The microbiological, parasitological and physicochemical studies of waste refuse dumpsites in University of Benin (Ugbowo Campus), Benin City, Nigeria

Studi microbiologici, parassitologici e chimico-fisici su rifiuti di discariche effettuati all'Università di Benin (Ugbowo Campus), Benin City, Nigeria

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This paper examined the effect of the refuse waste on the microbiological, parasitological and physicochemical properties on the immediate University of Benin Community. The microbiological, parasitological and physicochemical assessments were studied using the basic microbiological and parasitological techniques. The total heterotrophic bacterial counts ranged from $1.1 \times 10^5 \pm 0.35$ cfu/g to $5.4 \times 10^5 \pm 0.56$ cfu/g and the total fungal counts ranged from $1.5 \times 10^4 \pm 0.42$ cfu/g to $1.9 \times 10^5 \pm 0.14$ cfu/g of the refuse dumpsites between August, 2011 and January, 2012. The bacterial counts were shown to be highest in Faculty of Agricultural Science refuse dumpsites in the month of September, 2011. The microbial isolates isolated and characterized includes nine bacterial genera and seven fungal genera, they include *Bacillus, Enterobacter, Staphylococcus, Proteus mirabilis, Micrococcus, Pseudomonas, Serratia, Arthrobacter, Citrobacter* and *Aspergillus, Penicillium, Mucor, Rhizopus, Fusarium, Cladiosporium, Trichoderma* respectively. The total mean value of the frequently isolated bacterial isolates were *Micrococcus* sp 400 (21.8%) and *Bacillus* sp 369 (20.1%) respectively. The most frequently isolated fungal isolates from the refuse dumpsites were *Aspergillus* spp and *Penicillium* spp with the mean values of 146 (23.4%) and 167 (26.8%) respectively. This study also showed the degree of dumpsites contamination with pathogenic parasites in the community. A total number of fourteen parasites were isolated and identified, they include *Ascaris lumbricoides* (4.1%), *Taenia* sp (33.8%), *Ancyclostoma* sp (5.0%), Tapeworm (25.7%), *Taenia saginata* (10.6%), *Hymenolepsis* sp (4.8%), *Hymenolepsis diminuta* (2.0%), *Fasciola hepatica* (6.2%), *Capillaria hepatica* (1.5%), *Capillaria* sp (3.2%), *Aspiculus* sp (0.5%), *Ornithobiharzia* sp (0.8%), *Trichostrongylus* (1.1%) and *Syphacia obvelata* (0.7%). The result of the physiochemical parameters showed the pH mean value of the pH range of the refuse dumpsites in the various Faculties for six months of sampling ranged between 5.21 ± 0.39 and 5.57 ± 0.24 with the highest pH was obtained in the Faculty of Management Sciences and the least pH mean value was obtained in the Faculty of Agricultural Sciences. The results of the metal contents were revealed to be within the permissible limit. Statistical analyses of the total heterotrophic bacterial counts and fungal counts revealed that there was significant difference ($P > 0.05$) for the counts obtained from the refuse dumpsites between August, 2011 and January, 2012. Thus, it is recommended that the proper waste management system are essential and the practice of wastes management practices like waste re-use and recycling should be encouraged.

Key words: *Waste refuse dump, University of Benin, microbial isolates, parasitological and physicochemical parameters*

Introduction

Wastes can be loosely defined as any material that is considered to be of no further use to the owner and is hence discarded. However, most discarded waste can be reused or recycled. Additionally, wastes are such items which people are require to discard, for example because of their hazardous properties [Ali, 1999]. Many items can be considered as waste e.g., household rubbish, sewage sludge, wastes from manufacturing activities, packaging items, discarded cars, old televisions, garden waste, old paint containers etc. Thus all our daily activities give rise to a large variety of different wastes arising from different sources [Ali, 1999]. Over 1.8 billion tonnes of waste are generated each year in Africa, this amount to 3.5 tonnes generated per person. This is mainly made up of waste coming from households, commercial activities (shops, restaurants, and hospitals), industry (pharmaceutical companies, clothes manufacturers), agriculture (slurry), construction and demolition projects, mining and quarrying activities and from the generation of energy. With such vast quantities of waste being produced, it is of vital importance that it is managed in such a way that it does not cause any harm to either human health or to the environment [Onibokun, 1999]. There are a number of different options available for the treatment and management of waste including prevention, minimisation, re-use, recycling, energy recovery and disposal. Landfilling is seen as the last resort and could only be used when all other options have been exhausted, that is only material that cannot be pre-

vented, re-used, recycled or otherwise treated should be landfilled [FEPA, 1991]. Waste is generated universally and is a direct consequence of all human activities. Wastes are generally classified into solid, liquid and gaseous. Gaseous waste is/are normally vented to the atmosphere, either with or without treatment depending on composition and the specific regulations of the country involved [Attahi, 1999]. Liquid wastes are commonly discharged into sewers or rivers, which in many countries is subjected to legislation governing treatment before discharge. In many parts of the world such legislation either does not exist or is not sufficiently implemented and liquid wastes are discharged into water bodies or allowed to infiltrate into the ground [Słomczynska, 2001]. Indiscriminate disposal of liquid wastes pose a major pollution threat to both surface and groundwater. Solid wastes are known as trash or garbage, refuse or rubbish wastes type consisting of everyday items that are discarded by the public. Solid waste composition, rate of generation and methods of treatment and disposal vary considerably throughout the world and largely determine the potential of waste to impair groundwater quality [Attahi, 1999]. The aims and objectives of this research were to determine the effects of the waste refuse dump in the receiving environment that is University of Benin, Ugbowo Campus, Benin City on the microbiological, parasitological and physicochemical qualities.

