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Bacterial Community of Leachate from a Waste-Dump and an Adjacent Stream

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ABSTRACT: A total of 48 water samples collected from raw leachate and an adjacent stream were examined for temperature, pH and for the frequency of occurrence of bacterial isolates. The mean temperature values of the leachate ranged from 24.8°C to 28.3°C while values for the stations of the stream ranged from 23.2°C to 25.5°C. The mean pH values of the leachate ranged from pH 6.3 to 7.2 and pH values for the stream ranged from pH 6.7 to 7.9. The mean total viable aerobic heterotrophic bacterial counts for the leachate and stream ranged from, 2.5 x 10^6 CFU/ml to 6.5 x 10^6 CFU/ml and from 1.2 x 10^6 CFU/ml to 1.20 x 10^7 CFU/ml respectively. The bacteria with their frequency of isolation from the leachate and stream during the investigation are; *Bacillus* (26.08%), *Citrobacter* (4.35%), *Enterobacter* (4.35%), *E. coli* (4.35%), *Klebsiella* (4.35%), *Nieseria* (4.35%), *Pseudomonas* (4.35%), *Shigella* (4.35%), *Staphylococcus* (13.04%), *Streptococcus* (26.08%) and *Vibrio* (4.35%). However, this frequency of isolation is quite different from their frequency of isolation from each station. Only *Bacillus* sp occurred in the leachate and all the stations of the stream. *Staphylococcus* and *Streptococcus* sp occurred in the leachate and some stations of the stream. *Klebsiella* and *Shigella* occurred only in the leachate while *Citrobacter, Enterobacter, E. coli, Neiseria, Pseudomonas* and *Vibrio* species occurred only in some stations of the stream. Statistical analysis using ANOVA (F-test) showed that there is no significant difference at 5% level among the bacterial counts (population) of the raw leachate and the stations of the stream. @ *JASEM*

Human activities create vast amount of various wastes and pollutants. The release of these materials into the environment sometimes causes serious health problems. The level of wastes produced by dense human and domestic animal population often exceeds the local ecosystem's biodegradative capacity, resulting in serious environmental pollution and epidemic outbreaks of disease (Ronald, 1988). Urbanization and non-advanced patterns of consumption increases the amount of solid waste generated and the ability to manage such wastes has long been beyond the resources of most administration in developing countries (WHO, 1994; Cilinskis and Zaloksnis, 1996; Khupe, 1996; Yaliang, 1996). Refuse is seldom collected systematically outside prime residential areas and wastes are often dumped in peri urban areas in the inside of squatter settlements increasing exposure to health hazard (WHO, 1994; Moffat and Linden, 1995; Yaliang, 1996). Refuse for dumping comes primarily from household and commercial solid wastes. When refuse in placed in a dump, it undergoes a number of biological, physical and chemical changes. Degradation of organic wastes is a biological process in which organic matter is reduced to humus involving bacteria, fungi and actinomycetes all of which are widely distributed in nature or may be contained in the waste. Factors such as moisture content, pH, temperature and oxygen availability will determine the type of microbial population that prevail and the appearance and disappearance is cyclic (Yen, 1974; Pavoni *et al.*, 1975). The biological degradation of organic compounds result in the generation of gases, aliphatic acids and other liquids (Qasin *et al.*, 1970; Riley, *et al.*, 1976). The percolation of water through a dumpsite results in a solution with high concentration of both organic and inorganic compounds, which is generally referred to as Leachate. Leachate consists of decomposed organic and inorganic matter, which are subsequently mixed with water to produce effluents. The composition of leachate is highly variable in nature depending on numerous environmental factors and the characteristics of the refuse (Chem *et al.*, 1974; Yen, 1974; Pavoni *et al.*, 1975). This wide variation in the composition of Leachate even in the same general area could be explained from the standpoint of variation in the action of microorganisms in their formation (Rich, 1963; Chem *et al.*, 1974). Uncontrolled dumping of refuse into undesignated sites has been a major source of pollution both for underground and surface waters. This is as a result of direct movement of such materials into surface waters or indirectly through leaching of decomposable components of such wastes into surface and underground water. This constitutes a serious health hazard because of the presence of potential pathogens and other microbes, which such wastes contain (Foster *et al.*, 1973; Miller, 1973; Chem *et al.*, 1974; Pavoni *et al.*, 1975). The relative density of pathogens and non-pathogens present in the waste will depend on a number of complex factors, therefore, it is difficult to say with any degree of assurance what the general pathogenic and non-pathogenic character of a particular waste will be (Mallman *et al.*, 1961; Foster *et al.*, 1973; Pavoni *et al.*, 1975). The association of these microorganisms with the leachate will depend on their survival time in the soil and in the case of pathogens, the ability to survive outside their host under a variety of environmental conditions (Mallman *et al.*, 1961; Foster *et al.*, 1973; Pavoni *et al.*, 1975).

