JASEM ISSN 1119 - 8362

J. Appl. Sci. Environ. Mgt. 2002
Vol. 6 (1) 78 - 83

Microbial Community of a Waste- Dump Site

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ABSTRACT: A total of 48 soil samples were collected fortnightly in the months of June, July and August 1995, from four different stations of a waste-dump site. The samples were examined for temperature, pH and for the frequency of isolation of viable aerobic heterotrophic bacteria and fungi. The mean temperature values of the soils ranged from 27°C to 28°C while the mean pH values ranged from pH 5.4 to 7.9. The mean total viable aerobic hetertrophic bacteria population ranged from 0.38×10^6 CFU/g soil to 2.00×10^6 CFU/g soil while the mean total viable fungal population ranged from 1.9×10^4 CFU/g soil to 7.1×10^4 CFU/g soil. The bacteria with their frequency of isolation from the waste-dump soils were: *Arthrobacter (4.7%), Bacillus (15.2%), Escherichia coli* (12.1%), *Klebsiella* (9.6%), *Micrococcus* (2.5%), *Proteus* (10.2%), *Pseudomonas* (5.4%), *Serratia* (2.5%), *Staphylococcus* (21%) and *Streptococcus* (16.8%). Only *Bacillus, E. coli, Staphylococcus* and *Streptococcus* were isolated from all the stations. The fungi with their frequency of isolation were; *Aspergillus* (25.3%), *Fusarium* (5.4%), *Mucor* (11.5%), *Penicillium* (12.6%), *Rhizopus* (2.5%) and *Saccharomyces* (42.8%). All the fungi were isolated from all the stations. Statistical analysis using ANOVA (F – test) showed that there were no significant differences in the bacterial and fungal populations between the four stations. However, there was significant difference at 5% level for fungal populations between different sampling periods. @ *JASEM*

Waste is any substance, solution, mixture or article for which no direct use is envisaged but which is transported for reprocessing, dumping, elimination by incineration or other methods of disposal (Yakowtz, 1988).

With urban industrialization, social development and population increases, solid waste production are growing rapidly, making garbage pollution a serious problem (Khupe, 1996; Yaliang, 1996).

A waste is said to be hazardous if it is infectious, meaning containing viable microorganisms or their toxins which are known or suspected to cause disease in animal or human (Yakowitz, 1988). Waste disposal poses threat to both man, animals and the soil. Like chemical hazards, aetiologic agents might be dispersed in the environment through water and wind. Poisonous plants, insects, animals and indigenous pathogens are biologic hazards that might be encountered at the waste site (Khupe, 1996).

Municipal solid waste generation in Port Harcourt, Nigeria (approximate 96,000 tons yr^{-1}) in an order of magnitude higher than industrial solid-waste generation. Port Harcourt does not have a sanitary landfill (Moffat and Linden, 1995). The composition of municipal solid waste in Port Harcourt is food waste, paper cardboard, faeces, screening residual, plastics, broken bottles, batteries, textiles, bones, glass, wood and leaves, ferrous metal, leather and rubber, non-ferrous metal, concrete, and ceramic and hazardous waste.

Waste management in developing countries is usually equated with land disposal or discharge into bodies of water (Cilinskis and Zaloksnis, 1996). This method of waste management is unscientific and causes nuisance to the public and constitute pollution and health hazards.

When waste is dumped on land, soil microorganisms including fungi and bacteria, readily colonise the waste carrying out the degradation and transformation of degradable (organic) materials in the waste (Stainer *et al.*, 1989). Microorganisms in waste dump use the waste constituents as nutrients. Thus detoxifying the materials as their digestive processes breakdown complex organic molecules into simpler less toxic molecules (Pavoni *et al.*, 1975). This metabolic activity can be attributed to their high growth rate, metabolism, and their collective ability to degrade a vast variety of naturally occurring organic materials (Stainer *et al.*, 1989).

Port Harcourt City does not have a sanitary landfill. Improper disposal of untreated municipal solid wastes is not only harmful to human's health but also constitute a threat to ecological environment (Yaliang, 1996). The future benefits of intervening are commensurably high (Moffat and Linden, 1995). In Nigeria, little information is available, on the types of microorganisms associated with waste dump sites. There is therefore the need to isolate, characterize and identify the types of bacteria and fungi associated with a waste dump site. The aim is to assess the potential health hazard that could result from indiscriminate dumping of waste around residential areas.