Materials and methods

Study area

The University of Benin (Uniben) is one of the country's major universities located in Benin City, Edo State, Nigeria 6°20.022'N, 5°36.009'E. The University was founded in 1970 by military government of Samuel Ogbemudia. It started as an Institute of Technology and was accorded the status of a full-fledged University by National Universities Commission (NUC) on 1st July 1971. On 1st April 1975, at the request of the State Government, the University was taken over by the Federal Government and became a Federal University.

Sample collection

The samples were collected from the major dump sites in the ten (10) Faculties in University of Benin, Benin City. The dumps were characterized by the presence of papers, nylons, stationeries, plastics, bottles, leaves, organic wastes and food components. These wastes are managed occasionally by burning which does little in reducing the volume of such wastes and sometimes evacuated by the University Health Workers and the contracted waste managers. Soils under the waste dumps were exposed with the use of clean sterile hand garden rake. The soil samples were taken at about 10cm depth by the use of hand trowel, and taken to the laboratory in labelled polyethylene bags for parasitological, microbiological and physicochemical analyses.

Microbiological analyses

Serial dilution processes were used in the process of isolation. Ten gram (10 g) of each soil sample was dispensed in a beaker and mixed thoroughly with 90 ml sterilized water. The soil sample was serially diluted from the stock sample and then transferred to the first tube containing 9 ml of distilled water to give 10^{-1} dilution, from which further dilutions up to 10^{-6} were made. The pour plate methods were used for the inoculation on a sterilized nutrient agar medium (Oxoid) for the enumeration and isolation of bacterial isolates. One millimetre (1 ml) of 10^{-6} dilution was inoculated on sterile Petri-dishes and the sterilized molten media were poured aseptically on the inoculated plates. The cultured media were incubated at 37°C for 24 h for the growth of bacterial isolates. After incubation, viable numbers of growth (between 30 and 300) were multiply by the reciprocal of the dilution factor and recorded as colony forming unit per gram (cfu/g) of waste. Individual colonies were selected for pure culture technologies, which were further characterized and identified. The isolation and identification of bacterial isolates were carried out in accordance with Bergey's Manual of Determinative Bacteriology [Buchanan, Gibbons, 1974; Gerhardt et al., 1994].

Fungal counts and identification

The procedure earlier described for bacterial counts was adopted for fungi counts of the waste and soil samples. However, the medium of choice was sabouraud dextrose agar, while incubation period was 4 to 5 days at room temperature. The fungal isolates were identified through observation of their colonies, microscopic examination of their respective spores and hyphal appendages. Wet - mount method was used, this involved picking fungi growth from an agar plate using a sterile dissecting needle. This was then teased out on clean grease-free slide, on which a drop of lactophenol cotton blue was placed. The smears were then covered with cover slide and examined under the microscope using low power objectives. The morphology in conjunction with the typical cultural characteristics was used for identification of the various fungi isolates [Barnett, Hunter, 1975].

Parasitological analysis

The parasitological study of the samples was carried according to the method described by Keigei [Keigei, 1992]. With a garden rake, the waste was removed to expose the soil under waste dump. A soil sample was collected from underneath the waste dump, out of which 200 g of the soil was weighed. The weighed soil was filtered through a coarse sieve of about 4 mm^2 pore sizes to remove the stones, grasses and other pebbles. The extract was transferred to a volumetric flask and then mixed with distilled water to a 100 ml mark on the flask. The coarse particle was sieved out by passing through a coarse mesh clothe into a centrifuge tube and centrifuged at 300 rpm for 2 minutes.

The floatation fluid in the test tube/centrifuge tubes was filled up to form a meniscus and then cover-slip the tubes for 5 minutes. The cover-slip was then lifted with a swift action and placed on a glass slide and examined microscopically for the presence of cyst, parasites and eggs of parasites. The physicochemical parameters studied were pH, conductivity, available phosphorus, total organic matter, total nitrogen, exchangeable acidity, calcium, ammonia, sodium, potassium, sand, clay, silt, iron, manganese, copper, zinc, chromium, ammonia nitrogen and sulphates. The physicochemical analyses of the soil samples were determined using the methods of Association of Official Analytical Chemist [AOAC, 1990; Ogunwale, Udo, 1986].

Determination of plasmid profile of the bacterial isolates

Confluent (one plate per DNA sample) lawns of the bacterial culture was collected by sweeping with a glass rod and was resuspended in 100 μl of PEB 1 (50 mM glucose- 10 mM Ethylene dinitrilo tetra-acetic acid (EDTA) at 0°C in a 1.5 ml Eppendorf tube. After 10 minutes, 200 μl of PEB II (0.2 N Sodium hydroxide- 1% Sodium Dodecyl Sulphate) at room temperature was added and mixed gently by inversion several times. After 5 minutes of incubation at 0°C, 150 μl of PEB III (3 M Potassium acetate- 1.8 M Formic acid) at room temperature was added, mixed gently several times, and incubated for 15 minutes at 0°C. The mixture was centrifuged at 12,000 rpm in a microfuge for 3 minutes at room temperature. Part of the supernatant (350 μl) was transferred to another 1.5 ml Eppendorf tube and 1.0 ml of cold 95% ethanol was added, held for 20 minutes at -20°C, and centrifuged at 12,000 rpm.
The supernatant to be discarded was aspirated with a fine tip glass pipette, and the DNA pellet was resuspended in 100 μl of DNA wash buffer (0.1 M Sodium acetate-50 mM Morpholine- propane sulfonic acid (MOPS) pH 8.0) and reprecipitated in 200 μl of cold ethanol for 20 minutes at -20°C. The DNA was centrifuged and the pellet was washed again in 100 μl of DNA wash buffer and reprecipitated in ethanol at -20°C [Bradbury et al., 1983]. After 20 minutes, the DNA was centrifuged and resuspended in 50 μl of water-50 μl of LiCl solution and mixed before standing for 30 minutes at 0°C. The solution was centrifuged and100 μl of the supernatant was transferred to a 0.5 ml Eppendorf tube; 200 μl of cold ethanol was added, and the mixture was held at -20°C for 20 minutes. The solution was centrifuged and the supernatant was discarded. The DNA pellet was resuspended in 50 μl of DNA wash buffer, reprecipitated and centrifuged. The washing step was repeated before the DNA was reprecipitated, supernatant was discarded, and the pellet was stored at -20°C until required for electrophoresis. In preparation for electrophoresis, the pellet was resuspended in 20 μl of water, and 5 μl of SB buffer (25% sucrose, 5 mM sodium acetate, 0.05% bromophenol blue, 0.1% sodium dodecyl sulphate) was added and mixed gently. Electrophoretic separation of the sample DNA was done at 30 - 40 voltage for 2 - 6 hours on a horizontal 0.7% agarose slab gel (0.5 by 13 by 20 cm) in one part TAE buffer (40 mM Tris-hydrochloride, 20 mM sodium acetate, 2 mM EDTA), stained for 1 hour in ethidium bromide (1 $\mu g \cdot ml^{-1}$), and photographed by filtered (Kodak no 25 red filter) UV illumination on Polariod P/N film [Bradbury et al., 1983].