The protection of our environment is one of the most important challenges facing today's society. Waste

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management is the current environmental strategy to minimize pollution problems (Cilinskis and Zaloksnis, 1996; Khupe, 1996; Yaliang, 1996). Nigeria does not have a sanitary Landfill or solid waste treatment plant (Moffat and Linden, 1995) and waste management is generally associated with mere dumping of the collected waste at the final dump site. The ecological health of arable soils, rivers and stream around such dump sites is therefore highly threatened. Unfortunately, no information is available on the types of micro-organisms associated with leachate from waste-dump sites.

The aim of this investigation is to determine the bacterial counts (population) and the types of bacteria associated with raw Leachate and an adjacent stream and to determine the frequency of isolation of such bacteria.

MATERIALS AND METHODS

Description of the study area and sampling stations: The study area is the Eagle Island; an Island located south-west of Port Harcourt City. It is bounded on the East by Nkpolu-Oroworukwo and surrounded by Elechi Creek. It has a mangrove vegetation. It has both an industrial and residential community. The leachate-sampling site is around a waste-dump close to a stream located within Eagle Island. The waste dumpsite is the site used by the Port Harcourt City Council and the Environmental Sanitation Authority for solid waste disposal. Most of the wastes disposed are mainly domestic and household wastes. The waste-dump has an area of 4,355sq m. The stream is immediately adjacent to the waste dump and leachate effluent from the waste dump is directly discharge into the stream. The stream is shallow at low tide and its flow is unidirectional because of the blockage at the source from the Eagle Island road by-pass reclamation. The stream is continuous with Eagle Island main stream (Elechi Creek) through a culvet across the Eagle Island - Agip link road. Four sampling stations designated A, B, C and D were chosen to cover the length of the stream from the waste dump to the point where it links the culvet across the road. Station A received raw undiluted leachate from the waste dump. Stations B and C are located along the stream and received leachate as it emerged from the topsoil. Station D is also located along the stream but some distance from Station C and close to the culvet.

Collection of Samples, and Temperature and pH determinations: Sample collection was carried out fortnightly for the months of June, July and August 1995. Samples were collected in duplicate at each sampling station with well labelled sterile containers at periods of low tide. The temperature of each sample was determined immediately after collection at each sampling station using a mercury thermometer. The pH of the Leachate and Water samples from the stream was determined with the aid of a pH meter (Model No. Kem-H-112) after reciprocal shaking. A total of 48 samples were collected during the period. Samples collected on each visit were treated the same day. Before analysis, sample containers were agitated for sometime by reciprocal shaking.

Cultivation and Enumeration of Bacteria in Leachate and in water samples from the stream: Serial dilutions of the leachate and water samples were made using sterile normal saline as the diluent. Aliquots (0.1 ml) of the forth dilution (10^{-4}) of each sample was aseptically transferred onto freshly prepared nutrient agar plates and spread with a sterile bent glass rod. The inoculated plates were inverted and incubated at 37°C for 48 hrs after which colonies, which developed, were counted and recorded as total viable aerobic heterotrophic bacteria in the sample.