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MATERIALS AND METHODS

Description of the study area

The study area was the Eagle Island, an Island located south-west of Port Harcourt City. It is bounded on the East by Nkpolu-Oroworukwo (at the University of Science and Technology) and surrounded by Elechi Creek. It has a mangrove vegetation. It is both an industrial and Residential area. The sampling site was a dump located within Eagle Island. It is a waste dump site used by the Port Harcourt City Council and Environmental Sanitation Authority for solid waste disposal. Most of the waste disposed were mainly domestic and household wastes. The waste dump had an area of 4,355 sq. m. Sampling stations were established on the waste dump site and were represented as stations A,B,C, and D. Stations A and D were close to the stream while stations B and C were away from the steam. A map of the study area is shown in Figure 1.

Collection and Treatment of Soil Samples

Sample collection was carried out fortnightly for the months of June, July and August, 1995. A total of 48 samples were collected during the six (6) sampling periods. At each sampling station, the surface debris was removed and subsurface soil dug to a depth of about 5cm were scooped from one foot square area into sterile duplicate sampling bottles and appropriately labeled. Eight samples, two from each site, were collected on each visit.

The temperature of each sample was determined immediately after collection at each station using a thermometer, after which the samples were transported to the laboratory. Samples were treated within 2 hours after collection.

Soil samples were air-dried and sieved through a 0.2mm wire mesh to obtain fine soil particles (U.S. EPA, 1978). Ten grams (10g) of each air-dried fine soil sample was mixed in a test-tube containing 25ml of sterile distilled water using a sterile glass rod, and pH was determined with a pH meter (Model No 7020. Electronic Instrument Ltd., Kent).

Cultivation and Enumeration of Bacteria

Each sample (10g) of previously air-dried fine soil was thoroughly shaken in 10ml of normal saline. An liquot (1.0ml) was transferred into the next test tube and diluted serially in one-tenth stepwise to 10^{-5} dilution (Paul and Clark, 1988). From the dilution of 10^{-4} of each soil sample, 0.1ml aliquot was transferred aseptically onto freshly prepared Nutrient agar plates and spread with a sterile bent glass rod (Paul and Clark, 1988; Harrigan and McCance, 1990). The dilution of 10^{-4} was used in plating for bacteria because the dilution of 10^{-3} gave a confluent growth while 10^{-5} gave fewer growth. The inoculated plates were inverted and incubated at 37°C for 24 - 48 hrs after which the plates were examined for growth. The discrete colonies which developed were counted and the average counts for duplicate cultures were recorded as total viable aerobic heterotrophic bacteria in the sample.

Isolation, Characterization and Identification of Bacteria in the Waste Dump Site

Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types which appeared on the cultured plates onto freshly prepared Nutrient agar plates which were incubated at 28°C for 24 hrs. Discrete bacteria colonies which developed were sub-cultured on Nutrient agar slopes and incubated at 28°C for 24hrs. These served as pure stock cultures for subsequent characterization tests. The following standard characterization tests were performed in duplicates: Gram staining, catalase test, coagulate test, sugar fermentation test, motility test, methyl red test Voges-Proskauer test, Indole test, and Citrate utilization test. The pure cultures 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).

Cultivation and Enumeration of Fungi in the Waste Dump Site

Each sample (10g) of previously air-dried fine soil was thoroughly shaken in 10ml of sterile distilled water. Aliquot (1.0ml) of it was transferred into the next test tube and diluted serially in one-tenth stepwise to 10^{-4} dilution. From the dilution of 10^{-3} of each soil sample, 0.1ml aliquot was transferred aseptically onto freshly prepared Sabouraud's dextrose agar plates to which 0.2ml of 0.5% Ampicillin has been added to inhibit the growth of bacteria and allowing the growth of fungi (Harrigan and McCance, 1990). The inoculum was spread with a sterile bent glass rod. The dilution of 10^{-3} was used in plating for fungi because the dilution of 10^{-4} gave fewer growth. The inoculated plates were inverted and incubated at 28°C (room temperature) for 5 to 7 days. The colonies which developed were counted and the average count for duplicate cultures were recorded as total viable fungi in the sampe.

Isolation, Characterization and Identification of Fungi in the Waste Dump Site

Pure cultures of fungi were obtained by subculturing discrete colonies onto freshly prepare Sabouraud's dextrose agar plates and inoculated at 28°C for 5 to 7 days. The fungal isolates which developed were further subcultured onto agar slopes and incubated at 28°C for 5 to 7 days. The isolates which developed were pure cultures which were stored in the refrigerator as stock cultures for subsequent characterization test. The following standard characterization tests were performed in duplicates: Macroscopic examination of fungal growth was carried out by observing the colony morphology – Diameter, colour (Pigmentation), texture and surface appearance. Microscopic examination was done by needle mount method (Harrigan and McCance, 1990) and observing sexual and asexual reproductive structures like sporangia, conidial head, arthrospores and the vegetative mycelium. Sugar (Glucose, Fructose, Lactose, Sucrose, Galactose, Maltose and Mannose) fermentation test were also carried out for species identification.

