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REGULAR ARTICLE

BACTERIOLOGICAL AND MINERAL STUDIES OF ROAD SIDE SOIL SAMPLES IN ADO-EKITI METROPOLIS, NIGERIA

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ABSTRACT

The bacteriological and heavy metal analyses of road side soil samples in Ado-Ekiti metropolis, Nigeria were investigated. Bacterial population of the soil samples were analyzed using serial dilution procedure and pour plate method for the isolation of organisms. Biochemical tests like catalase, citrate were used to identify the isolated organisms. Atomic absorption spectrophotometry method was used to analyze the heavy metal contents in the soil samples. The mean total bacterial count ranged between 1.60 x 10^2 CFU.g^{-1} and 14.5 x 10^3 CFU.g^{-1}; while the mean total coliform count ranged between 0.3 x10^2 CFU.g^{-1} and 2.0 x 10^2 CFU.g^{-1}. There was significant difference at 0.05 level between the mean total bacteria and total coliform counts of the soil samples at 40meters distance to the major road. There were significant difference within the group of total bacteria and coliform counts at 0.05 level. *Staphylococcus* spp have the highest frequency of occurrence of 41.5% while *Proteus* spp have the least frequency of occurrence of 1%. The heavy metal contents of the road side soil samples ranged as follows: zinc (8.45 - 325.22 mg.kg^{-1}), lead (0.25 - 174.21 mg.kg^{-1}), copper (1.56 - 40.33 mg.kg^{-1}), cobalt (0.37 - 140.33 mg.kg^{-1}), and mercury (0.01 - 4.52 mg.kg^{-1}). Conclusively, heavy metals contents had negative effect on the organisms in the soil and also on the environment.

Keywords: Bacteriological, Roadside soil, Total bacterial, Coliform, Ado-Ekiti

INTRODUCTION

Ado-Ekiti as the state capital of Ekiti is full of commercial and public service activities. For this reason, Ado-Ekiti metropolis is densely populated. Ado-Ekiti attracts the activities of inhabitants of neighbouring towns and villages migrating into it using various automobiles. Vehicular discharge of numerous gaseous and trace metals contaminants due to incomplete combustion of petroleum fuel adversely affects the microbial population and their activities in soil **(Weckwerth, 2001)**. A strong evidence of this phenomenon is the roadside soil that often shows a high degree of heavy metal contamination that can be attributed to motor vehicles **(Weckwerth, 2001)**.

The pollution of soils by heavy metals from automobile sources is a serious environmental issue. Heavy metals are widely distributed in the environment and are not biodegradable but can be transformed into different chemical forms, often with different valance states. Some of the transformation processes result from activities related to industrialization, including combustion of fuels, or other temperature driven reactions associated with motor vehicle performance **(Sawyer, 1998)**. These metals are released during different operations of the road transport such as combustion, component wear, fluid leakage and corrosion of metals **(Khalid *et al*., 2006)**. Lead, cadmium, copper, and zinc are the major metal pollutants of the roadside environments and are released from fuel burning, wear out of tyres, leakage of oils, and corrosion of batteries and metallic parts such as radiators etc. Therefore it is commonly known that high concentrations of these heavy metals occur within 100m of major road highways and near urban centres, hence directly impacting on surrounding soils.

Heavy metals concentrations in soil are associated with biological and geochemical cycles and are influenced by anthropogenic activities such as agricultural practices, industrial activities and waste disposal methods **(Eja *et al.,* 2003; Zauyah *et al.,* 2004)**. Contamination and subsequent pollution of the environment by toxic heavy metals have become an issue of global concern due to their sources, widespread distribution and multiple effects on the ecosystem **(Nriagu, 1990)**. Heavy metals are generally present in agricultural soils at low levels. Due to their cumulative behaviour and toxicity, however, they have a potential hazardous effect not only on crop plants but also on human health **(Das *et al.,* 1997)**.

Very few studies have been carried out in developing countries such as Nigeria and data on pollutant metal concentrations and distribution in such areas are extremely scarce. Therefore, this study was initiated to assess the level of contamination of surface roadside soil

by some heavy metals along a major traffic highway and their effect on microbial population, since there have been no studies about the extent of contamination of the roadside ecosystem by priority heavy metal pollutants. It also considered the antibiotic resistance pattern of isolated bacteria to know the health implication of antibiotic resistant pathogens on the community.

