International Journal of Microbiology and Mycology | IJMM
pISSN: 2309-4796
http://www.innspub.net
Vol. 1, No. 1, p. 23-30, 2013
Open Access RESEARCH PAPER

Microbiological and physicochemical analyses of top soils obtained from four municipal waste dumpsites in Benin City, Nigeria

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Keywords: Microbiological, physico chemical, municipal waste dumpsites, soils, Benin City.

Publication date: September 10, 2013

Abstract

Several methodologies were utilized to evaluate the microbiological and physico chemical properties of top soil samples bored from four municipal waste dumpsites and a farmland (control sample) all located in Benin City, Edo State. The soil samples were obtained during the month of January, 2013. The mean aerobic bacterial counts for the soil samples ranged from 9.7×10^3 cfu/g for the control soil to 1.80×10^4 cfu/g for the soil sourced from the dump site at Ikheuniro. The mean heterotrophic fungal counts varied from 7.0×10^2 cfu/g for capitol dumpsite to 3.3×10^3 cfu/g for the control soil. Ten (10) microbial isolates were characterized and identified; *Bacillus* sp., *Pseudomonas* sp., *Aeromonas* sp., *Enterobacter* sp., *Klebsiella* sp. and *Staphylococcus* sp., *Aspergillus* sp., *Mucor* sp., *Saccharomyces* sp. and *Fusarium* sp. respectively. Both *Bacillus* sp. and *Pseudomonas* sp. were the most dominant amongst the bacterial isolates whilst *Staphylococcus* sp. was the least occurring bacterial isolate. *Aspergillus* sp. was the highest occurring fungal isolate while the least isolated fungal culture was *Saccharomyces* sp. The physico chemical results showed values which ranged from 5.60 to 8.08, 164.00 µS/cm to 540.00 µS/cm, 2.378 mg/kg to 3.444 mg/kg, 0.009 mg/kg to 0.016 mg/kg for pH, electrical conductivity, sulphate and cadmium. Despite the positive impacts of the dumped municipal wastes on the microbial and organic properties of the analyzed soils, disposal of municipal wastes in open dump sites is an archaic and unsustainable option in the management of municipal wastes.

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Introduction

The disposal of domestic, commercial and industrial garbage in the world is a problem that continues to grow with human civilization and no method so far is completely safe. Experience has shown that all forms of waste disposal have negative consequences on the environment, public health, and local economies (Abduls-Salam, 2009). Solid wastes are sources of environmental pollution through introduction of chemical substances above their threshold limit into the environment (Obasi *et al.*, 2012). Dumpsite is an old traditional method of waste disposal similar to landfill method of waste management. Dumpsites are often established in disused quarries, mining or excavated pits away from residential areas (Abduls-Salam, 2009). Designated government agency, corporate bodies and some individuals collect wastes routinely into these dumpsites (Abduls-Salam, 2009). In Benin City in particular and in Nigeria in general, modern landfill facilities are not found in these municipal dumpsites, hence the sorting-out of solid wastes into degradable, non-degradable and recyclable precious materials cannot be achieved. Poor management of dumpsites could create a number of adverse environmental impacts, including wind-blow litter, attraction of mice and pollutants such as leachate, which can pollute underground soil bed, and / or aquifer (Abduls-Salam, 2009). Landfill gas mostly composed of methane and carbon (IV) oxide is produced through biodegradation of such waste (Abduls-Salam, 2009). Leachate from dumpsites is of particular interest when it contains potentially toxic heavy metals. These metals are known to bio accumulate in soil and have long persistence time through interaction with soil component and consequently enter food chain through plants or animals (Dosumu, 2003). Household and industrial garbage may contain toxic materials such as lead, cadmium, mercury, manganese from batteries, insect sprays, nail, polish, cleaners, plastics polyethylene or PVC (polyvinyl chloride) made bottles and other assorted products (Abduls-Salam, 2009). Soil microorganisms can degrade organic contaminants, while metals need immobilization or physical removal because metals at higher concentrations are toxic and can cause oxidative stress by formation of free radicals (Henry, 2000) and thus may render the land unsuitable for plant growth and destroy the biodiversity. When waste is dumped on land, soil microorganisms including fungi and bacteria, readily colonize 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). However, municipal solid wastes are known to contain large amount of persistent organic pollutants (Minh *et al*., 2006). The concentrations and transformations of heavy metals in solid municipal wastes lead to accumulation in the food web (Gimmler *et al*., 2002). The municipal waste dump sites in Benin City are of environmental interest because of the closeness of these dumpsites to residential houses as a consequence of urbanization and the lack of pre-classification and sorting-out of wastes prior to disposal. The aims of this study were to evaluate the microbial and physicochemical qualities of top soils collected from four municipal waste dumpsites located at several areas in Benin City, Edo State, Nigeria.

