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EFFECTS OF HEAVY METAL CONTAMINANTS FROM WASTE DUMPSITE ON INCIDENCE OF ANTIMICROBIAL RESISTANCE AMONG *ENTEROCOCCUS FEACALIS*

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ABSTRACT
This study investigates the effects of heavy metal contaminants from waste dumpsite on the incidence of antimicrobial resistance among *Enterococcus faecalis.* Soil samples were collected at a depth of 10-20cm from 20 dumpsites at some reference locations in Ado metropolis. The samples were aseptically mixed to form composite samples in triplicates. Strains of *Enterococcus feacalis* were isolated by serial dilution and pour plate methods. Antibiotic susceptibility test was done using 8 palets of gram positive disc; Gentamycin (10µg), Cotrimozazole (25 µg), Chloraphenicol (10µg), Augumentin (30 µg), Streptomycin (10µg), Erythromycin (5 µg), Tetracyclin (10µg) and Cloxacillin (5µg). The resistance pattern in each location for different antibiotics was documented. Soil samples for heavy metal analysis were analyzed using Atomic Absorption Spectrophotometer. Concentration of each heavy metal was compared with WHO guideline limit. Cconcentration of Zn, Pb, Cu, Co and Hg measured (in mg/g) in the soil samples of all the dumpsites in comparison with the reference site ranged from 5.77 to 38.51, 0.54 to 18.33, 1.39 to 11.28, 0.36 to 6.30 and 0.05 to 1.10 respectively. The concentrations of the heavy metals are above the WHO limits for heavy metals in the soil. *Enterococcus feacalis* showed 100.0% resistance to Chloraphenicol, Erythromycin and Tetracyclin; Augumentin (99.0%), Streptomycin (97.0%), Cloxacillin (94.0%), Cotrmozazole (93.0%), and the lowest was Gentamycin with 79.0% resistance. Heavy metal contamination may be attributed to antibiotic resistance capacity in *E. feacalis* present in the dumpsite. Waste management education with a view to promoting waste recycling and recovery from materials with heavy metal contents is advocated.

KEYWORDS: Municipal dumpsite, heavy metals, soil contamination, disc diffusion, antibiotic resistance.

INTRODUCTION
An estimated 7.6 million tons of municipal solid waste is produced per day in developing countries (Adefehinti, 2001). These wastes are disposed in open dumps creating considerable nuisance and constitutes risk to environment and public health. Municipal solid waste (MSW) includes waste generated from residential, commercial, industrial, institutional, construction and demolition processes. These wastes are disposed-off in uncontrolled dumpsites or burnt polluting soil, air and water resources (Odunaiya, 2002). 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. Designated government agency, corporate bodies and some individuals collect wastes routinely into these dumpsites. In Nigeria, modern landfill facilities are not found in these dumpsites; consequently sorting-out of 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 rodents and pollutants such as leachate, which can pollute underground soil bed, and aquifer (Abdus-Salam, 2009). Leachate from dumpsites is of particular interest when it contains potentially toxic heavy metals. Heavy metal is a general term applied to the group of metals and metalloids with an atomic density greater than 5gcm-3 (Ajibosun, 2002). The inorganic pollutants from metals, metal salts, mineral substances, solid particulate matter and other synthetic chemical compound and their by-products pollute soil in the dumpsite (Alloway, 1996; Sharma and Thapaliya, 2009). The occurrence of various heavy metals such as Mn, As, Cr, Cd, Ni, Zn, Co, Cu, and Fe in MSW dumpsites was reported by many workers (Amusan *et al.*, 2005; Esakku *et al.*, 2003; Hoffmann *et al.*, 1991; Ogundiran and Osibanjo, 2008). Introduction of certain concentrations of heavy metals into the environment kills majority of microflora, thereby selecting for a few cells that would have evolved resistance mechanisms to the metals (Awolesi, 2000).