MATERIAL AND METHODS

Collection of samples and sampling site design

Thirty six (36) soil samples were collected from seven major streets across Ado-Ekiti metropolis, Nigeria. These streets are Adebayo Street, Ajilosun Street, Basiri road, Poly road, Ilawe road, Okesa Street, Old and New garage road. At two different points (80 meters apart), three samples were collected from the road into the street (Figure 1). The soil samples were collected for a season of three months (August- October) in the year 2011.

The sampling points were: A_1 (Adebayo roadside II), A_2 (50m away from A_1), A_3 (100m away from A_1). B_1 (Adebayo roadside I), B_2 (50m away from B_1), B_3 (100m away from B_1). C_1 (Basiri roadside I), C_2 (50m away from C_1), C_3 (100m away from C_1). D_1 (Basiri roadside II), D_2 (50m away from D_1), D_3 (100m away from D_1). E_1 (Ilawe roadside I), E_2 (50m away from E_1), E_3 (100m away from E_1). F_1 (Ilawe road I), F_2 (50m away from F_1), F_3 (100m away from F_1). G_1 (Old Garage roadside), G_2 (50m away from G_1), G_3 (100m away from G_1). H_1 (New garage roadside), H_2 (50m away from H_1), H_3 (100m away from H_1). I_1 (Ajilosun roadside I), I_2 (50m away from I_1), I_3 (100m away from I_1). J_1 (Ajilosun roadside II), J_2 (50m away from J_1), J_3 (100m away from J_1). K_1 (Polytechnic roadside I), K_2 (50m away from K_1), K_3 (100m away from K_1). L_1 (Polytechnic roadside II), L_2 (50m away from L_1), L_3 (100m away from L_1). Fifty grams of surface soil of each location were collected into a sterile polythene bag, sealed and labeled accordingly.

Microbiological analysis

The soil samples were serially diluted and sub-cultured on nutrient agar using pour plate techniques and the total bacterial counts determined after 24 hours of incubation at ambient temperature using methods as described by **Olutiola *et al.* (2000)**. Slant agar was prepared in Bijou bottles using nutrient agar. After 24 hours of incubation, the plates were