The complete identification of fungal isolates was done by comparing the result of their cultural, morphological and biochemical characteristics with those of known taxa (Haley and Callaway, 1978; Olds, 1983).

RESULTS AND DISCUSSION

The mean temperature (°C) and P^H values of soil samples of the four stations on the dump site are as shown in Table1 and 2 respectively

Table 1: *Mean Temperature (°C) values of soil of the four stations on the waste-dump site.*

	STATION			
Sampling (bi-weekly)	A	B	C	D
1.	27	27	28	27
2.	27	28	28	27
3.	28	28	27	28
4.	27.5	27	28	28
5.	28	28	27.5	28
6.	28	28	27.85	28
TOTAL	165.5	166	166	166
MEAN	27.58	27.66	27.66	27.66

Table 2: Means pH values of soils of the four stations on the waste-dump site.

	STATION			
Sampling (bi-weekly)	A	B	C	D
1.	7.7	6.8	5.9	7.8
2.	6.5	7.9	7.3	7.6
3.	7.3	6.5	7.8	6.5
4.	7.9	5.9	6.8	7.9
5.	7.4	7.7	5.4	6.3
6.	6.8	6.3	7.9	6.9
TOTAL	43.6	41.1	41.1	43
MEAN	7.26	6.85	6.85	7.16

Generally, the temperature values ranged from 27°C to 28°C in all the stations. The mean temperature values for station A was 27.5 while mean values for stations B, C and D was 27.6.

The pH values ranged from pH 6.5 to 7.9 in Station A; pH 5.9 in Station B; pH 5.4 to 7.9 in Station C and pH 6.3 to 7.9 in Station D. The mean pH for stations A,B,C and D. during the sampling period was 7.2, 6.8, 6.8, and 7.1 respectively.

The temperature of the soil samples from Eagle Island waste dump site ranged from between 27°C and 28°C. The falls within the mesophilic range of temperatures which is between 20°C and 45°C. Most microbial species are mesophilic.Hagerty *et al.*, (1973) reported that, during initial composting development, the mesophilic flora predominate and are responsible for most of the metabolic activities that occurs. This increased microbial activity elevates the temperature of the compost, with the subsequent replacement of mesophilic population by thermophilic flora such as *Bacillus, Aspergillus,* and *Mucor* reported in this study. The degree of acidity (pH), reported in this study for all the stations of the waste dump site ranged from between pH 5.4 and 7.9. Hagerty *et al.*, (1973) reported that, the initial pH of solid waste is between pH 5.0 and 7.0 for refuse which is about 3 days old. In the first 2 to 3 days of composing, the pH drops to 5.0 or less and then begins to rise to about 8.5 for the reminder of the aerobic process (Pavoni *et al.*, 1975).

The present investigation reveals the degree of acidity (pH) of the soil samples obtained from the waste dump site. According to the soil classification by Odu *et al.*, (1985) the degree of acidity for soil from stations A and D ranged from slightly acidic to basic while the soil from stations B and C ranged from moderately acidic to basic.

The mean value of total aerobic heterotrophic bacteria per gram soil ranged from 0.42 x 10^6 CFU to

1.60 x 10^6 CFU in station A, 0.50 x 10^6 CFU to 1.46 x 10^6 CFU in Station B, 0.38 x 10^6 CFU to 1.25 x 10^6 in station C, and 0.56 x 10^6 CFU to 2.00 x 10^6 CFU in station D. Station D recorded the highest total number of 6.75 x 10^6 CFU while Station B recorded the lowest total number of 5.12 x 10^6 CFU during the sampling period.

The mean value of total viable fungi per gram soil ranged from 1.9 x 10^4 CFU to 7.1 x 10^4 CFU in Station A, 2.5 x 10^4 CFU to 3.9 x 10^4 in Station B, 2.1 x 10^4 CFU to 4.8 x 10^4 CFU in Station C, and 2.3 x 10^4 CFU to 5.2 x 10^4 CFU in Station D. Station A recorded the highest total number of 2.50 x 10^5 CFU while Station C recorded the lowest total number of 2.22 x 10^5 CFU during the sampling period.

The types of bacteria and their frequency of isolation (indicated as percentages of total viable heterotrophic bacterial count) in the four stations of the waste dumpsite are as shown in Table 3.