Isolation, characterization and Identification of Bacteria in Leachate and Water samples: Pure cultures of bacteria were obtained by aseptically streaking representative discrete colonies of different morphological types, which appeared, on the cultured plates onto freshly prepared nutrient agar plates. The plates were incubated at 37°C for 24 hrs. Discrete bacterial colonies, which developed, were further sub-cultured onto nutrient agar slopes and incubated at 37°C for 48 hrs. These served as pure stock cultures for subsequent characterization tests. The following standard characterization tests were performed in duplicates. Gram staining, Catalase, Coagulase, Sugar fermentation, Motility, Methyl red, Voges Proskauer, Indole and Citrate utilization. The pure cultures of bacterial isolates were identified on the basis of their cultural, morphological and physiological characteristics in accordance with methods described by Cruickshank *et al.*, (1975), and with reference to Holt (1977).

RESULTS AND DISCUSSION

The mean temperature (°C) and pH values of the raw leachate (Station A) and of water samples from Stations B, C and D of the stream are as shown in Table 1 and Table 2 respectively. The temperature values ranged from 24.8°C to 28.3°C in Station A, 23.5°C to 25.3°C in Station B, 23.2°C to 25.4°C in Station C and 23.3°C to 25.5°C in Station D. Generally, the temperature values for stations B, C and D ranged from 23.2°C to 25.5°C.

Table 1: Mean Temperature (°C) values of the raw leachate and stream

Sampling (bi - weekly)	Station Leachate ←	Stream		→
	A	B	C	D
1.	26.7	24.5	25.4	25.5
2.	28.3	25.3	23.2	23.4
3.	25.7	23.9	24.6	24.7
4.	24.9	23.5	23.2	23.3
5.	24.8	24.4	23.6	23.5
6.	26.7	24.7	24.5	23.8
Total	157.1	146.3	144.5	144.2
Mean	26.18	24.38	24.08	24.03

Table 2: Mean pH values of the raw leachate and stream

Sampling (bi - weekly)	Station Leachate ←	Stream		→
	A	B	C	D
1.	6.8	6.7	7.2	7.8
2.	6.9	7.3	7.5	7.4
3.	6.3	6.9	7.9	7.3
4.	7.2	7.5	7.6	7.6
5.	6.5	6.8	7.2	7.7
6.	6.6	7.2	7.5	7.5
Total	40.3	42.4	44.9	45
Mean	6.71	7.06	7.48	7.5

The pH values ranged from pH 6.3 to 7.2 in station A; pH 6.7 to 7.5 in Station B, pH 7.2 to 7.9 in Station C and pH 7.3 to 7.8 in station D. The result showed that, the temperature of the raw leachate and of all the stations of the stream studied was within the mesophilic range. However, the temperature of the leachate was higher than the temperature of the stream. The microbial activity within the dump, which resulted in the formation of the leachate, may have resulted in the elevated temperatures of the leachate. The present investigation also showed that, the degree of acidity (pH) for the raw Leachate (Station A) is slightly acidic while those of the stream ranged from slightly acidic to basic in station B and basic or alkaline in stations C and D. The order of decrease in acidity (i.e. increase in pH) among the stations is Station A > B > C > D. The products of microbial activity within the dump must have resulted in the acidic nature of the raw leachate in the present investigation. According to Qasin *et al.*, (1970) and Riley *et al.*, (1976), anaerobic decomposition within a dump yields large amount of aliphatic acids in a raw leachate.

The passage of the leachate in the present investigation through soils in stations B, C and D coupled with dilution of water of the stream may have resulted in the observed increase in pH of these stations of the stream.