Materials and methods

Sample collection

Top soil samples were collected from four dumpsites in Benin City. The sites were; Bypass (Ikhueniro), NITEL, NIFOR (CBN) and Capitol. Two of these municipal dumpsites; Bypass (Ikueniro) and NIFOR (CBN) are approved and managed by the Edo State Ministry of Environment. A control soil was obtained from a farmland in the Ugbowo Campus of the University of Benin, Benin City.

The top soil samples were collected at a depth of 2 cm - 20 cm with the aid of a soil auger. Prior to sampling the surface debris of the soils were removed. At each boring about 100 g of the respective soils were obtained and the soils were collected in duplicates. All the soil samples were obtained during the month of January, 2013 and the samples were dispensed into sterile containers and labeled.

Enumeration and isolation of heterotrophic soil microflora using general purpose media

One (1) gram of the respective fresh soil samples were weighed and dissolved into 99 ml of sterile prepared peptone water diluent under aseptic conditions (Harley and Prescott, 2002). Serial fold dilutions were then made up to 10^{-6} and aliquots of each dilution were cultured on plates of Nutrient Agar for mean heterotrophic bacterial count and Potato Dextrose Agar (PDA) for mean heterotrophic fungal count respectively by pour plate method (Aneja, 2003). Prior to pouring, all the respective prepared culture media were autoclaved at 121°C for 15min. Plating was done in duplicates and the culture plates were swirled, allowed to solidify and incubated at 35^0C for 48 hr and ambient room temperature (28 ± 2^0C) for 5 days in respect of the mean aerobic bacterial and heterotrophic fungal counts respectively.

Characterization of the soil microbiota

Unique representative bacterial and fungal colonies were sub-cultured on freshly prepared nutrient agar and potato dextrose agar plates. These plates were incubated at 35°C for 24 hr and room temperature (28 ± 2^0 C) for 3 days for bacterial and fungal cultures respectively. The colonial characteristics of the sub-cultured bacterial colonies were recorded. The bacterial isolates were further identified by the identification schemes of Holt *et al*. (1989) and Aneja (2003). Isolated bacteria were also cultured on nutrient agar slants and stored at 2^0 C. The sub cultured fungal isolates were identified on the basis of their morphological and microscopic features.

Their microscopic attributes were examined using the wet mount technique (Sharma, 2009). Both lactophenol cotton blue and distilled water were used respectively as mountants (Enabulele and Obayagbona, 2013). The microscopic structures observed were recorded and compared to illustrations stated by Barnett and Hunter (1972). The fungal isolates were also transferred to potato dextrose agar slants and stored in aerated sterile cabinets which served as stock cultures (Obayagbona 2012).

Physicochemical analyses of the soil samples

The physiochemical properties of the various soil samples were determined. With the exception of moisture content analysis, the respective soil samples were placed on large wooden trays and air-dried for 72 hr. Lumps of moist soil samples were broken by hand prior to air drying of the samples. The air dried samples were also sieved using a 2mm mesh. Parameters which included moisture content, pH, electrical conductivity and particle size distribution were ascertained using procedurees described by Kalra and Maynard (1991) Also, metals such as Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg) and Manganese (Mn) and the particle size distrubtion were determined according to methods stated by Radojevic and Bashkin, (1999). The Nitrate (NO^{3--}), Ammonium (NH_4^+), Total Nitrogen (N_2), Phosphate ($PO^{3-}{}_4$) content were evaluated using methods described by Onyeonwu (2000). The respective trace metals; (Zinc (Zn), Iron (Fe), Lead (Pb), Copper (Cu), Cobalt (Co), Vanadium (V), Nickel (Ni), Alumium (Al), Chromium (Cr) and Cadmium (Cd) content of the soil samples were also evaluated in accordance with procedures stated by Onyeonwu (2000) using a Digester (Gerhardt digester, UK.), and Atomic Absorbance Spectrophotometer (AAS) (Buck Scientific model 210 VGP USA) . The Total Organic Carbon (TOC) content of the respective samples was elucidated according to proceduree described by Bremmer and Mulvaney (1982).