Statistical analysis

Statistical analysis of the variance of the microbial mean counts was conducted using SSPS version 16 and the significant differences in the mean counts were detected with the aid of Duncan multiple range test.

Results

A total number of thirteen (13) parasites were isolated from the refuse dumpsites, the parasites includes *Ascaris lumbricoides, Taenia* sp., *Ancyclostoma* sp., *Taenia saginata*, *Hymenolepsis* sp, *Hymenolepsis diminuta, Fasciola hepatica, Capillaria hepatica, Capillaria* sp., *Aspiculus* sp., *Ornithobiharzia* sp., *Trichostrongylus* and *Syphacia obvelata* (Table 1). The frequency of occurrence of the parasitic forms found in the dumpsites and the percentage frequency of distribution of the parasites are showed in Table 1; *Ascaris lumbricoides* (5.6%), *Taenia* sp. (45.5%), *Ancyclostoma* sp. (6.7%), *Taenia saginata* (14.3%), *Hymenolepsis* sp (6.5%), *Hymenolepsis diminuta* (2.7%), *Fasciola hepatica* (8.3%), *Capillaria hepatica* (4.3%), *Capillaria* sp. (2.0%), *Aspiculus* sp. (0.7%), *Ornithobiharzia* sp. (1.0%), *Trichostrongylus* (1.5%) and *Syphacia obvelata* (0.9%) between August, 2011 and January, 2012. The most frequently isolated parasite from the refuse dumpsites was *Taenia* sp. (45%) followed by *Aspiculus* sp. (0.7%), as the least frequently isolated parasite between August, 2011 and January, 2012.
The frequency of occurrence of the parasites was observed to be influenced by the wet period of the year. It was discovered that the population decreases along the months due to decreases in moisture. Tables 2 and 3 showed the mean values of the total heterotrophic bacterial counts which ranged from $1.1 \times 10^5 \pm 0.35$ cfu/g to $5.4 \times 10^5 \pm 0.56$ cfu/g and the mean values of the total fungal counts ranging from $1.5 \times 10^4 \pm 0.42$ cfu/g to $1.9 \times 10^5 \pm 0.14$ cfu/g from the refuse dumpsites between August, 2011 and January, 2012. Tables 4 and 5 showed the percentage frequency of occurrence of bacterial and fungal isolates, *Bacillus* sp. (20.1%), *Enterobacter* sp. (11.2%), *Staphylococcus* sp. (11.6%), *Proteus mirabilis* (2.2%), *Micrococcus* sp. (21.8%), *Pseudomonas* sp. (13.6%), *Serratia* sp. (1.4%), *Arthrobacter* sp. (8.7%), *Citrobacter* sp. (9.4%), *Aspergillus* spp. (23.4%), *Aspergillus niger* (3.0%), *Penicillium* spp. (26.8%), *Mucor* sp. (7.8%), *Rhizopus* sp. (15.0%), *Fusarium* sp. (10.7%), *Cladiosporium* sp. (5.4%) and *Trichoderma* sp. (7.9%) respectively from the refuse dumpsites. The absence of the common bacterial isolates such *E. coli*, *Streptococcus faecalis* and clostridia is not surprising, because the waste

were not connected with sewage nor with animal and waste faces, which are primary sources of these organisms. Figure 1 showed the result of the plasmid profile analysis of the bacterial isolates from the refuse dumpsites. The plasmid profile screening showed the presence of plasmid 25.1 kb in Lanes 1, 2, 3, 4, 9, 10 and 12 with reference to bacterial isolates such as *Pseudomonas aeruginosa*, *Micrococcus* sp, *Arthrobacter* sp, *Bacillus* sp, *Bacillus* sp and *Bacillus* sp respectively. The plasmids revealed the survival capabilities of the isolates, specifically the bacterial isolates Pseudomonas aeruginosa across the refuse collection points. The presence of plasmids was thus influenced by the composition of refuse collection points. The results of the summary of the physicochemical parameters studied are presented in Table 6.

The result of the pH recorded ranged from 5.21 ± 0.39 to 5.57 ± 0.24. The highest mean exchangeable ions in meq/100 g of the refuse dumpsites obtained were 12.56 ± 8.04, 1.42 ± 1.19, 0.59 ± 0.10 and 1.56 ± 1.33 for Ca, Mg, K and Na respectively. The average mean values of heavy metals, iron, copper and chromium of the respective soil samples from the dumpsites for six months of sampling ranged from 92.95 ± 61.07 to 196.39 ± 264.5 ppm, 5.22 ± 1.24 to 7.77 ± 2.99 ppm and 0.32 ± 0.15 to 1.42 ± 1.45 ppm for iron, copper, and chromium respectively. The highest percentage mean value of organic carbon was recorded in the refuse dumpsites in Faculty of Physical Sciences (0.91 ± 0.31%) and Faculty of Life Sciences (0.91 ± 0.23%) and the lowest mean value of 0.67 ± 0.30% was recorded in Faculty of Engineering.