Enterococcus feacalis is a Gram-positive commensally bacterium inhabiting gastrointestinal tracts of human and other mammals. It can cause life threatening infections in humans. The naturally high levels of antibiotic resistance found in *E. feacalis* contribute to its pathogenicity. The ability of *E. feacalis* to cause serious infections has been linked to the intrinsic ruggedness of the bacterium which allows the organisms to persist in the environment, particularly in waste dumpsites and survive many host defenses. The combined expression of metal tolerance and antibiotic resistance is caused by selection, resulting from the metals present in the environment (Hussein *et al.*, 2005). The genes that code for antibiotic resistance trait and genes that code for metal resistance are often carried on the same plasmid or mobile genetic elements (Yilmaz *et al.*, 2004). This shows that there is a close association between metal resistance and antibiotic resistance. A correlation between heavy metal tolerance and antibiotic resistance in *Escherichia coli* and *Staphylococcus* sp. (Spain, 2003). Alonso *et al.* (2000) implicated a cluster of genes to be involved in antibiotic and heavy-metal resistance of a clinical isolate of Gram-negative bacterium, *Stenotrophomonas (Xanthomonas) maltophilia.* Heavy metal contamination and antimicrobial resistance in the environment are threat to public health. Natural and anthropogenic sources account for this contamination. Many approaches have been used to assess the risk posed by the contaminating metals in soils, water bodies etc, but there is dearth of information about the tolerance of soil bacteria to heavy metals, hence this study intends to determine the antibiotic resistance and heavy metal tolerance of *E. feacalis* isolated from municipal dumpsites in Ado Ekiti, Nigeria.

MATERIALS & METHODS

Study area

Ado-Ekiti the state capital of Ekiti State is a city in Southwest Nigeria. The city has long been a commercial and industrial centre among cities, towns and villages in Ekiti state. According to Nigeria 2006 National Census figure, Ado-Ekiti is the largest city in Ekiti state with estimated population of about 2.3 million people (Odeyemi *et al.*, 2010). Ado-Ekiti is situated at 7.62° North latitude, 5.22° East longitude and 456 meters elevation above the sea level. Fig. 1 shows the Ado -Ekiti city and the major roads.

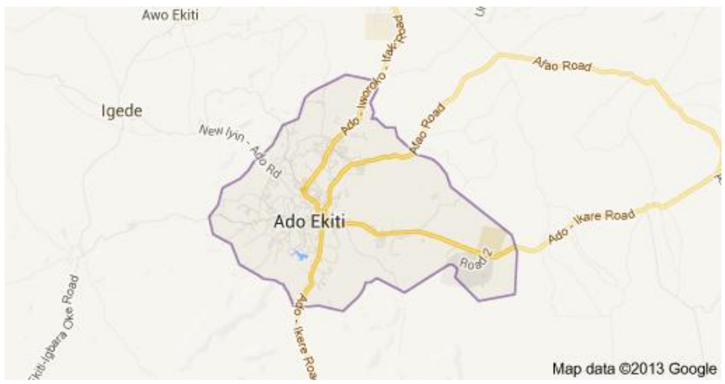

FIGURE 1: Showing map of Ado-Ekiti and the major roads.

Collection of samples

Soils samples were purposively collected within and around Ado-Ekiti metropolis, taking from different directions and also from centre of dumpsites (10-20 cm) at each site. A total of twenty sites (Table 1) were selected. Grab soil samples were mixed to form composite samples in triplicates in each direction and at the centre of dumpsites. A total of 5 samples for each site were analyzed. After bulky materials such as cloth, plastic, glass, rubber, and metal had been sorted out manually, the samples were air dried and ground to pass through a 2-mm sieve. The soil samples were kept in polythene packets for heavy metal analysis.