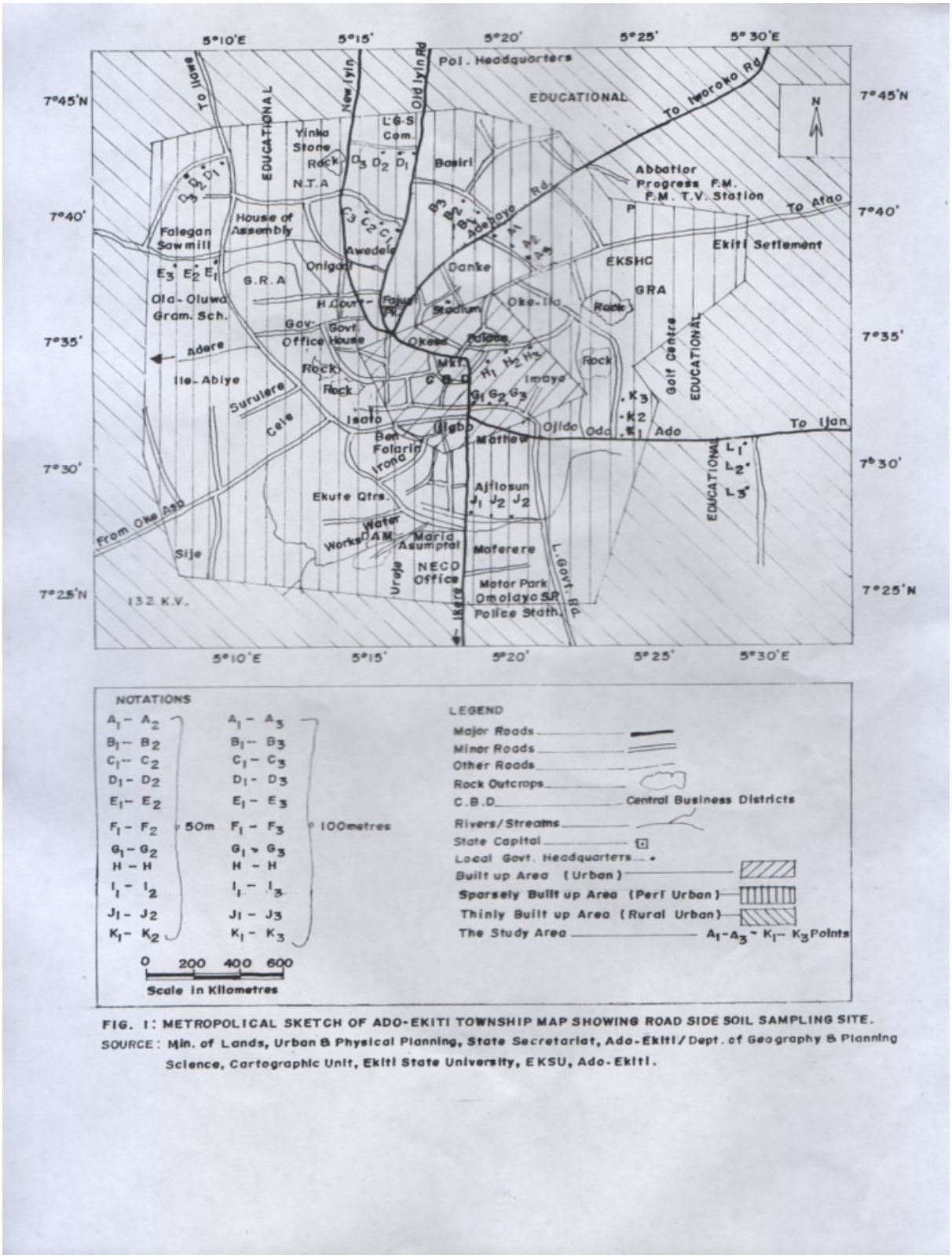

FIG. 1: METROPOLICAL SKETCH OF ADO-EKITI TOWNSHIP MAP SHOWING ROAD SIDE SOIL SAMPLING SITE.
SOURCE: Min. of Lands, Urban & Physical Planning, State Secretariat, Ado-Ekiti / Dept. of Geography & Planning Science, Cartographic Unit, Ekiti State University, EKSU, Ado-Ekiti.

Figure 1 Metropological sketch of Ado-Ekiti township map showing road side soil sampling site

examined for growth and distinct colonies were picked on the incubated plates and sub-cultured on freshly prepared nutrient agar to obtain pure strains which were kept in the sterile slant agar. Pure cultures of isolates were kept on nutrient agar slants at 12°C until used. The isolates were identified on the basis of cellular morphology following Gram stain, and results of biochemical testing, including catalase production, growth in 6.5% NaCl broth, haemolytic activity and motility **(Devriese *et al*., 1992)**.

Antibiotic susceptibility test

The antibiotics susceptibility of the isolates was determined by the disk diffusion method on Mueller-Hilton agar according to **CLSI (2005)**. The bacterial isolates were tested against seven ABTEK disc antibiotics which comprised gram positive antibiotic discs : Cetfazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Lincomycin (2µg), Oxacillin (10µg), Cloxacillin(5µg), Ofloxacin (5µg), Augmentin(30µg), while the gram negative antibiotic discs were: Nitrofurantoin (30g), Cefotaxime (30µg), Gentamicin (10µg), Cetazidime (30µg), Cefuroxime (30µg), Augmentin (30µg), Ofloxacin (5µg), and Amoxicillin (30µg). The inoculum was standardized by adjusting its density to equal the turbidity of a Barium sulphate ($BaSO_4$) (0.5 McFarland turbidity standard), and incubated at 35^{o}C for 18 hours. The diameter of inhibition zone (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted on the basis of CLSI guideline **(CLSI, 2005)**.

Soil digestion

All reagent used were of analytical grade and double distilled water was used in all preparation except otherwise stated. The method of **Ho and Tai (1988)** was used for sample digestions. Samples were sealed in polythene bag and air dried. The samples were grinded using an acid pre-washed mortar and pestle sieved by passing them through a 1 mm mesh. One gram of soil of each of the samples was accurately weighed and treated with 10 ml aliquots of high purity conc. HNO_3. The mixture was on a hot plate until the sample is almost dry and then cooled. This procedure was repeated with another 10 ml conc. HNO_3 followed by 10 ml of 2 M HCl. The digested soil samples were then warmed in 20 ml of 2 M HCl to redissolved the metal salts. Extract were filtered through filter papers and the volume was

then adjusted to 25 ml with doubled distilled water. Metal concentrations were determined by UNICAM SOLAR 32 Data station V7.15 AAS model.

Mineral analysis

Metal analyses were carried out using flame atomic absorption spectrophotometer (GBC Avanta version 1.31). The calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. Average values of three replicates were taken for each determination.