Table 3: Types of bacteria with frequencies of isolation (indicated as Percentages of total viable heterotrophic bacteria) in the four stations on the waste-dump site.

	STATION				
Type of Bacterium	A	B	C	D	A+B+C+D/ 4 (mean)
Arthrobacter sp.	9.09	10	0	0	4.77
Bacillus sp.	9.09	10	25	16.16	15.19
Escherichia coli	9.09	10	12.5	16.67	12.07
Klebsiella sp.	9.09	0	12.5	16.67	9.56
Micrococcus sp.	0.0	10	0	0	2.5
Proteus sp.	18.18	10	12.5	0	10.17
Pseudomonas sp.	9.09	0	12.5	0	5.4
Serratia sp	0	10	0	0	2.5
Staphylococcus aureus	9.09	0	12.5	16.67	9.56
Staphylococcus sp	9.09	20	0	16.67	11.44

The frequency of isolation of the bacterial isolates ranged from 0% to 18.18% in Station A, 0% to 20.00% in Station B, 0% to 25.00% in Station C, and 0% to 33.34% in Station D. Among the bacteria isolated, only *Bacillus* spp., *Escherichia coli, Staphylococcus* spp. and *Streptococcus* spp. were isolated from all the stations.

The types of fungi and their frequencies of isolation (indicated as percentages of total viable fungal count) in the four stations of the waste dumpsites are as shown in Table 4.

Table 4: Types of fungi-moulds and yeast,with frequencies of isolation (indicated as Percentages of total viable fungi) in four stations on the waste-dump site.

	STATION				
Type of Fungus	A	B	C	D	A+B+ C+D/4 (mean)
Aspergillus flavus	4.4	11.5	4.8	7.2	6.975
Aspergillus fumigatus	27.6	8.9	11.7	9.0	14.3
Aspergillus niger	4.0	4.2	3.7	4.1	4.0
(Total for Asp. Spp.)	**36.0**	**24.6**	**20.2**	**20.3**	**25.275**
Fusarium sp.	5.6	5.2	5.3	5.4	5.375
Mucor sp.	14.0	13.1	7.5	11.2	11.45
Penicillum sp.	8.4	11.0	17.0	14.0	12.6
Rhizopus sp.	3.2	2.6	2.1	2.2	2.525
Saccharomyces sp.	12.0	14.7	21.3	21.2	17.3
Saccharomyces sp.	20.8	28.8	26.6	25.7	25.48
(Total for *Sacch.* Spp.)	32.8	43.5	47.9	46.9	42.775

Generally, all the fungal isolates occurred in all the stations and their frequencies ranged from 3.2% to 36.0% in Station A, 2.6% to 43.5% in Station B, 2.1 to 49.9% in Station C and 2.2% to 46.9% in Station D.

The total viable aerobic bacterial count was highest in station A, and lowest in station B. The order of decrease in the bacterial counts in the stations was A>D>C>B. The total viable fungal count was also highest in station A, but lowest in station C. The order of decrease in fungal counts in the stations was A>D>B>C. Generally the bacterial counts were higher than the corresponding fungal counts in all the stations. This is not surprising since the degree of acidity (pH) reported for the soil (pH 5.4 to 7.9) would favour the proliferation of bacteria than that of fungi. Other factors, which affect the microbial population of soil, include amount and type of nutrient, temperature, and pH of the soil (Marshall and Devinny, 1988). The bacterial and fungal counts were higher in stations A, and D than in stations B, and C. Stations A and D are close to the stream while stations B and C are away from the stream. Being that the study was carried out in the months of June, July, and August, (rainy season), organisms may have been washed from stations B and C towards stations A and D and into the stream due to the waste dump sloping to the stream.

The present investigation has also shown that seasonal influence can affect microbial proliferation. The second sampling in the month of June (which

had a mean monthly rainfall value of 147.0mm) which is the beginning of the heavy rains, recorded the lowest number of total viable aerobic heterophic bacteria (2.67×10^6) but recorded the highest number of total viable fungi (2.10×10^5) for all the stations, while the fourth and sixth samplings carried out in the months of July and August (which had mean monthly rainfall value of 318-517mm) considered as peak of the rainy season, recorded the highest number of total viable aerobic heterotrophic bacteria (5.07×10^6) and the lowest number of total viable fungi (9.4×10^4) for all the stations, respectively. This showed that, bacteria and fungi of the waste dump site respond differently to seasonal influence. Variations of climatic conditions such as distinct wet and dry seasons, may selectively favour certain physiologjical types (Marshall and Devinny, 1988). Statistical analysis, using analysis of variance. (ANOVA) for the data obtained in the present investigation showed that, there was no significant difference in the number of bacteria and fungi at 5% level among the four stations of the waste dumpsite. There were however significant differences in the number of fungi among the different sampling periods (season) at 5% level.