Methods of lsolation

One hundred (100) strains of *Enterococcus feacalis* were isolated through the combination of serial dilution technique and pour plate methods. One gram of soil sample from the dump site was crushed and slightly heated; it was then transferred into test tube containing 9 ml of distilled water to give a dilution of 0.1ml of inoculum. A serially diluted inoculum of dumpsite sample with a known dilution factor was pipetted into the petri dish and cooled molten bile esculin agar at 45ºC was poured on it. The agar and the inoculums were mixed together by gently swirling the content of the plate. After the agar has solidified, it was incubated for 48 hours at 37ºC to aid maximum growth of the colonies. A distinct cell that grows after the incubation period were aseptically picked with a wire loop and transferred into a slant of nutrient agar to store as pure culture of the isolates.

Antibiotic susceptibility test

The isolates from the slants were first activated in nutrient broth. The nutrient broth were inoculated with the isolates and incubated at 37ºC until the broth becomes turbid. Swab sticks were used to spread the turbid inoculum containing the activated isolate on the nutrient agar plate. The disc diffusion method was used for susceptibility testing as described by the Clinical and Laboratory Standards Institute (2005). The isolates were tested against 8 different commercial antibiotic disks with their concentrations (in µg), Gentamycin (10), cotrimozazole (25), chloraphenicol (10), augumentin (30), streptomycin (10), erythromycin (5), tetracycline (10) and cloxacillin (5). The antibiotic discs were carefully and firmly placed on the inoculated plates using a sterile pair of forceps. The plates were inverted and incubated for 370C for 24 hours. The diameter of the zone of inhibition was measured in millimeters (mm) using a meter rule.

Heavy metals assessment

Total metal concentrations of heavy metals such as copper (cu), zinc (zn), cobalt (co), mercury (Hg) and Lead (Pb) were analyzed. One gram sample of soils were digested separately in the presence of HNO_3 following standard procedures (Ogundiran and Osibanjo, 2008). Atomic Absorption Spectrophotometer (AAS, ECIL-4141) was used to analyze total metal concentrations of digested soil samples. Procedural blanks and international standards were also used where appropriate.

RESULTS & DISCUSSION

The total metal concentrations of selected heavy metals at each dumpsites are given in Table 1. Concentrations differ for each metal across the dumpsite locations. There was an increase in the concentration of the heavy metals in the soil samples from the different dumpsites. In all the dumpsites sampled, Zn has the highest concentrations; 38.51 mg/g-dumpsite D, 37.42 mg/g-dumpsite K and 35.20 mg/g-dumpsite H. This is because the dumpsites are enriched with zinc through metals deposition. High concentrations of zinc have been observed to be specifically toxic to an aquatic insect *Ranatra elongate (*Shukla *et al.*, 1983).

Mean concentration (mg/g) of the heavy metals in all the dumpsites were 18.69 ± 2.3, 4.99 ± 1.2, 6.63 ± 0.7, 1.59 ± 0.4 and 0.1 ± 0.5 for Zn, Pb, Cu, Co and Hg respectively. However, such high variations in concentration had been reported by previous researchers (Nweke *et al.*, 2007).

TABLE 1: The concentration (mg/g) of heavy metals in each dumpsite locations

S/N	Dumpsites	Zn	Pb	Cu	Co	Hg
1	A	15.71	3.25	2.82	1.43	N.D
2	B	19.50	1.22	2.95	N.D	N.D
3	C	10.33	N.D	8.11	2.40	0.05
4	D	38.51	10.10	11.33	6.30	1.10
5	E	13.43	8.31	9.90	2.00	0.14
6	F	20.36	4.25	8.78	N.D	N.D
7	G	27.31	N.D	8.45	4.00	N.D
8	H	35.20	1.11	7.46	1.39	0.25
9	I	27.30	N.D	6.77	N.D	N.D
10	J	9.75	8.02	10.10	5.67	N.D
11	K	37.42	11.36	8.54	1.28	0.50
12	L	20.34	12.36	5.88	N.D	N.D
13	M	25.61	18.33	11.28	3.28	N.D
14	N	18.50	10.15	8.74	N.D	N.D
15	O	5.77	2.35	4.22	1.21	N.D
16	P	8.93	0.54	3.28	0.36	N.D
17	Q	9.48	1.53	6.72	1.06	N.D
18	R	10.37	1.45	1.39	N.D	N.D
19	S	9.58	3.29	2.48	0.52	N.D
20	T	10.50	2.20	3.47	N.D	N.D
	Mean value	18.69±2.3	4.99±1.2	6.63 ±0.7	1.59±0.4	0.1±0.5