RESULTS AND DISCUSSION

The soil samples collected along the roadside on the major streets of Ado-Ekiti were bacteriologically analyzed. Table 1 shows the mean total bacterial counts and total coliform counts of the various soil samples. The mean values for the total bacterial and coliform counts of the sampling site ranged as 2.1×10^2 CFU/g – 8.5×10^3 CFU/g and 0.2×10^2 CFU/g – 1.5×10^3 CFU/g respectively. The results obtained for the bacteria counts of the road side soil samples in this study ranged between 10^2-10^3 CFU/g of soil, this fell within range as reported by earlier workers **(Ogunwonyi *et al.*, 2008)**. The soil samples were collected for a season of three months (August- October), and from the obtained results, no marked fluctuation was noticed in the total bacterial and coliform counts of the soil samples irrespective of the seasonal variations as reported in the findings of **Ferando (1994)**.

The bacterial counts increased as the samples were collected further into the streets (i.e. the counts increased with increasing distance from the road). This correspond to the work of **Ali (2003)**, when he studied the effect of lead contamination on soil and its organisms in the soils of Razan-Hamadan highway in Iran. This increase in microbial load with increase in distance from the road may be attributable to factors like increased vegetation, reduced traffic, and increased human activities. Presence of vegetation has a great effect on the microbial load present in the particular soil. Root exudates release nutrients into the soil; also there is interaction between plants and soil bacteria and fungi. Some rhizosphere bacteria adhere tightly to soil fungal hyphae, whereas others are directly associated with root surfaces **(Amir, 1998; Bianciotto *et al.*, 2001,)**. Decay of dead plants also increases the organic matter content of the soil, which in turn increase the microbial content of the soil. According to some earlier

researchers, bacteria and other soil microorganisms are cleaners/decomposers **(Anderson, 2005; Prescott, 2008)**.

Table 1 Duncan multiple range test (DMRT) showing the mean bacterial counts of roadside soil samples from Ado-Ekiti metropoli, Nigeria.

Sites	Total bacteria Count (10^2)	Total bacteria Count (10^3)	Total coliform Count (10^2)	Total Coliform Count (10^3)
Adebayo road I	2.1 ± 0.86^{d}	1.6 ± 0.55^{d}	0.3 ± 0.30^{b}	0.2 ± 0.21^{ab}
Adebayo road II	7.1 ± 3.04^{bcd}	4.7 ± 3.12^{bcd}	0.9 ± 0.51^{ab}	0.7 ± 0.12^{b}
Basiri road I	4.8 ± 1.63^{bcd}	3.8 ± 1.39^{bcd}	1.1 ± 1.21^{ab}	0.5 ± 0.46^{ab}
Basiri road II	5.0 ± 2.57^{bcd}	3.1 ± 0.25^{cd}	0.9 ± 0.74^{ab}	0.3 ± 0.42^{ab}
Ilawe road I	5.2 ± 2.41^{bcd}	3.5 ± 1.27^{cd}	1.6 ± 0.59^{ab}	1.2 ± 0.59^{ab}
Ilawe road II	3.8 ± 2.84^{cd}	1.7 ± 1.10^{d}	1.1 ± 0.93^{ab}	0.4 ± 0.55^{ab}
Old garage	9.5 ± 3.72^{b}	5.3 ± 2.08^{abcd}	1.6 ± 1.44^{ab}	0.7 ± 0.64^{ab}
New garage	8.0 ± 2.04^{bcd}	5.7 ± 2.89^{abc}	2.0 ± 0.92^{a}	1.0 ± 0.95^{ab}
Ajilosun road I	6.8 ± 1.61^{bcd}	4.3 ± 2.37^{bcd}	0.2 ± 0.40^{b}	0.03 ± 0.58^{b}
Ajilosun road II	7.3 ± 2.91^{bc}	3.2 ± 1.86^{cd}	0.5 ± 0.42^{ab}	0.3 ± 0.31^{ab}
Poly road I	14.5 ± 4.96^{a}	8.5 ± 2.12^{a}	1.3 ± 0.85^{ab}	0.6 ± 0.98^{ab}
Poly road II	8.5 ± 3.14^{bc}	7.4 ± 2.89^{ab}	1.2 ± 1.06^{ab}	1.5 ± 1.50^{a}

Legend: Groups with the same letter within the columns are not significantly different at 0.05 level.

Human activities increase as we move further into the streets, because of high number of residential houses. The activities of residents that increase soil microbial load include discharge of domestic waste water on roads; presence of open dump site, etc. open dump site by road side is a common phenomenon in Ado-Ekiti. These organisms decompose/degrade these domestic wastes. This increases the organic matter content of the soil, and thus the decomposers present in the soil. This was also explained in the report of **Akpor and Okor (2006)**.