Table 1: Percentage frequency of occurrence of parasites found in refuse dump sites in UNIBEN between August, 2011 and January, 2012

Parasites isolated	MONTHS OF SAMPLING						Total	Percentage frequency of Occurrence (%)
	August 2011	*September 2011*	*October 2011*	*November 2011*	*December 2011*	*January 2012*		
Taenia sp	150	90	33	20	17	28	338	45.5
Taenia saginata	63	25	18	-	-	-	106	14.3
Hymenolepsis sp	27	15	3	-	-	3	48	6.5
Hymenolepsis diminuta	5	-	2	13	-	-	20	2.7
Fasciola hepatica	8	10	13	10	5	16	62	8.3
Capillaria hepatica	13	-	2	-	-	-	15	2.0
Capillaria sp	10	8	1	-	6	7	32	4.3
Ascaris lumbricoides	13	10	5	10	-	3	41	5.6
Aspiculus sp	2	-	3	-	-	-	5	0.7
Ancyclostoma sp	17	20	8	-	-	5	50	6.7
Ornithobiharzia sp	8	-	-	-	-	-	8	1.0
Trichostrongylus	6	5	-	-	-	-	11	1.5
Syphacia obvelata	7	-	-	-	-	-	7	0.9

Table 2: Mean values of the total heterotrophic bacterial counts of the dump sites (refuse collection points) in UNIBEN from August, 2011 to January, 2012 (cfu/g)

Sampling Sites	*August 2011*	*September 2011*	*October 2011*	*November 2011*	*December 2011*	*January 2012*
Control	$1.05\times10^5\pm0.71$	$2.60\times10^5\pm0.14$	$2.6\times10^5\pm0.14$	$3.2\times10^5\pm0.42$	$2.15\times10^5\pm0.35$	$4.25\times10^5\pm0.49$
College of medical Sciences	$3.5\times10^5\pm0.00$	$3.6\times10^5\pm0.84$	$3.1\times10^5\pm0.14$	$1.85\times10^5\pm0.21$	$1.1\times10^5\pm0.35$	$2.3\times10^5\pm0.28$
Faculty of Physical Sciences	$1.3\times10^5\pm0.42$	$1.95\times10^5\pm0.21$	$1.95\times10^5\pm0.70$	$1.9\times10^5\pm0.28$	$1.75\times10^5\pm0.35$	$2.3\times10^5\pm0.28$
Faculty of Education	$3.35\times10^5\pm0.49$	$3.10\times10^5\pm0.14$	$2.95\times10^5\pm0.70$	$2.4\times10^5\pm0.84$	$2.2\times10^5\pm0.70$	$2.85\times10^5\pm0.49$
Faculty of Arts	$4.1\times10^5\pm0.14$	$4.2\times10^5\pm0.42$	$3.85\times10^5\pm0.21$	$3.3\times10^5\pm0.35$	$2.75\times10^5\pm0.35$	$3.5\times10^5\pm0.70$
Faculty of Agric. Science	$4.95\times10^5\pm0.35$	$5.4\times10^5\pm0.56$	$4.4\times10^5\pm0.56$	$5.05\times10^5\pm0.07$	$3.35\times10^5\pm0.21$	$5.10\times10^5\pm0.14$
Faculty of Life Sciences	$3.0\times10^5\pm0.00$	$2.9\times10^5\pm0.14$	$2.65\times10^5\pm0.21$	$3.4\times10^5\pm0.14$	$3.0\times10^5\pm1.05$	$3.75\times10^5\pm0.07$
Faculty of Engineering	$2.7\times10^5\pm0.28$	$2.4\times10^5\pm0.56$	$2.85\times10^5\pm0.21$	$3.8\times10^5\pm0.42$	$2.75\times10^5\pm0.35$	$4.05\times10^5\pm0.07$
Faculty of Pharmacy	$1.9\times10^5\pm0.14$	$1.65\times10^5\pm0.21$	$2.4\times10^5\pm0.56$	$2.3\times10^5\pm0.56$	$1.75\times10^5\pm0.14$	$2.70\times10^5\pm0.21$
Faculty of Management Sciences	$3.2\times10^5\pm0.14$	$3.05\times10^5\pm0.70$	$3.1\times10^5\pm0.14$	$2.0\times10^5\pm0.28$	$1.9\times10^5\pm0.14$	$2.45\times10^5\pm0.77$
Faculty of Social Sciences	$4.25\times10^5\pm0.49$	$4.4\times10^5\pm0.42$	$4.25\times10^5\pm0.21$	$3.6\times10^5\pm0.14$	$3.1\times10^5\pm0.91$	$4.3\times10^5\pm0.14$

Table 3: Mean values of the total fungal counts of the dump sites (refuse collection points) in UNIBEN from August, 2011 to January, 2012 (cfu/g)