Cu=copper, Zn=zinc, Co= cobalt, Hg= Mercury, Pb= lead, N.D=Not detected

Generally, this observation suggests high levels of heavy metal accumulation in the dumpsite. In this regard, the dumpsite represents a significant source of heavy metal contamination of soils, vegetation and underground water in the surrounding environment. Most of the values obtained for the heavy metals in these study locations are above the threshold limit of the recommended WHO guidelines (reported by Alloway in 1996) for heavy metals in the soil, which could be traced to the development of antibiotics resistance potential among the isolated organisms. On average, the concentration of mercury was observed to be below the permissible limt of heavy metals in the soil as recommended by WHO. The investigation of the concentrations of heavy metals in the soil of dumpsites was restricted to the top. This is because the surface of the soil is better indicators of metallic burdens (lderiah *et al.*, 2005).

Also this study confirms that *Enterococcus feacalis* showed 100% resistance to Cloxacillin, Erythromycin and Tetracycline. The high rate of resistance among the organisms could be as a result of widespread use of antibiotics for prophilaxis and therapeutic (as shown in table 2a and 2b) in the neighbourhoods.The prevalence of these erythromycin and tetracycline-resistant enterococci could also be traced to its frequent usage. Uncontrolled community use of antibiotics for empirical treatment of infectious diseases has been implicated as a cause of high prevalence of erythromycin resistant enterococci (Arvanitidou *et al.*, 2001; Odeyemi *et al.*, 2010; Phillips *et al.*, 1990). High prevalence of resistance to erythromycin and tetracycline as presented in this study was also demonstrated by previous studies (Odeyemi *et al.*, 2010; Ferria da Silva *et al.*, 2006; Mondino *et al.*, 2003). Gentamycin showed 79% resistance which proves to be slightly more effective compared to other antibiotics used for this study (Figure 2)..However, our observations is in agreement with the report of Niemi *et al.* (1983) that demonstrated low incidence of antibiotic resistance to gentamycin.

TABLE 2a: Antibiotics resistance pattern of the isolates in each dumpsites location

Location	Resistance pattern	Cot	Chl	Cxc	Ery	Gen	Aug	Str	Tet
A	R	5	5	5	5	5	5	5	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
B	R	5	5	5	5	3	5	5	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	2	0	0	0
C	R	5	5	5	5	4	5	5	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	1	0	0	0
D	R	3	5	5	5	0	4	5	5
	I	2	0	0	0	1	1	0	0
	S	0	0	0	0	0	0	0	0
E	R	4	3	5	5	4	5	5	5
	I	1	1	0	0	0	0	0	0
	S	0	1	0	0	1	0	0	0
F	R	5	5	5	5	4	5	5	5
	I	0	0	0	0	1	0	0	0
	S	0	0	0	0	0	0	0	0
G	R	4	4	5	5	4	5	4	5
	I	0	0	0	0	0	0	0	0
	S	1	1	0	0	1	0	1	0
H	R	3	5	5	5	5	5	5	5
	I	2	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
I	R	5	4	5	5	2	5	5	5
	I	0	0	0	0	1	0	0	0
	S	0	1	0	0	2	0	0	0
J	R	4	5	5	5	4	5	5	5
	I	0	0	0	0	0	0	0	0
	S	1	0	0	0	1	0	0	0

R=Resistance. I=Intermediate, S=Susceptible,
Cot= Cotrimozazole, Chl=Chloraphenicol, Cxc= Cloxacillin, Ery=Erythromycin, Gen=Gentamycin, Aug=Augumentin, Str=Streptomycin, Tet=Tetracyclin.