There is thin traffic flow with increased distance from the major roads. As shown in Table 1, the number of microorganisms at points 1 through 3 increased (i.e. number of microbes in A1<A2<A3). Reduced traffic is the reason for this. The heavy traffic flow at the major road points, industrialization and road construction activities in Ado-Ekiti are the reasons for reduced number of microorganisms at the road points. **Garcia and Millan (2002)**

reported the same phenomenon in Spain when they tested the heavy metals contents from the road side soils on the soil microorganisms. The correlation in their findings between soil contamination by heavy metals and the soil microorganisms was that the heavy traffic areas had reduced microbial population while the zones with thin/low traffic had increased number of microorganisms. This is attributable to the factor of release of heavy metals into the soil in the heavily trafficated areas; and these heavy metals are toxic to the microbes. Only the microorganisms that can withstand the toxic effects of these heavy metals can grow in these contaminated soils. Various authors have reported isolating spores of some bacteria like Bacillus and arbuscular mycorrhizal fungal taxa as Gigaspora, associated with heavy metal polluted soils **(Raman *et al.*, 1993; Raman and Sambandan, 1998)**. This, as opposed to the research of some other authors that found no bacteria in heavy metal polluted soil; but found more metal tolerant yeast and fungal cells like *Aureobasidium pullulans*, *Chaetomium* sp., etc **(Monge-Najera *et al.*, 2002; De Jager *et al.*, 2001)**.

The percentage distribution of bacteria isolated from the road side soil samples were *Escherichia coli* 2.83%, *Staphylococcus* spp. 41.5%, *Enterococcus* spp. 12.3%, *Klebsiella* spp. 1.89%, *Proteus* spp. 1%, *Bacillus* spp. 32.01%, *Pseudomonas*, 4.72% and *Serratia* 3.77% (Figure 2). The bacterial species isolated in this study were identified to be similar to those commonly encountered in soil samples as reported by earlier workers **(Ogunmwonyi *et al.*, 2008; Mohan Raj and Ravichadran, 2010)**. Out of the eight genera of organisms isolated, *Staphylococcus* and *Bacillus* species had the highest frequency of occurrence and this corresponds with the work of **(Akpor *et al*., 2006; Abou *et al*., 2008)**. Their works reflected *Bacillus*, *Staphylococcus*, and *Pseudomonas* spp in high pesticide content soil and heavy metal polluted soils. The abundance of these bacteria was typical of soil environment with high species richness and functional diversity.