Sampling Sites	*August 2011*	*September 2011*	*October 2011*	*November 2011*	*December 2011*	*January 2012*
Control	$6.0\times10^4\pm0.14$	$1.0\times10^5\pm0.28$	$6.0\times10^4\pm0.14$	$1.05\times10^5\pm0.07$	$9.0\times10^4\pm0.14$	$1.10\times10^5\pm1.14$
College of medical Sciences	$1.25\times10^5\pm0.35$	$1.30\times10^5\pm0.14$	$1.05\times10^5\pm0.07$	$9.0\times10^4\pm0.14$	$5.0\times10^4\pm0.07$	$9.5\times10^4\pm0.21$
Faculty of Physical Sciences	$9.0\times10^4\pm0.28$	$6.5\times10^4\pm0.21$	$9.0\times10^4\pm0.14$	$4.0\times10^4\pm0.21$	$4.0\times10^4\pm0.21$	$7.5\times10^4\pm0.35$
Faculty of Education	$1.25\times10^5\pm0.21$	$1.05\times10^5\pm0.07$	$8.6\times10^4\pm0.07$	$1.25\times10^5\pm0.35$	$1.00\times10^5\pm0.00$	$1.30\times10^5\pm0.14$
Faculty of Arts	$1.70\times10^5\pm0.42$	$1.40\times10^5\pm0.56$	$1.10\times10^5\pm0.14$	$1.10\times10^5\pm0.14$	$9.0\times10^4\pm0.14$	$2.35\times10^5\pm0.21$
Faculty of Agric. Science	$9.5\times10^4\pm0.35$	$1.90\times10^5\pm0.14$	$1.10\times10^5\pm0.14$	$1.10\times10^5\pm0.56$	$9.0\times10^4\pm0.21$	$1.60\times10^5\pm0.56$
Faculty of Life Sciences	$1.05\times10^5\pm0.07$	$8.5\times10^4\pm0.07$	$6.5\times10^4\pm0.07$	$1.50\times10^4\pm0.42$	$1.9\times10^5\pm0.14$	$1.70\times10^5\pm0.42$
Faculty of Engineering	$1.35\times10^5\pm0.21$	$1.45\times10^5\pm0.07$	$1.30\times10^5\pm0.14$	$5.5\times10^4\pm0.07$	$6.0\times10^4\pm0.28$	$1.0\times10^5\pm0.07$
Faculty of Pharmacy	$8.5\times10^4\pm0.21$	$5.5\times10^4\pm0.07$	$9.0\times10^4\pm0.14$	$6.5\times10^4\pm0.49$	$6.0\times10^4\pm0.07$	$1.1\times10^5\pm0.14$
Faculty of Management Sciences	$1.50\times10^5\pm0.42$	$6.0\times10^4\pm0.14$	$5.5\times10^4\pm0.07$	$2.5\times10^4\pm0.07$	$3.0\times10^4\pm0.07$	$6.5\times10^4\pm0.21$
Faculty of Social Sciences	$1.75\times10^5\pm0.35$	$1.45\times10^5\pm0.07$	$9.0\times10^4\pm0.14$	$6.0\times10^4\pm0.14$	$6.0\times10^4\pm0.21$	$7.5\times10^4\pm0.07$

Table 4: Mean values of the total fungal counts of the dump sites (refuse collection points) in UNIBEN from August, 2011 to January, 2012 (cfu/g)

Parasites isolated	**MONTHS OF SAMPLING**						**Total**	**Percentage frequency of Occurrence (%)**
	August 2011	*September 2011*	*October 2011*	*November 2011*	*December 2011*	*January 2012*		
Bacillus sp	57	66	61	80	30	74	369	20.1
Enterobacter sp	39	40	0	0	60	59	206	11.2
Staphylococcus sp	42	37	0	47	55	31	212	11.6
Proteus mirabilis	15	0	0	8	0	17	40	2.2
Micrococcus sp	56	61	78	65	55	85	400	21.8
Pseudomonas sp	55	67	77	50	0	0	249	13.6
Serratia sp	11	0	9	0	0	5	25	1.4
Arthrobacter sp	46	54	58	0	0	0	158	8.7
Citrobacter sp	0	0	29	45	34	65	173	9.4
Total	321	325	312	295	234	331		100

Table 5: Frequency of occurrence of the fungal isolates from refuse dump sites in UNIBEN between August, 2011 and January, 2012

Parasites isolated	**MONTHS OF SAMPLING**						**Total**	**Percentage frequency of Occurrence (%)**
	August 2011	*September 2011*	*October 2011*	*November 2011*	*December 2011*	*January 2012*		
Bacillus sp	57	66	61	80	30	74	369	20.1
Enterobacter sp	39	40	0	0	60	59	206	11.2
Staphylococcus sp	42	37	0	47	55	31	212	11.6
Proteus mirabilis	15	0	0	8	0	17	40	2.2
Micrococcus sp	56	61	78	65	55	85	400	21.8
Pseudomonas sp	55	67	77	50	0	0	249	13.6
Serratia sp	11	0	9	0	0	5	25	1.4
Arthrobacter sp	46	54	58	0	0	0	158	8.7
Citrobacter sp	0	0	29	45	34	65	173	9.4
Total	321	325	312	295	234	331		100

The mean values of total nitrogen (%) and ammonium nitrogen, NH_4N, (mg/kg) recorded ranged from 0.14 ± 0.07% to 0.25 ± 0.12% and 11.88 ± 4.17 to 15.14 ± 3.62 mg/kg for all the sampling refuse dumpsites between August, 2011 and January, 2012. Exchangeable acidity mean value recorded for six months of sampling from the refuse dumpsites ranged from 0.28 ± 0.11 to 0.46 ± 0.13 meq/100 g while the highest mean value observed for available phosphorus was obtained in Faculty of Arts, 21.44 ± 15.83 mg/kg and the lowest value 11.56 ± 6.69 mg/kg was recorded in the refuse dumpsites soil samples.