Riley *et al.*, (1976) also stated that, passage of leachate through soil stripped the odorous acids resulting in a substantial rise in pH. The mean values of total aerobic heterotrophic bacteria in raw leachate and in water samples of the stream are as shown in Table 3. The mean values per ml of the samples ranged from 2.5 x 10^6 CFU to 6.5 x 10^6 CFU in Station A, (Leachate) 2.4 x 10^6 CFU to 7.8 x 10^6 CFU in Station B, 2.1 x 10^6 CFU to 7.3 x 10^6 CFU in Station C, and 1.2 x 10^6 CFU to 1.20 x 10^7 CFU in Station D, Station C recorded the highest total mean value of 4.5 x 10^6 CFU while Station B recorded the lowest total mean value of 4.18 x 10^6 CFU during the sampling period. Statistical analysis of the data obtained using ANOVA (F-test) gave a correction term (C.T) value of 45153.375 and calculated F-value of 0.0160. This shows that samples from all the stations have high counts of total viable aerobic heterotrophic bacteria indicating that the leachate and the stream were generally rich in available organic matter. The mean total viable aerobic heterotrophic bacteria was highest in station C and lowest in station B. The order of decreasing mean total viable heterotrophic bacteria among the stations is; Station C > A > D > B.

Table 3: Mean Densities of Aerobic Heterotrophic Bacteria (x 10^6 CFU/ML) in the Leachate and Stream, Samples

Sampling	Station Leachate ←	Stream		→
	A	B	C	D
1.	2.7	2.4	3.4	4.0
2.	3.7	4.5	4.2	12.0
3.	2.5	2.5	7.3	4.0
4.	6.5	7.8	2.1	2.2
5.	6.0	4.7	5.0	1.2
6.	4.7	3.2	5.0	2.5
Total	26.1	25.1	27.0	25.9
Mean	4.35	4.18	4.5	4.31

The acidic nature of Station A as observed in the present investigation must have exerted a bacteriostatic effect on the bacteria in Station A (i.e. leachate).

In addition to the slightly acidic to alkaline nature of Station B, it received leachate as it emerged from the top soil. Passage of the leachate through the top soil may also have stripped it of some bacteria. That is, bacteriostatic and filterable effects must have resulted in Station B recording the lowest total bacterial count among the stations.

On the other hand, though station C also received leachate as it emerged from the top soil, the alkaline or basic pH range of station C and D must have favoured the proliferation of bacteria in these stations. The counts of total aerobic heterotrophic bacteria for stations A and D were close. The difference may be due to distance from the waste-dump site and dilution by the stream water. Statistical analysis of the data obtained using analysis of

variance - ANOVA (F-test), showed that there is no significant difference in the bacterial counts among the stations at 5% level. This supports the argument of Oppenheimer *et al.*, (1980) that numerical data without statistical analysis is sufficient to analyse changes in a dynamic system such as aquatic environments as investigated in this study.
The types of bacteria with their frequency of isolation (indicated as percentages of total viable aerobic heterotrophic bacteria) in the leachate and water samples of stations from the stream are as shown in Table 4.

Table 4: Types of bacteria with frequency of isolation from the raw leachate and the stream

Type of Bacterium	Station Leachate ← Stream →				Frequency of Isolation during the investigation
	A	B	C	D	
Bacillus sp	16.6	25	25	40	26.08
Citrobacter sp	0	12.5	0	0	4.35
Enterobacter sp	0	0	0	20	4.35
E. coli	0	0	0	20	4.35
Klebsiella sp	16.6	0	0	0	4.35
Neiseria sp	0	0	25	0	4.35
Pseudomonas sp	0	0	0	20	4.35
Shigella sp	16.6	0	0	0	4.35
Staphylococcus sp	33.3	12.5	0	0	13.04
Streptococcus sp	16.6	50	25	0	26.08
Vibrio sp	0	0	25	0	4.35