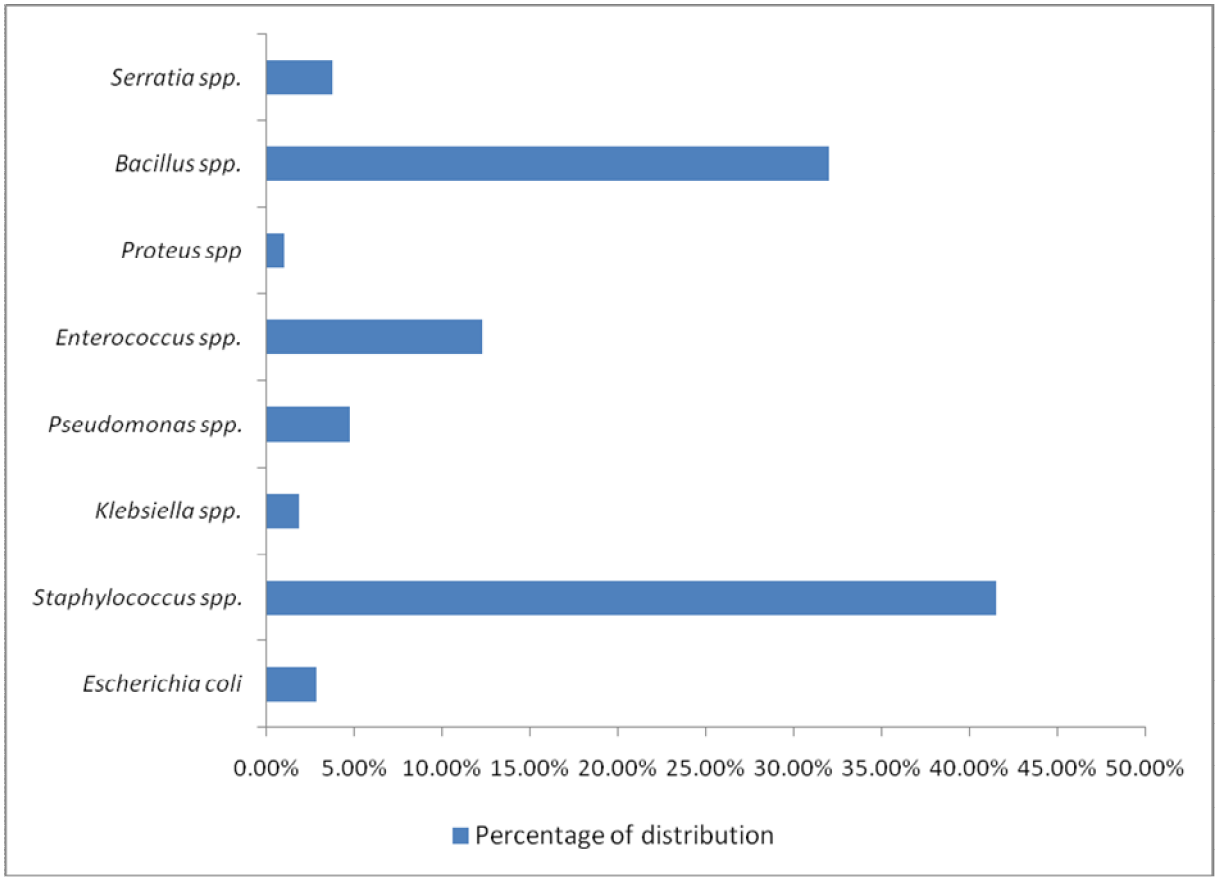

Figure 2 Percentage distribution of bacterial isolates from roadside soil samples from Ado-Ekiti metropoli, Nigeria

Table 2 shows the antibiotics resistant pattern of fifteen tested Gram negative isolates. One organism had 2 antibiotics it was resistant to, 3 organisms were resistant to 3 antibiotics, 4 organisms to 4 antibiotics, 3 organisms to 5 antibiotics, 3 organisms to 6 antibiotics and only one organism showed high resistance pattern to 7 antibiotics. Augmentin had the highest percentage of resistant organisms (100%), as well as cefuroxime (100%), ceftazidime (60%), nitrofurantoin (53.3%), gentamicin (6.6%), amoxicillin (20%), and ofloxacin had the least number of resistance (0%). This implies that ofloxacin was effective on all the test organisms; gentamicin also had quite a great effect, as well as amoxicillin. Table 3 shows the resistance pattern of fourty-four (44) *Staphylococcus* strains isolated from the road side soil samples. Out of 44 isolates of *Staphylococcus* strains, 18 were resistant to ceftazidime, 36 to cefuroxime, 3 to gentamicin, 40 to lincomycin, 38 to oxacillin, 39 to cloxacillin, 40 to augmentin, but none to ofloxacin. Ofloxacin was the best effective antibiotic against the isolates, with no resistance at all to the *Staphylococcus* strains; while lincomycin and augmentin showed the highest frequency of resistance (90.91%). Table 3 also revealed the

antibiotics effectiveness towards Gram positive bacteria. All the 13 *Enterococcus* isolates showed 100% resistance to lincomycin, cloxacillin, and augmentin. Twelve of the 13 organisms were resistant to both cefuroxime and oxacillin, thus showing 92.31% frequency of resistance. Gentamicin and ofloxacin showed the least percentage frequency (15.38% & 7.70%). Twenty nine of the 34 *Bacillus* spp. were resistant to both ceftazidime and cefuroxime (85.30%). 33 organisms to cloxacillin, 32 organisms to lincomycin, 28 to oxacillin, 3 to gentamicin and none to ofloxacin. It can thus be said that cloxacillin had the highest frequency of resistance, while ofloxacin had the least resistance frequency.

The results of the antibiotics resistance noted that there's wide spread of resistance to antibiotics among the isolated organisms. Of all the antibiotics used, ofloxacin and gentamicin proved to be the most effective against the test organisms, as they showed the least frequency of resistance. Ceftazidime, cefuroxime, oxacillin, cloxacillin, augmentin and lincomycin all showed high resistance. As explained in the research of **Christian *et al*., (2004)** and **Odeyemi *et al*., (2010)** that, most antibiotics were gotten from soil organisms, as such; these organisms have derived means to detoxify the effects of these antibiotics, thus having little or no effect on them. She also reported that transfer of antibiotic resistance genes from one organism to the other is a reason for the high antibiotic resistance pattern in these organisms. These antibiotic resistance genes were identified by analyzing the sequences that contain open reading frames (ORFs) that resemble enzymes that confer resistance by inactivation of these antibiotics.

