shah *et al*., 2008; Muthukumar and Veerappapillai, 2015; Ahmed *et al*., 2018). Other bacteria isolated in this soil could be associated with the degradation of plastics.

The soils of metal and bottle wastes had four bacterial species each. *Salmonella*, *Streptomyces* and *Bacillus* species were present in both soil types. While *Escherichia coli* was present in bottle wastes, *Streptococcus* was present in metal wastes. *Bacillus* spp. have been demonstrated to have glass fiber degrading abilities (Xu *et al*., 2018). *Bacillus* and *Streptomyces* species are documented metal degraders (Meenambigai *et. al*., 2016; Jin *et al*., 2018). Microorganisms degrade recalcitrant environmental contaminants through various mechanisms which are dependent on the pollutant or waste type. These mechanisms involve enzymatic depolymerization, biosorption, and transformation of the pollutant (Muthukumar & Veerappapillai, 2015; Liu *et al*., 2016).

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CONCLUSION

Waste production and management continues to be a serious issue worldwide. The search for the best solutions of waste management, with the least cost and effect to both humans and the environment is usually the best alternative. The use of natural sources (microorganisms and plants) to degrade environmental pollutants is the most acceptable clean up method for the environment. The study has highlighted various genera of bacteria which could be potential sources of degraders of wastes that are seemingly resistant in the environment (plastics, bottles and metals), in addition to general organic wastes. These microorganisms could be specific in their degradation of the waste type and should be explored for further application.

FUTURE RESEARCH

Degradative studies on the abilities of the individual microorganisms or in combinations on the different waste types should be undertaken for best results. Molecular identification methods would be beneficial to ascertain the true identity of the microorganisms.

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Table 1: Physicochemical properties and total heterotrophic bacterial counts of soils of the different types of wastes

Location and Type of waste	Physicochemical properties and THB of soils						
	Temperature (°C)	pH	EC (mS/m)	Moisture (%)	PS (g/cm)	TOC	THB (10^5cfu/g)
L1, Plastics	29.52 ± 0.16a	7.70 ± 0.14bc	203.17 ± 0.14e	7.53 ± 0.05d	72.57 ± 0.16c	2.92 ± 0.05c	4.22 ± 0.08c
L2, Bottles	29.52 ± 0.05a	7.40 ± 0.09a	167.33 ± 1.03c	1.67 ± 0.02a	75.50 ± 1.05d	2.62 ± 0.02b	6.34 ± 0.06d
L3, Metals	29.57 ± 0.10a	7.75 ± 0.10c	185.67 ± 1.63d	5.55 ± 0.02c	66.72 ± 0.12b	2.64 ± 0.02b	4.12 ± 0.18b
L4, General wastes	29.57 ± 0.08a	7.58 ± 0.10b	152.17 ± 2.14b	9.22 ± 0.02e	91.48 ± 0.15e	5.52 ± 0.05d	7.91 ± 0.05e
L5, Control	29.60 ± 0.09a	7.40 ± 0.13a	63.50 ± 1.05a	4.53 ± 0.02b	63.22 ± 0.15a	0.89 ± 0.04a	1.60 ± 0.11a

Key: L, location; pH, hydrogen ion concentration; EC, electrical conductivity; PS, particle size; TOC, total organic carbon; THB, total heterotrophic bacteria.

*Numbers with different alphabets on the same column represented values with statistical significant differences ($P<0.05$)

Table 2: Occurrence of the bacterial isolates in the various types of wastes

Bacterial Isolate	L_1P	L_2B	L_3M	L_4GW	L_5C	Total (%)
Eikelboom type 0092	1	-	-	1	1	3 (9.38%)
Vibrio spp.	1	-	-	3	1	5 (15.62%)
Escherichia coli	1	2	-	2	1	6 (18.75%)
Salmonella spp.	-	1	1	2	1	5 (15.62%)
Streptomyces spp.	1	1	1	1	-	4 (12.50%)
Streptococcus spp.	-	-	1	1	-	2 (6.25%)
Bacillus spp.	2	1	1	2	1	7 (21.88%)
Total	6	5	4	12	5	32 (100%)

Key: L_1P, Plastics; L_2B, Bottles; L_3M, Metals; L_4G.W = L_4 General waste;

L_5C = L_5 C, Control.

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Table 3: Cultural, morphological and biochemical properties of the isolates

Isolate	Gram's reaction	Cultural and Biochemical Properties						
		NA	LB	MAC	Oxi	Cit	Cat	Coa
Type 0092	- curved filaments with dashed lines,	Filamentous	Whitish, filamentous growth	No growth	-	+	+	+
Vibrio spp.	- short rods	Moist, translucent colonies	Grayish, translucent, tiny colonies	Colorless colonies	+	+	+	+
Escherichia coli	- rods	Grayish-white, smooth, opaque colonies	Grayish-white, opaque, round colonies	Pink, flat colonies	-	-	+	-
Salmonella spp.	- rods	Grayish-white, moist colonies	Tiny grayish colonies	Pale, colorless colonies	-	+	+	-
Streptomyces spp.	+ rods in filaments	Light brown filaments	White, round colonies	No growth	+	-	+	+
Streptococcus spp.	+ cocci in chains	Mucoid colonies	No observable growth	No growth	-	+	-	-
Bacillus spp.	+ rods	Grayish, granular colonies	Dry, flat, opaque and white colonies, with irregular and lobate margins	No growth	-	+	+	-

Key: - negative; + positive; NA, nutrient agar; LB, Luria Bertani; MAC, MacConkey agar; Oxi, oxidase; Cit, citrate; Cat, catalase; Coa, coagulase.

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