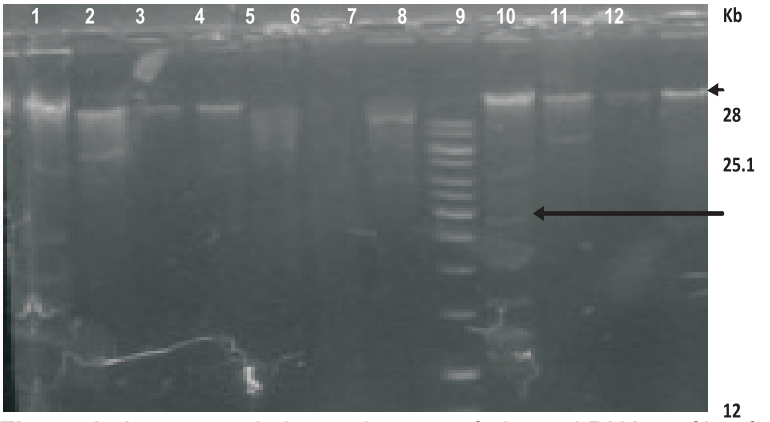

Figure 1: Agarose gel electrophoresis of plasmid DNA profile of bacterial isolates from waste refuse
Lane1 *Pseudomonas aeruginosa*, Lane 2 *Micrococcus* sp., Lane 3 *Arthrobacter* sp., Lane 4 *Bacillus* sp., Lane 5 *Proteus mirabilis*, Lane 6 *Serratia* sp., Lane 7 *Proteus mirabilis*, Lane 8 = 10 kb DNA ladder; Lanes 9 *Bacillus* sp., Lane 10 *Bacillus* sp., Lane 11 *Micrococcus* sp., Lane 12 *Enterobacter* sp.

Discussion

Solid wastes constitute an environmental and public health nuisance in major cities all over the world. The government consider solid waste management as an essential social service where budgetary provision is made in line with the population projection [Eja et al., 2010]. Solid waste management technology consists of the following stages such as, collection, transportation and disposal of the waste. The microbiological, parasitological and physicochemical qualities of the refuse dumpsites to the receiving environment, University of Benin, Ugbowo Campus, Benin City were studied between August, 2011 and January, 2012. The results obtained for the parasitological analyses showed that a total number of thirteen (13) parasites were isolated from the waste refuse dumpsites between August, 2011 and January, 2012. Among the parasites isolated, *Taenia* sp. was recorded to be the most frequently isolated parasites at 45.5% frequency of occurrence between August, 2011 and January, 2012. While the least frequently isolated parasite was *Aspiculus* sp. (0.7%). The occurrence of this parasite may be as a result of its ability to adapt to various environmental conditions [Adeyeba, Okpala, 2000]. *Syphacia obvelata* was only found in the month of August, 2011 and only in four of the sampling refuse dumpsites. Adeyeba and Okpala, [Adeyeba, Okpala, 2000] stated that parasitic agents isolated from refuse dumpsites are potential causes of infection to the population present in the community. The result obtained also showed the effect of climatic conditions on the occurrence of the parasites isolated from the refuse dumpsites between August, 2011 and January, 2012. Parasites such as *Syphacia obvelata*, *Trichostrongylus*, *Ornithobiharzia* sp. and *Aspiculus* sp. were mainly isolated during the rainfall season of August to September, 2011 and slightly in October, 2011, which showed the effect of season on the distribution of occurrence of the parasites isolated from the refuse dumpsites. This result is similar to the report of Smyth, [Smyth, 1996], who reported that, the survival of intestinal parasites is dependent on the favourable conditions of both environmental and climatic factors. The highest number of parasites isolated from the refuse dumpsites was recorded in the month of August, 2011, during which rainfall was recorded high. The least number of parasites was recorded in the month of December, 2011 and January, 2012, which could be attributed to the low rainfall in the dry season or practically no rainfall. The highest total heterotrophic bacterial counts was recorded from refuse dumpsite in Faculty of Agricultural Science in the month of September, 2011 while the least total heterotrophic bacterial counts was recorded from refuse dumpsite in College of Medical Sciences in the month of December, 2011.

The highest total fungal counts was recorded from refuse dumpsite in Faculty of Arts in the month of January, 2012 while the least total fungal counts was recorded from refuse dumpsite in Faculty of Management Sciences in the month of December, 2011. Eja et al. [Eja et al., 2010], reported similar results that the total bacterial and fungal counts from decomposing solid waste, soil at waste the dumpsite and leachate were generally high during the four months of sampling from January to April, 2010, which poses high risk of contacting microbial infection.

The results of the microbiological analysis showed the frequency of occurrence of the bacterial isolates between August, 2011 and January, 2012, which showed *Micrococcus* sp. (21.8%) and *Bacillus* sp. (20.1%) as the most frequently isolated bacterial isolates. The microbial isolates isolated and characterized includes nine bacterial genera and seven fungal genera among which are *Bacillus*, *Enterobacter*, *Staphylococcus*, *Proteus mirabilis*, *Micrococcus*, *Pseudomonas*, *Serratia*, *Arthrobacter*, *Citrobacter* for the bacterial isolates and *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Fusarium*, *Cladiosporium*, *Trichoderma* for the fungal isolates.

Table 6: The mean values of the physicochemical parameters of refuse collection points in Uniben from August, 2011 - January, 2012 (± STANDARD DEVIATION)