Table 2 Antibiotic resistance pattern of isolated Gram negative bacteria from roadside soil samples from Ado-Ekiti metropoli, Nigeria

Isolates	AUG			AMX			GEN			OFL		
	R	S	I	R	S	I	R	S	I	R	S	I
Escherichia spp	3(100%)	-	-	3(100%)	-	-	1(33.3%)	2(66.7%)	-	-	3(100%)	-
Proteus spp	1(100%)	-	-	1(100%)	-	-	-	1(100%)	-	-	1(100%)	-
Serratia spp	4(100%)	-	-	3(75%)	-	1(25%)	-	4(100%)	-	-	4(100%)	-
Klebsiella spp	2(100%)	-	-	2(100%)	-	-	-	2(100%)	-	-	2(100%)	-
Pseudomonas spp.	5(100%)	-	-	4(80%)	-	1(20%)	-	5(100%)	-	-	2(40%)	3(60%)

Isolates	NIT			CAZ			CRX			CTX		
	R	S	I	R	S	I	R	S	I	R	S	I
Escherichia spp	3(100%)	-	-	1(33.3%)	-	2(66.7%)	3(100%)	-	-	1(33.3%)	2(66.7%)	-
Proteus spp	-	1(100%)	-	-	-	1(100%)	1(100%)	-	-	-	1(100%)	-
Serratia spp	-	4(100%)	-	1(25%)	-	3(75%)	4(100%)	-	-	-	4(100%)	-
Klebsiella spp	2(100%)	-	-	2(100%)	-	-	2(100%)	-	-	2(100%)	-	-
Pseudomonas spp.	3(100%)	-	2(40%)	5(100%)	-	-	5(100%)	-	-	3(60%)	2(100%)	-

Legend: Resistance = R Sensitivity = S Intermediate = I

AUG- augentin NIT- nitrofurantoin CAZ- ceftazidime CRX- cefuroxime

GEN- gentamycin CTX- cefutaxime OFL- ofloxacin AMX- amoxicillin

Table 3 Antibiotic resistance pattern of isolated Gram positive bacteria from roadside soil samples from Ado-Ekiti metropoli, Nigeria

Isolates	AUG			LIN			OXC			OFL		
	R	S	I	R	S	I	R	S	I	R	S	I
Staphylococcus spp	40(90.9%)	1(2.27)	3(6.82%)	40(90.9%)	1(2.27%)	3(6.82%)	38(86.4%)	2(4.55)	4(9.09%)	-	42(95.5%)	2(4.55%)
Bacillus spp	30(88.2%)	2(5.88%)	2(5.88%)	32(94.1%)	-	2(5.88%)	28(82.4)	2(5.88%)	4(11.8%)	-	32(94.1%)	1(2.94%)
Enterococcus spp	13(100%)	-	-	13(100%)	-	-	12(92.3%)	-	1(7.69%)	1(7.69%)	11(84.6%)	1(7.69%)

Isolates	CXC			GEN			CAZ			CRX		
	R	S	I	R	S	I	R	S	I	R	S	I
Staphylococcus spp	38(86.4%)	2(4.55%)	4(9.09%)	3(6.82%)	40(90.9%)	1(2.27%)	18(40.9%)	11(25%)	15(34.1%)	37(84.1%)	1(2.27%)	6(13.6%)
Bacillus spp	33(97.1%)	-	1(2.94%)	3(8.82%)	31(91.2%)	-	29(85.3%)	1(2.94%)	4(11.8%)	29(85.3%)	-	5(14.7%)
Enterococcus spp	13(100%)	-	-	2(15.4%)	11(84.6%)	-	8(61.5%)	3(23.1%)	2(15.4%)	12(92.3%)	1(7.69%)	-

Legend: Resistance = R Sensitivity = S Intermediate = I

CAZ- ceftazidime CRX- cefuroxime LIN- lincomycin OXC- oxacillin

CXC- cloxacillin OFL- ofloxacin AUG- augmentin GEN- gentamycin

The analysis of the heavy metal of the road side soil samples at the main road points revealed that zinc values ranged between 76.4-325.2 $mg.kg^{-1}$ of soil, with a mean of 167.0 $mg.kg^{-1}$. Lead had a range of 76.5- 174.2 $mg.kg^{-1}$ with a mean value of 167.0 $mg.kg^{-1}$. Cobalt ranged between 28.5-140.0 $mg.kg^{-1}$ and a mean value of 104.3 $mg.kg^{-1}$. Mercury had a range of 1.38-4.52 $mg.kg^{-1}$ and a mean value of 0.69 $mg.kg^{-1}$, and copper had a range of 11.3-40.3 $mg.kg^{-1}$ of soil and a mean value of 22.6 $mg.kg^{-1}$ (Table 4).