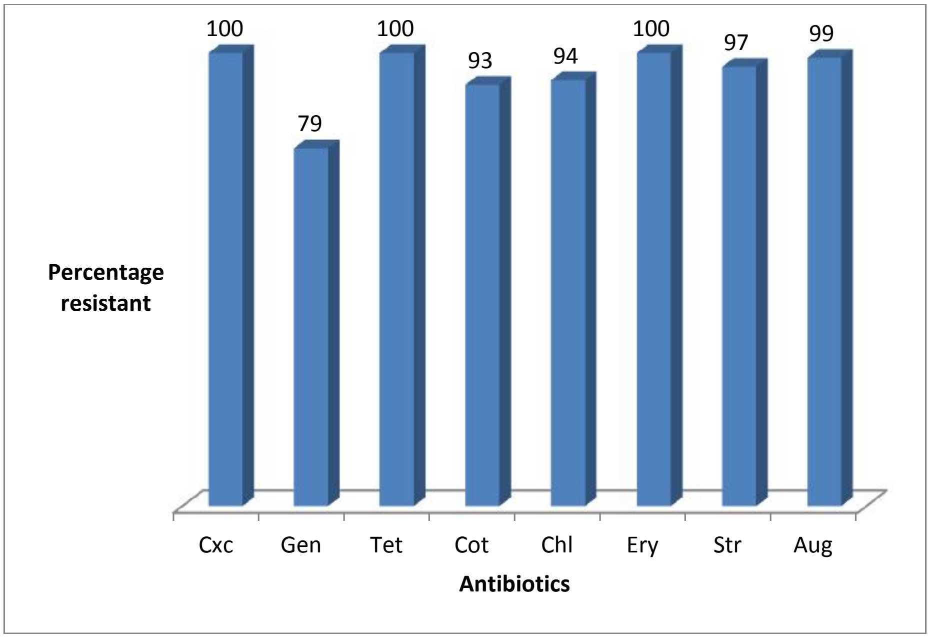

FIGURE 2: Percentage resistant of *E.feacalis* to antibiotics

TABLE 2b: Antibiotics resistance pattern of the isolates in each dumpsites location

Location	Resistance pattern	Cot	Chl	Cxc	Ery	Gen	Aug	Str	Tet
L	R	5	5	5	5	3	5	5	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	2	0	0	0
M	R	5	5	5	5	5	5	5	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
N	R	5	5	5	5	4	5	4	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	1	0	1	0
O	R	5	5	5	5	5	5	5	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
P	R	5	5	5	5	5	5	4	5
	I	0	0	0	0	0	0	1	0
	S	0	0	0	0	0	0	0	0
Q	R	5	5	5	5	5	5	5	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
R	R	5	5	5	5	3	5	5	5
	I	0	0	0	0	1	0	0	0
	S	0	0	0	0	1	0	0	0
S	R	5	5	5	5	5	5	5	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
T	R	5	5	5	5	5	5	5	5
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
U	R	5	3	5	5	4	5	5	5
	I	0	1	0	0	0	0	0	0
	S	0	1	0	0	1	0	0	0

R=Resistance. I=Intermediate, S=Susceptible

Cot= Cotrimozazole, Chl=Chloraphenicol, Cxc= Cloxacillin, Ery=Erythromycin, Gen=Gentamycin, Aug=Augumentin, Str=Streptomycin, Tet=Tetracyclin.