Results

The mean aerobic bacterial counts for the soil samples ranged from 9.7×10^3 cfu/g for the control soil to 1.80×10^4 cfu/g for the soil sourced from the dump site at Ikheuniro (Table 1). The mean heterotrophic fungal counts varied from 7.0×10^2 cfu/g for capitol dumpsite to 3.3×10^3 cfu/g for the control soil (Table 1). Six bacterial and four fungal isolates were characterized and identified from the various dumpsite and control soils. The bacterial isolates were; *Bacillus* sp., *Pseudomonas* sp., *Aeromonas* sp., *Enterobacter* sp., *Klebsiella* sp. and *Staphylococcus* sp. (Figure 1) while the fungal isolates were; *Aspergillus* sp., *Mucor* sp., *Saccharomyces* sp. and *Fusarium* sp. respectively (Figure 2). Both *Bacillus* sp. and *Pseudomonas* sp. were the most dominant amongst the bacterial isolates whilst *Staphylococcus* sp. was the least occurring bacterial isolate (Figure 1). *Aspergillus* sp. had the highest frequency of isolation while the least isolated fungal culture was *Saccharomyces* sp. (Figure 2). The physico chemical results showed values which ranged from 5.60 to 8.08, 164.00 µS/cm to 540.00 µS/cm, 2.378 mg/kg to 3.444 mg/kg, 8.803 mg/kg to 12.852 mg/kg, 0.009 mg/kg to 0.016 mg/kg for pH, electrical conductivity, sulphate, phosphate and cadmium (Table 2).

Table 1: Total aerobic bacterial and heterotrophic fungal counts for the top soils

Samples	***Mean aerobic bacterial count (cfu/g)***	***Mean fungal count (cfu/g)***
Control	9.7×10^3	3.3×10^3
Capitol dumpsite	1.20×10^4	7.0×10^2
Bypass (Ikheuniro) dumpsite	1.80×10^4	2.1×10^3
NIFOR (CBN) dumpsite	1.55×10^4	7.0×10^2
NITEL dumpsite	1.85×10^4	9.0×10^2

Discussion

Intact soil is a continuum of mineral particles, organic materials, pore spaces, and organisms (Frey, 2007). The microbial bio load recovered from the control soil was comparatively lesser than that recorded for the soils collected from the dump sites (Table 1). This trend is collaborated by the higher organic carbon and nitrogen content of the dump site soils in comparison to the control soil (Table 2). This phenomenon might be the result of the increased availability of biodegradable organic and inorganic substrates from the variety of municipal wastes continuously being dumped at these sites. The isolation of *Bacillus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Staphylococcus* sp., *Aspergillus* sp., *Mucor* sp., *Fusarium* sp. and *Saccharomyces* sp. from the soil samples (Fig. 1 and 2), was similar to a report by Obire *et al.*, (2002) which stated the presence of these microorganisms in soils collected from a waste dumpsite located at Eagle island, Rivers State, Southern Nigeria. All the microbial isolates identified from the soil samples (Fig.1 and 2), have been reported to be associated with wastes and waste biodegradation (Obire *et al.*, 2002). Gray (1967), reported the association of *Bacillus* and *Pseudomonas* species with waste. *Aspergillus, Fusarium, Mucor, Penicillium Rhizopus* and a variety of yeasts have also been reported to be associated with waste biodegradation (Ekundayo, 1977). With the exception of NIFOR soil sample, the other analyzed soils were acidic at varying pH levels (Table 2).

This observation is similar to reports by Abduls-Salam (2009) and Ogbonna *et al.*, (2009) which reported the acidic characteristics of top soils sourced from several municipal waste dumpsites in Ilorin, Central Nigeria and Port Harcourt, Southern Nigeria. Itanna, (1998) and Bhattacharya *et al*., (2002) stated that pH, TOC and particle size distribution are among several components of soil that affect the availability, retention and mobility of metals with increasing pH. Soils with acidic pH levels tend to have an increased micronutrient solubility and mobility as well as increased heavy metal concentration (Odu *et al*., 1985), thus rendering the particular soil unsuitable for waste land filling (Ogbonna *et al*., 2009).

Table 2. Physico chemical properties of the top soils.