PARAMETERS	SAMPLING SITES										
	Control	College of Medical Sciences	Faculty of Physical Sciences	Faculty of Education	Faculty of Arts	Faculty of Agric. Science	Faculty of Life Sciences	Faculty of Engineering	Faculty of Pharmacy	Faculty of Management Sciences	Faculty of Social Sciences
pH	5.29 ± 0.59	5.48 ± 0.29	5.44 ± 0.46	5.21 ± 0.39	5.23 ± 0.34	5.32 ± 0.39	5.25 ± 0.13	5.28 ± 0.24	5.35 ± 0.36	5.57 ± 0.45	5.49 ± 0.43
Conductivity	150.7 ± 0.85	213.77 ± 109.9	221.72 ± 102.9	245.63 ± 118.9	246.5 ± 123.7	251.2 ± 122.3	257.16 ± 114.4	225.5 ± 90.64	218.7 ± 93.82	230.5 ± 95.88	246.8 ± 123.0
NH_4N (mg/kg)	9.79 ± 5.55	13.83 ± 4.94	14.20 ± 5.60	14.34 ± 4.93	11.88 ± 4.17	15.00 ± 4.41	15.41 ± 3.62	13.97 ± 4.38	14.41 ± 5.25	15.37 ± 5.30	14.17 ± 6.45
NO_3 (mg/kg)	9.45 ± 4.24	13.56 ± 6.56	12.61 ± 6.24	12.48 ± 6.03	12.41 ± 4.93	12.53 ± 4.85	12.7 ± 5.28	13.3 ± 6.98	12.24 ± 6.14	11.81 ± 5.27	12.95 ± 6.70
SO_4 (mg/kg)	19.87 ± 4.76	27.71 ± 6.58	29.99 ± 7.60	27.17 ± 8.77	28.35 ± 8.20	25.75 ± 7.60	31.63 ± 6.35	25.59 ± 10.30	26.57 ± 7.79	31.34 ± 8.48	26.94 ± 8.26
Avail. P (mg/kg)	8.76 ± 3.80	11.56 ± 6.69	18.18 ± 13.7	20.97 ± 14.97	21.44 ± 15.83	21.19 ± 17.06	21.07 ± 14.82	17.55 ± 13.43	12.42 ± 7.94	17.77 ± 12.89	11.98 ± 7.47
Na (meq/100g)	0.63 ± 0.23	1.29 ± 1.08	1.56 ± 1.33	1.34 ± 1.05	1.85 ± 1.74	1.36 ± 0.94	1.34 ± 1.15	1.31 ± 1.16	1.21 ± 0.99	1.40 ± 1.18	1.17 ± 0.77
K (meq/100g)	0.21 ± 0.07	0.48 ± 0.20	0.42 ± 0.05	0.48 ± 0.02	0.53 ± 0.17	0.59 ± 0.10	0.65 ± 0.16	0.19 ± 0.10	0.48 ± 0.21	0.34 ± 0.15	0.29 ± 0.06
Ca (meq/100g)	6.83 ± 2.92	11.38 ± 9.04	12.24 ± 9.08	12.15 ± 8.91	11.62 ± 8.84	11.82 ± 8.22	12.44 ± 8.12	11.73 ± 7.87	12.56 ± 8.04	11.6 ± 9.00	11.84 ± 8.41
Mg (meq/100g)	0.41 ± 0.40	0.69 ± 0.57	1.25 ± 1.08	1.14 ± 1.06	1.11 ± 0.95	1.27 ± 0.92	1.42 ± 1.19	0.79 ± 0.68	0.86 ± 0.86	1.15 ± 1.09	0.91 ± 0.81
Sand (%)	87.05 ± 3.77	90.46 ± 0.97	90.82 ± 0.83	90.99 ± 0.31	91.18 ± 0.46	91.32 ± 0.14	90.86 ± 0.55	91.48 ± 0.46	91.02 ± 0.58	90.54 ± 0.98	91.13 ± 0.29
Silt (%)	2.58 ± 1.44	3.33 ± 0.73	3.27 ± 0.80	3.73 ± 0.73	3.95 ± 0.56	3.53 ± 0.77	3.21 ± 0.66	3.54 ± 0.56	3.24 ± 0.80	3.72 ± 0.68	3.28 ± 0.47
Clay (%)	10.38 ± 5.09	6.05 ± 1.66	6.09 ± 0.97	5.06 ± 0.87	5.06 ± 0.92	5.41 ± 0.92	5.94 ± 1.12	4.99 ± 0.92	5.75 ± 1.16	5.69 ± 1.59	5.64 ± 0.91
Fe (ppm)	111.28 ± 8.58	102.7 ± 60.60	92.95 ± 61.07	139.8 ± 111.7	173.34 ± 168.2	95.19 ± 61.97	196.39 ± 264.5	183.8 ± 210.5	134.1 ± 113.01	163.1 ± 150.8	101.9 ± 69.94
Mn (ppm)	13.11 ± 2.87	27.26 ± 13.64	28.73 ± 19.03	35.87 ± 25.98	37.57 ± 27.58	21.9 ± 7.21	37.33 ± 80.15	21.43 ± 7.01	23.09 ± 10.19	27.54 ± 18.72	19.76 ± 6.15
Zn (ppm)	5.59 ± 4.50	9.45 ± 4.60	8.41 ± 2.37	8.71 ± 3.68	9.34 ± 4.82	8.84 ± 4.14	10.61 ± 5.11	8.02 ± 2.68	8.19 ± 2.61	8.25 ± 2.84	8.31 ± 2.68
Cu (ppm)	5.38 ± 0.87	5.75 ± 1.54	5.52 ± 0.86	6.19 ± 1.45	7.06 ± 2.80	6.63 ± 1.61	7.77 ± 2.99	6.65 ± 1.01	6.75 ± 0.97	5.80 ± 3.57	5.22 ± 1.24
Cr (ppm)	0.10 ± 0.13	0.60 ± 0.78	0.51 ± 0.62	0.89 ± 1.05	0.98 ± 0.98	1.08 ± 1.25	1.42 ± 1.45	0.32 ± 0.15	0.68 ± 0.84	0.90 ± 1.13	0.77 ± 1.11
Total Nitrogen (%)	0.20 ± 0.16	0.25 ± 0.12	0.23 ± 0.11	0.18 ± 0.08	0.17 ± 0.07	0.20 ± 0.08	0.14 ± 0.07	0.16 ± 0.06	0.15 ± 0.05	0.14 ± 0.04	0.19 ± 0.06
Exangeable Acidity (meq/100g)	0.47 ± 0.04	0.29 ± 0.05	0.33 ± 0.09	0.32 ± 0.10	0.28 ± 0.11	0.39 ± 0.15	0.33 ± 0.12	0.39 ± 0.14	0.46 ± 0.13	0.31 ± 0.09	0.38 ± 0.16
Organic carbon (%)	0.71 ± 0.03	0.80 ± 0.24	0.91 ± 0.31	0.76 ± 0.32	0.79 ± 0.34	0.79 ± 0.38	0.91 ± 0.23	0.67 ± 0.30	0.74 ± 0.33	0.72 ± 0.26	0.79 ± 0.25