The present study shows the types of bacteria and fungi and their frequency of isolation from the waste dump site in Eagle Island. The bacteria isolated from the dump site include, *Arthrobacter* spp., *Bacillus* spp., *Escherichia coli, Klebsiella* spp., *Micrococcus* spp., *Proteus* spp., *Pseudomonas* spp., *Serratia* spp., *Staphylococcus aureus, Staphylococcus* spp., and *Streptococcus* spp. Only *Bacillus, Escherichia coli, Staphylococcus,* and *Streptococcus* were isolated from the stations while *Micrococcus* spp. and *Serratia* sp. were isolated only in station B. The other bacterial isolates occurred in two or three stations. Of the different genera of bacteia isolated from the waste dump site, *Proteus, Staphylococcus* and *Streptococcus* had the highest frequency of isolation in station A, *Staphylococcus* and *Streptococcus* in station B, *Bacillus* in station C, and *Staphylococcus* in station D.

The order of decreasing frequency of isolation is *Staphylococcus* > *Streptococcus* > *Bacillus* > *Micrococcus* and *Serratia.*

All the bacterial isolates reported in this study have been reported to be associated with waste and waste biodegradation. Faecal coliforms and streptococci have been reported to be associated with waste (Ekundayo, 1977). *Arthrobacter, Bacillus* and *Pseudomonas* species were reported by Gray (1967); to be associated with waste. *Bacillus, E. coli, Klebsiella,* and *Pseudomonas* were also reported by Cook *et al.,* (1964). Liu and Chen (1980) reported *Enterobacter, E. coli* and *Serratia* among others. Ekundayo (1977) reported *Bacillus, E. coli, Proteus, Pseudomonas, Micrococcus, Serratia, Staphylococcus, Streptococcus* among others. *Pseudomonas* has been widely reported to be associated with waste (Sabry,1992)

The fungi isolated from the Eagle Island waste dump site include, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium,* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp. and two different yeast species which are both *Saccharomyces* spp. Generally, *Rhizopus* spp. recorded the lowest frequency of isolation in all the stations. *Aspergillus* recorded the highest frequency of isolation, in station A while *Saccharomyces* recorded the highest frequency of isolation in station B, C and D. Among the moulds, *Aspergillus* recorded the highest frequency of isolation in each station, followed by *Penicillium* in station C and D; while *Mucor* recorded the second highest frequency of isolation in stations A and B The order of decreasing frequency of isolation of fungal genera in all station is, *Saccharomyces* > *Aspergillus* > *Pencillium* > *Mucor* > *fusarium* > *Rhizopus. Aspergillus, Fusarium, Mucor, Penicillium Rhizopus* and a variety of yeasts have been reported to be associated with waste biodegradation (Ekundayo, 1977). *Aspergillus* and *Saccharomyces* were reported by Ikpedu (1980), while *Saccharomyces* and *Rhizopus* were reported by Sanni (1980). *Aspergillus fumigatus* recorded the highest frequency of isolation among the *Aspergillus* species isolated from the waste-dump site. Thomas (1973) reported that *Aspergillus fumigatus* is the most commonly recovered species among the *Aspergillus* species recovered species from clinical forms of aspergilliosis.

The present investigation has revealed the presence of various bacteria, fungi and yeast known to be associated with waste biodegradation and their frequency of isolation. The activities of these bacteria, fungi and yeast if properly harnessed can be used in future treatment plants in Nigeria in accelerating the bioconversion of waste compost into organic fertilizer for use in gardening, agriculture and horticulture.

All the bacterial genera reported in this study with the exception of *Arthrobacter* have been reported by Cook *et al* (1964) and Monica Chesborough (1985) as potential pathogens. That is, they are capable of causing disease. Also, all the fungal genera reported in this study with the exception of *Penicillium* are potential pathogens (Thomas, 1973; Manson-Bahr and Apted, 1982). Pavoni *et al* (1975) reported that truly pathogenic forms may survive in waste. The

presence of these potential pathogens reported in the present investigation may be attributed to the disposal of raw human faecal discharges and other human wastes at the waste-dump site.

The health hazard associated with the indiscriminate dumping of waste around residential areas and other ecologically sensitive areas such as rivers and streams and arable land cannot therefore be under-estimated.

Nigeria should therefore direct her efforts towards the treatment of waste before disposal as to minimize the health hazards associated with dumping of waste.

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