PARAMETERS (UNITS)	***CONTROL***	***NIFOR***	***NITEL***	***CAPITOL***	***BYPASS***
Clay (%)	17.7	13.5	16.4	20.6	19.4
Sand (%)	62.6	70.4	63.8	56.4	67.0
Silt (%)	19.7	16.1	19.8	23.0	13.6
pH	5.60	8.08	7.63	7.70	6.39
Electrical Conductivity EC (µS/cm)	184.00	164.00	387.00	540.00	261.00
Sulphate SO_4^{2-} (mg/kg)	3.444	3.010	2.624	2.346	2.378
Phosphate PO_4^{3-} (mg/kg)	12.852	11.233	9.792	8.803	8.874
Nitrate NO^{3-} (mg/kg)	5.208	4.552	3.968	3.476	3.596
Ammonium NH_4^+ (mg/kg)	1.008	0.881	0.768	0.641	0.696
Sodium Na (meq/100g)	8.652	7.571	6.592	5.850	5.974
Potassium K (meq/100g)	6.787	6.048	5.171	4.641	4.686
Calcium Ca (meq/100g)	5.922	5.123	4.512	3.996	4.089
Magnesium Mg (meq/100g)	3.372	2.879	2.569	2.398	2.328
Aluminum Al (meq/100g)	0.672	0.599	0.512	0.439	0.464
Iron Fe (mg/kg)	3.536	3.094	2.694	2.411	2.442
Zinc Zn (mg/kg)	2.234	1.994	1.702	1.472	1.543
Manganese Mn (mg/kg)	1.109	0.959	0.845	0.739	0.766
Chromium Cr (mg/kg)	0.094	0.079	0.072	0.059	0.065
Lead Pb (mg/kg)	0.028	0.025	0.021	0.015	0.019
Copper Cu (mg/kg)	0.353	0.305	0.269	0.233	0.244
Nickel Ni (mg/kg)	0.202	0.181	0.154	0.133	0.139
Cadmium Cd (mg/kg)	0.016	0.014	0.012	0.009	0.011
Cobalt Co (mg/kg)	<0.001	<0.001	<0.001	<0.001	<0.001
Vanadium V (mg/kg)	0.260	0.228	0.198	0.187	0.180
Total Org. Carbon TOC (%)	1.03	1.42	1.56	1.37	1.05
Total Nitrogen N_2 (%)	0.11	0.15	0.17	0.15	0.11

Soil pH influences a number of factors affecting microbial activity, like solubility and ionization of inorganic and organic soil solution constituents, and these will in turn affect soil enzyme activity (Voroney, 2007). All the top soil samples were sandy (Table 2). This phenomenon is in agreement with reports by Eneje and Lemoha (2013), Oyedele *et al.*, (2008) and Ideriah *et al.*, (2010), which observed the sandy nature of top soils sourced from several municipal dump sites in Owerri, Eastern Nigeria,

Ile-Ife, Western Nigeria and Port Harcourt, Southern Nigeria. However, this trend contrasted with a report by Ogbonna *et al.*, (2009), which indicated that a majority of top soil samples collected from waste dump sites in Port Harcourt, Rivers State, Nigeria were silty in nature. Oyedele *et al.*, (2008) stated that the textural class of a particular soil is mainly inherited from the soil forming materials.

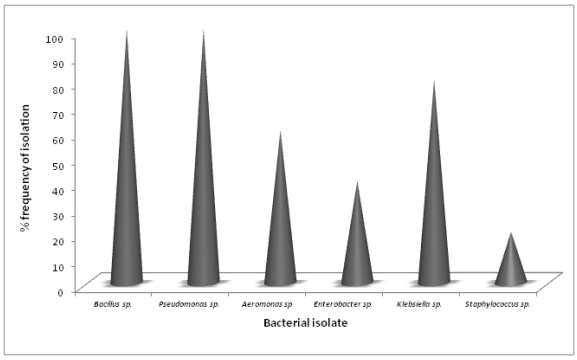

Fig. 1. Frequency of isolation (%) of the respective bacterial isolates.

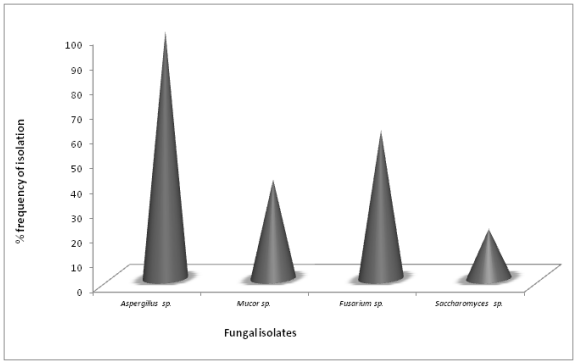

Fig. 2. Frequency of isolation (%) of the respective fungal isolates.