In this study, there is a correlation between the prevalence of multiple resistances among environmental isolates of enterococci and level of heavy metals from municipal dumpsites. The genetic determinant responsible for the heavy metal resistance often resides on plasmids which mediate antibiotic resistance (Sha-Ato *et al.*, 2007). Resistance plasmid enable bacteria to degrade or inactivate antibiotics used to halt bacterial growth by converting it into a less toxic form. Resistance plasmids are currently a topic of intense research because of growing problems with disease causing bacteria that are resistant to penicillin, vancomycin and other commonly used antibiotics (Perez *et al.*, 2008).

The persistence and proliferation of antibiotic resistance in bacterial pathogens represents a considerable public health concern. Subsequent measures to control the emergence and propagation of antibiotic resistance have encountered limited success, and it persists in spite of the restricted use of several key antibiotics, which indicates that there are components governing the evolution, dissemination, and perpetuation of these resistance systems that are yet to be understood (Piccolo and Mbagwu, 1997).

CONCLUSION

Persistent multiple drug resistance of isolated *Enterococcus faecacalis* to appropriate antibiotics of choice are of great public health concern and calls for periodic monitoring of microbial isolates to detect possible changing patterns. Microbiological significance of high resistance among the isolated organisms to antibiotics indicates that the infections acquired from such areas cannot be treated with antibiotics that were used in this study. This could play a significant role in the emergence of resistant bacteria.

Hence, these underlined the need to establish a regular waste monitoring and waste management agencies to recycle the disposed waste to useful products such as biogas, compost etc. Waste management education with a view to promoting the health status of the residents living close to the designated dumpsites will also be important.

REFERENCES

Abdus-Salam, N. (2009) Assessment of Heavy Metals Pollution in Dumpsites in Ilorin Metropolis. *Ethiopia Journal of Environmental Studies and Management*. 2 (2), 92-99.

Adefehinti, O.A. (2001) Toxicity testing of stimulated leachates of solid waste from Olusosun landfill, Ojota, Lagos.

M.sc Thesis, Chemistry Department, University of Ibadan, Oyo State, Nigeria.Pp-104-105.

Ajibosun, T.O. (2002) Sequential extraction of heavy metal in soil from dumpsites in lagos. M.Sc Thesis, Chemistry Department, University of Lagos, Nigeria. Pp 63.

Alloway, B. J. (1996) Heavy metal in the soils. Halsted press,John willey & Sons lnc. London Pp 291.

Alonso, A., Sanchez, P. & Martinez, J.L. (2000) Stenotrophomonas maltophilia D457R contains a cluster of genes from Gram-positive bacteria involved in antibiotic and heavy metal resistance. *Antimicrob. Agents Chemother.* 44, 1778–1782.

Amusan, A.A., Ige, D.V., Olawale, R., (2005) Characteristics of soils and crops' uptake of metals in municipal waste dump site in Nigeria, *Journal of Human Ecology,* 17(3), pp 167-171.

Arvantidou, M., Katsouyannopoulous, V., Tsakris, A. (2001) Antibiotic resistance patterns of enterococci isolated from coastal bathing waters. *J. Med. Microbiol.* 50:1000-1005.

Awolesi A. K. (2000) Chacterization and Toxicity testing in stimulated leachates of soil waste in Olusosun landfill, Ojota, Lagos. M.Sc Thesis, Chemistry Department, University of Ibadan, lbadan. Pp 96.

Esakku, S., Palanivelu, K., Joseph, K., (2003) Assessment of heavy metals in a municipal solid waste dumpsite, Workshop on Sustainable Landfill Management. 3–5 December, Chennai, India, pp 139-145.

Ferreira da Silva, M., Tiago, I., Veríssimo, A., Boaventura, R.A., Nunes, O.C. & Manaia, C.M. (2006) Antibiotic resistance of enterococci and related bacteria in an urban wastewater treatment plant. *FEMS Microbiol. Ecol*., 55:322-329.