The total heterotrophic bacterial counts ranged from $1.1\times10^5 \pm 0.35$ cfu/g to $5.4\times10^5 \pm 0.56$ cfu/g while the total fungal counts ranged from $1.5\times10^4 \pm 0.42$ cfu/g to $1.9 \times 10^5 \pm 0.14$ cfu/g between August, 2011 and January, 2012. PAI [PAI, 1982] reported that as long as solid wastes disposal is essentially of the simple dumping type, its land pollution effects need to be strongly stressed. The presence of *Staphylococcus* sp. and *Micrococcus* sp. isolated from the dump sites points to health risk associated with solid waste [Eja et al., 2010]. However, the disposal sites were an open dump where aerial pollution may be very high. This implies that the air will be highly polluted and serves as potential sources of air borne infections. The absence of the common bacterial isolates such *E. coli*, *Streptococcus faecalis* and clostridia is not surprising, because the waste were not connected with sewage nor with animal and waste faces, which are primary sources of these organisms. Statistically, the result obtained showed a high significant difference in the total heterotrophic bacterial counts between the sources of sampling, refuse dumpsites from the ten Faculties, and between the months of sampling, August, 2011 to January, 2012. Contrary to the result obtained by Eja et al. [Eja et al., 2003], that there is no significant difference between the sources of samples and between the months of sampling for the total heterotrophic bacterial counts. The Mean total bacterial counts obtained in Faculty of Agricultural Sciences dumpsites showed the highest microbial population across the months of sampling from August, 2011 to January, 2012. The difference in the range of the bacterial isolates can be traced to the composition of the refuse dumpsites and the ability of the microorganisms to survive at these dumpsites [Bowman et al., 1997]. The fungal counts recorded in the Faculties of Physical, Pharmacy, Medical Sciences, Management Sciences and Social Sciences dumpsites showed relatively low fungal population compared to the refuse dumpsites in Faculties of Education, Life Sciences, Arts, Engineering and Agricultural Science. Least significant difference (LSD) test at 5% level of probability showed that, the dumpsites in Faculty of Arts had the highest fungal counts while the least recorded fungal counts were obtained in Faculty of Management Sciences in the month of December, 2011. The results showed that the bacterial strains isolated from refuse dump sites in Uniben carried a plasmid size of 25.1 kb and 12 kb. Lanes 1 - 4 showed the presence of plasmid of size 25.1 kb in *Pseudomonas aeroginosa*, *Micrococcus* sp., *Arthrobacter* sp., and *Bacillus* sp. Lane 5 and 6 containing *Proteus mirabilis* and *Serratia* sp. respectively showed that there are no plasmids present. The plasmids revealed the survival capabilities of the isolates, specifically the bacterial isolates *Pseudomonas aeruginosa* across the refuse collection points. The presence of plasmids was thus influenced by the composition of refuse collection points. The mean values of the physicochemical analyses of the refuse dumpsites are showed in Table 6. It was observed that the pH of the control site or uncontaminated soil was low and acidic compared to the pH of the refuse dumpsites. The mean value of the pH range of the refuse dumpsites in the various Faculties for six months of sampling ranged between 5.21 ± 0.39 and 5.57 ± 0.24 with the highest pH was obtained in the Faculty of Management Sciences and the least pH mean value was obtained in the Faculty of Agricultural Sciences. It has been reported that strongly acidic soils (pH 4 - 5) usually exhibits high concentrations of soluble aluminium and manganese, which are toxic to many plants [Manahan, 1994]. Generally, the pH of the soil tends to increase across the months of sampling from August, 2011 to January, 2012. The percentage concentration of sand is relatively high with mean level of 91.48% in December, 2012 compared to clay with mean level 6.09%. The poorly sorted nature of the various particle sizes may indicate that these soils were not formed from the natural process of weathering of the underlying parent material but rather, from the deposited wastes [Okoronkwo et al., 2006]. Soils with high sand and low clay content have high pollutant leaching potentials. It could therefore be deduced that the underground water in this dumpsites could suffer from pollution as reported by Nyles and Ray [Nyles and Ray, 1999]. The metal contents of Mn, Zn, Cu and Cr found in the refuse dumpsites between August, 2011 and January, 2012, ranged from 19.70 ± 6.15 - 37.57 ± 27.58, 8.02 ± 2.68 - 9.45 ± 4.60, 5.22 ± 1.24 - 7.77 ± 2.99 and 0.32 ± 0.15 - 1.42 ± 1.45 ppm respectively. These results were within the permissible limits according to Pendias and Pendias [Pendias, Pendias, 1992], CCME [CCME, 1999]. According to Welch [Welch, 1980], metals such as Zn, Mn, Cu and Cr are well known toxicants that could occur in a variety of wastes. The mean values of the metal contents relatively increases across the months of sampling indicating the effect of time and season on the dumpsites.

Conclusion

Wastes (refuse collection points) are known to be potent source of contamination of soil and groundwater or even surface water due to leaching and run-off during rains from accumulations of organic matters and toxic sludge.
The result of the study showed the effect of refuse dump waste (refuse collection points) on the microbiological, parasitological and physicochemical qualities on the receiving environment and its potential impact on public health in the University community. The open waste dump at the dumpsites in the institution could constitute sources of microbial and toxic chemical contamination of the dumpsite soil. The effect of the refuse dumps as revealed in the study could be a source of health risk and destruction of biodiversity in the terrestrial and aquatic ecosystems.
It is revealed that, the poor waste management system in the institution could constitute good channels for disease transmission by vectors such as flies, mosquitoes that could result to outbreak of diseases. This study revealed that the composition, storage and disposal of solid waste

(refuse collection points) in the University community potentially poses serious environmental and public health implications, hence the need for proper waste management must be stressed and encouraged.

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