The particle size distribution is also known to have an influence on the bacterial diversity of soils (Faoro *et al*., 2010). Sessitsch *et al.*, (2001) reported that the clay fraction has a more diverse bacterial community than do silt or sand fractions. The high sand content of these soils recovered from the vicinities of the municipal waste dump sites could permit the usage of these open waste dump sites as land fill sites. Ogbonna *et al.*, (2006), reported that waste dumpsites with low sand fractions (<40%) are not suitable for waste land filling since they are rapidly permeable and could allow large quantities of leachate from the wastes to invade the deposited refuse and finally to the groundwater resources.

The heavy metal levels of both the control and MSW soils were at trace concentrations (Table 2). Eneje and Lemoha (2013) and Ideriah *et al.*, (2010), also observed similar trace levels of zinc, iron, copper and lead in soils collected from several soils obtained from MSW sites located in Owerri, Imo State and Port Harcourt, Rivers State, Nigeria. However this trend was at variance with a report by Ogbonna *et al.*, (2009) which indicated substantial amounts of several heavy metals; lead, cadmium, copper and zinc in soils collected at several depths from MSW sites in Port Harcourt, Rivers State, Nigeria. The low concentrations of these trace metals in the analyzed soils could be the result of an interplay of several factors which include; the acidic pH and sandy nature of the soils which can result in the increased mobilization, precipitation and infiltration of these heavy metals in the affected soils.

Despite the positive impacts of the dumped municipal wastes on the microbial and organic properties of the analyzed soils, disposal of municipal wastes in open dump sites is an archaic and unsustainable option in the management of municipal wastes. The onus is on the Edo State Government in particular and the Nigerian Government in general to create a conducive environment for interested companies or public-private partnerships to invest on a large scale on the management of municipal wastes using sustainable alternatives such as recycling and energy generation from municipal wastes.

References

Abduls- Salem, N. 2009. Assessment of heavy metal pollution in dump sites in Ilorin metropolis. Ethiopian Journal of Environmental Studies and Management **2(2),** 92 – 99.

Aneja KR. 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. Fourth Edition. New Delhi: New Age Pub. 606 pp.

Barnett HL, Hunter BB. 1972. Illustrated Genera of Imperfect Fungi. Third edition. New York: Burgess. 225 pp.

Bhattacharya P, Mukherjee AB, Jacks J, Nordqvist S. 2002. Metal contamination experimental studies on remediation. The Science of Total Environment **290**, 165 – 180.

Bremmer IM, Mulvaney CS. 1982. Total Nitrogen. In: American Society of Agronomy and Soil Science (eds). Methods of soil analysis. Agronomy Monograph 9. Madison: American Society of Agronomy and Soil Science, 595-627.

Dosumu OO, Salami N, Adekola FA. 2003. Comparative study of trace element levels. Bulletin of the Chemical Society of Ethiopia **17(1),** 107 – 112.

Ekundayo JA. 1977. Environmental consequences of the pollution of the Lagos Lagoon. Bulletin of the Science Association of Nigeria **3(2),** 290 - 299.

Enabulele OI, Obayagbona ON. 2013. Biodegradation potentials of mycoflora isolated from auto mobile workshop soils on flow station crude oil sludge. International Research Journal of Biological Sciences **2(5),** 1-6.

Eneje RC, Lemoha KT. 2012. Heavy metal content and physicochemical properties of municipal solid waste dump soils in Owerri, Imo State. International Journal of Modern Engineering Research **2(5),** 3795 – 3799.

Faoro H, Alves AC, Souza E M, Rigo IV, Cruz IM, Al-Janabi SM, Monteiro RA, Baura VA, Pedrosa FO. 2010. Influence of soil characteristics on the diversity of bacteria in the southern Brazilian Atlantic forest. Applied and Environmental Microbiology **76 (14),** 4744 – 4749.

Frey SD. 2007. Spatial distribution of soil organisms. In: Eldor AP, (Ed.) Soil Microbiology, Ecology and Biochemistry. Third edition. New York: Elsevier, 283 – 300.