Hoffmann, L., Christiansen, K., Petersen, C. (1991) Lead and cadmium contamination of compostable waste derived from source-separated household waste, In: Farmer JG, editor, International conference on heavy metals in the environment, vol. 2. Edinburgh: CEP Consultants Ltd, pp.264–267.

Hussein S., Farag, K., Kandil, H. and Moawad, H. (2005) Tolerance and uptake of heavy metals by Pseudomonads. *Process Biochemistry*, 40:955-961.

lderiah, T.J.K., Braide, S.A., Briggs. A.O. (2005) Distribution of lead and total hydrocarbon in tissues of periwinkles (*Tympanotonus fuscatus* and *Pachymelinia aurita*) in the upper Bonny River, Nigeria. *J. Appl. Sci. and Environ. Mgt* 10 (2): 135- 143.

Mondino, S.S.B., Castro, A.C.D., Mondino, P.J.J., Carvalho, M.G.S., Silva, K.M.F., Teixeira, L.M. (2003) Phenotypic and genotypic characterization of clinical and intestinal *enterococci* isolated from inpatients and outpatients in two Brazilian hospitals. *Microbial. Drug Resist*. 9: 167- 174.

Niemi, M., Sibacov, M., Niemela, S. (1983) Antibiotic resistance among different species of fecal coliforms isolated from water samples. *Appl. Environ. Microbiol.* 45: 79-83.

Nweke, C.O., Ntinugwa, C., Obah, I. F., Ike, S. C., Eme, G. E., Opara, E. C., Okolo, J.C. & Nwanyanwu, C.E. (2007) *In vitro* effects of metals and pesticides on dehydrogenase activity in microbial community of cowpea (*Vigna unguiculata*) rhizoplane *African Journal of Biotechnology* Vol. 6 (3), pp. 290-295.

Pérez Rodríguez, L. (2008) Carotenoid based ornamentation as a dynamic but consistent individual trait. *Behav. Ecol. Sociobiol*. 62, 995-1005.

Phillips, G., Parrat, D., Orange, G.V., Harper, I. McEwan, H,, Yound, N. (1990) Erythromycin-resistant Streptococcus pyogenes. *J. Antimicrobial Chemither*. 25: 723-724.

Odeyemi, A. T., Dada, A.C., Ogunbanjo, O.R. and. Ojo M.A. (2010) Bacteriological, physicochemical and mineral studies on Awedele spring water and soil samples in Ado Ekiti, Nigeria. *African Journal of Environmental Science and Technology* Vol. 4(6), pp. 319-327.

Odunaiya, C.O. (2002) Repositioning solid waste management practices in cosmopolitan Lagos. Lagos state Polythecnic lnaugural lecture Series No 6.Pp 66.

Ogundiran, M.B., Osibanjo, O. (2008) Heavy metal concentrations in soils and accumulation in plants growing in a deserted slag dumpsite in Nigeria, *African Journal of Biotechnology*, 7(17), pp 3053–3060.

Piccolo, A. & Nbagwu J.S.C. (1997) Exogenous humic substances as condition for rehabilitation of degraded soils. Agro-foods lndustry Health Technology Pp 21-28.

Sha Ato, R., Aboho, S.Y., Oketunde, F.O., Eneji, I.S., Unazi, G and Agwa, S. (2007) Survey of solid waste generation and composition in a rapidly growing urban area in central Nigeria. Waste management 27:352-358.

Sharma, M. & Thapaliya, H.P. (2009) Antibiotic profiling of heavy metal resistant bacterial lsolates fro the effluent of a Garment industry in Lalitpul, Nepal. Our Nature 7:203-206

Shukla, G.S. and Omkar, M.R. (1983) Toxicology Letters, 15, 39-41.

Spain, A. (2003) Implications of microbial heavy metal tolerance in the environment. Rev. Undergrad. Res., 2: 1-6.

Yilmaz, E.L. (2003) Metal tolerance and biosorption capacity of Bacillus circulans strain EBI" *Research in Microbiology* 154:409-415