The frequency of isolation of the bacterial isolates obtained during the investigation ranged from 0 to 33.3% in Station A, 0 to 50% in Station B, 0 to 25% in Station C and 0 to 40% in station D. Generally, the types of bacteria and their frequency of isolation during the investigation are *Bacillus* sp (26.08%), *Citrobacter* sp (4.35%), *Enterobacter* sp (4.35%), *E. coli* (4.35%), *Klebsiella* sp (4.35%), *Neiseria* sp (4.35%), *Pseudomonas* sp (4.35%), *Shigella* sp (4.35%), *Staphylococcus* sp (13.04%), *Streptococcus* sp (26.08%) and *Vibrio* sp (4.35%). The present investigation showed the type of bacteria and their frequency of isolation from the leachate and stream. The various bacteria isolated include species known to be involved in the degradation of organic matter. Among the bacteria isolated, *Staphylococcus* sp (33.3%), *Streptococcus* sp (50%) and *Bacillus* sp (40%) had the highest frequency of isolation in Stations A, B, and D respectively. However, *Bacillus* sp, *Neiseria* sp, *Streptococcus* sp and *Vibrio* sp had an equal frequency of isolation (25%) in Station C. Generally, *Bacillus* and *Streptococcus* species had the highest frequency of isolation (26.08%). However, *Bacillus* sp was isolated in all the stations while *Streptococcus* sp was isolated from stations A, B and C. *Staphylococcus* sp was also isolated in stations A and B. That is, this investigation has shown that, *Bacillus* sp, *Staphylococcus* sp, and *Streptococcus* sp occurred in the leachate and in the stream. *Klebsiella* sp and *Shigella* sp occurred only in station A (i.e. the leachate). *Citrobacter* sp occurred only in station B while *Neiseria* sp and *Vibrio* sp occurred only in station C. On the other hand, *Enterobacter* sp, *E. coli*, and *Pseudomonas* sp occurred only in station D. The frequency of occurrence of the bacterial isolates in the various stations showed that, the bacteria must have originated from the leachate. Cook *et al.*, (1964) reported *Bacillus* sp, *E. coli* and *Streptococcus* sp to be associated with leachate. Foster *et al.*, (1973) reported, *Shigella* sp and *Vibrio* sp and Ekundayo (1977) reported *Bacillus* sp, *Enterobacter* sp, *E. coli*, *Pseudomonas* sp, *Streptococcus* sp and *Vibrio* sp. The present investigation has also shown that environmental factors such as temperature, pH and passage of leachate through soil must have also influenced the distribution or occurrence of the bacteria in the stations. The presence of *Klebsiella* sp and *Shigella* sp in the leachate and their absence in the stream may be due to their survival time and the passage of leachate through soil which resulted in the absence of these organisms in the stream. The proliferation of *Bacillus* sp, *Citrobacter* sp, *Staphylococcus* sp and *Streptococcus* sp continued even after the passage of leachate through soil in Station B. On the other hand, the high temperatures recorded in Station A and the slightly acidic conditions of Stations A and B must have had a bacteriostatic effect on the proliferation of *Enterobacter* sp, *E. coli*, while the cooler and more alkaline conditions in Stations C and D of the stream favoured their proliferation. Shuval *et al.*, (1981) reported that there is a regrowth of enteric bacteria in the cooler exterior of the dump so that a population of pathogenic organisms continues to survive. The present investigation shows that serious health hazards could result from the contamination of aquatic environment by leachate. All the bacteria (with the exception of *Citrobacter* sp) isolated during this investigation have been reported by Cook *et al.*, (1964) and Monica Cheesborough (1985) as potential pathogens. The presence of these potential pathogens in the leachate and stream during the present investigation may be attributed to the disposal of raw human faecal discharges and other human wastes at the waste-dump site of the leachate. It has also been reported that truly pathogenic forms or microorganisms may survive in waste or leachate (Mallman *et al.*, 1961; Cook *et al.*, 1964; Foster *et al.*, 1973; Pavoni *et al.*, 1975; Ekundayo, 1977). It was also reported by Ekundayo (1977) that the access of faecal matter to water may add a variety of intestinal pathogens and that the most easily and frequently isolated include *E. coli*, *Salmonella*, *Shigella* and *Vibrio* species. From the results of the present investigation, the health hazards associated with the contamination of aquatic environment by untreated solid waste or leachate cannot therefore be under-estimated.

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