Gimmler H, Carandang J, Boots A, Reisberg E, Woitke M. 2002. Heavy metal content and distribution within a woody plant during and after seven years continuous growth on municipal solid waste MSW bottom slag rich in heavy metals. Journal of Applied Botany **76**, 203-217.

Gray KR. 1967. Accelerated Composting. Compost Science **7(3),** 29 - 32.

Harley JP, Prescott LM. 2002. Laboratory Exercises in Microbiology. Fifth edition. New York: Mac Graw Hill. 449 pp.

Henry JR. 2000. An overview of phytoremediation of Lead and Mercury. Washington D.C: NNEMS Report, p. 3 – 9.

Holt JG, Krieg NR, Sneath PHA. 1989. Bergey's Manual of Determinative Bacteriology (Vol. 4). London: Cambridge University Press 2493 pp.

Ideriah JJK, Harry FO, Stanley HO, Igbara J K. 2010. Heavy metal contamination of soils and vegetation around solid waste dumps in Port Harcourt, Nigeria. Journal of Applied Science and Environmental Management **14(1),** 101- 109.

Itanna F. 1998. Comparative study on soil pollution with toxic substances on farmlands close to old and new industrial sites in Ethiopia. Bulletin of Chemical Society of Ethiopia **12(2),** 105 - 112.

KalraYP, Maynard DG. 1991. Methods Manual for Forest soil and Plant Analysis. Edmonton, Canada: Minster of supply and services. 125 pp.

Minh NH, Minh TB, Kajiwara N, Kunisue T, Subramanian A, Iwata H, Tana TS, Baburajendran R, Karuppiah S, Viet PH, Tuyen BC, Tanabe S. 2006. Contamination by persistent organic pollutants in dumping sites of Asian developing countries: Implication of emerging pollution sources. Environmental Contamination and Toxicology **50(4),** 474-481.

Obasi NA, Akubugwo EI, Ugbogu OC, Otuchristian G. 2012. Assessment of physicochemical properties and heavy metals bioavailability in dump sites along Enugu- Port Harcourt expressways, South East, Nigeria. Asian Journal of Applied Sciences **5(6),** 342-356.

Obayagbona ON. 2012. Biodegradation potentials of mycoflora isolated from auto mechanic workshop soils on flow station crude oil sludge. MSc Thesis, University of Benin, Nigeria, 172 pp.

Obire O, Nwabueta O, Adue SBN. 2002. Microbial community of a waste dump site. Journal of Applied Science and Environmental Management **6 (1),** 78 – 83.

Odu CTI, Esuruoso OF, Nwoboshi LC, Ogunwale JA. 1985. Environmental Study of the Nigerian. Agip Oil Company, Operational Area. Soil and Fresh Water Vegetation. Milan: Union Graft Publishing, 21 – 25.

Ogbonna DN, Igbenijie M, Isirimah NO. 2006. Microbiological and Physicochemical Characteristics of the soils of waste collection sites in Port Harcourt city, Nigeria. Nigeria Journal of Soil Science **16,**162 – 167.

Ogbonna DN, Kii BC, Youdeowei PO. 2009. Some physicochemical and heavy metal levels in soils of waste dump sites in Port Harcourt municipality and environs. Journal of Applied Science and Environmental Management **13 (14),** 65 – 70.

Onyeonwu RO. 2000. Manual for Waste/Wastewater, Soil/ Sediment, Plant and Fish analysis. Benin City: MacGill Environmental Research Laboratory Manual. 81 pp.

Oyedele DJ, Gasu MB, Awotoye OO. 2008. Changes in soil properties and plant uptake of heavy metals on selected municipal solid waste dump sites in Ile-Ife, Nigeria. African Journal of Environmental Science and Technology **3(5),** 107 – 115.

Pavoni JL, Heer Jr. JE, Hagerty DL. 1975. Handbook of Solid Waste Disposal, Materials and Energy Recovery. New York: Van Nostrand Reinhold Company.

Radojevic M, Bashkin VN. 1999. Practical Environmental Analysis. Cambridge: The Royal Society of Chemistry, p.466.

Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E. 2001. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Applied Environmental Microbiology **67,** 4215–4224.

Sharma P. 2009. Manual of Microbiology, tools and techniques. New Delhi: Ane books. Pvt. Ltd. 405 pp.

Voroney RP. 2007. The soil habitat. In: Eldor AP, (Ed.) Soil Microbiology, Ecology and Biochemistry. Third edition. New York: Elsevier, 25 – 49.