Table 5 shows the heavy metal analysis values of road side soil samples 100m away from the road points. Zinc had a range of 9.26-30.3 $mg.kg^{-1}$ of soil and a mean value of 16.2 $mg.kg^{-1}$. Lead ranged from 0.25-4.35 $mg.kg^{-1}$ with a mean value of 22.2 $mg.kg^{-1}$. Copper had a range of 1.56-7.50 $mg.kg^{-1}$ of soil and a mean value of 4.42 $mg.kg^{-1}$. Cobalt ranged between 0.37-2.54 $mg.kg^{-1}$ of soil with a mean value of 1.24 $mg.kg^{-1}$. Mercury ranged between 0.01-0.10 $mg.kg^{-1}$ of soil, and a mean value of 0.01 $mg.kg^{-1}$.

The results of the heavy metal analysis indicated higher amount of heavy metals in the soil samples by the road-side. The amount of zinc in the road side soil ranged from 76.4-325.2 $mg.kg^{-1}$ and a mean value of 167.0 $mg.kg^{-1}$. **Khalid *et al.* (2006)** reported the amount of zinc in the road side soils of northern Europe to range from 56.7-480 $mg.kg^{-1}$. Normal concentrations of zinc in soils range from 1-900 $mg.kg^{-1}$ **(Alloway, 1995; Sparks, 2003)**. **McGrath and Loveland (1992)** reported that zinc concentrations in soils of Wales and England ranged from 5-3648 $mg.kg^{-1}$ with a median value of 82 $mg.kg^{-1}$. Zinc values of soil samples within the streets ranged from 8.45-30.3 $mg.kg^{-1}$ with a mean value of 16.2 $mg.kg^{-1}$. From the normal range of 1-900 $mg.kg^{-1}$, the results of this present study fall within range while there was a significant correlation between this study and previous studies. The road points showed high values of zinc than the points 100m away from the road. This is attributable to release of this metal into the soil from the wear of car tyres, road construction activities and the use of petrol/diesel driven vehicles **(Fakayode *et al.*, 2003)**.

Table 4 Heavy metal content ($mg.kg^{-1}$) of roadside soil samples from Ado-Ekiti metropoli, Nigeria

Soil samples	Minerals				
	Zn	Pb	Cu	Co	Hg
A_1	97.7	105.3	25.3	ND	ND
B_1	76.4	86.2	32.4	ND	ND
C_1	272.0	128.4	20.3	28.6	ND
D_1	173.5	100.3	20.9	113.2	4.52
E_1	295.2	121.4	23.4	123.2	1.38
F_1	98.8	78.5	40.3	115.3	ND
G_1	325.2	174.2	11.3	ND	ND
H_1	145.7	99.4	18.4	140.0	ND
I_1	123.4	103.2	27.3	113.2	ND
J_1	140.3	76.5	22.5	103.5	2.33
K_1	122.5	89.7	11.5	120.3	ND
L_1	133.5	88.5	16.9	107.5	ND
Mean value	167.0	104.3	22.6	80.4	0.69

Legend: Zn: zinc, Pb: lead, Cu: copper, Co: cobalt, Hg: mercury, ND: not detected

A-L = Soil samples; 1= Roadside sampling point

Table 5 Heavy metal content (mg.kg^{-1}) of soil samples 100metres away from roadside, Ado-Ekiti metropoli, Nigeria

Soil samples	Minerals				
	Zn	Pb	Cu	Co	Hg
A_3	11.7	1.31	3.32	1.20	ND
B_3	12.4	0.54	4.50	2.25	ND
C_3	19.5	0.25	1.56	ND	ND
D_3	21.3	2.31	2.82	0.37	0.01
E_3	23.6	1.24	5.21	1.32	ND
F_3	30.3	8.21	6.33	2.10	0.01
G_3	23.6	4.35	7.50	1.32	0.10
H_3	12.7	2.18	3.45	0.38	0.02
I_3	10.5	1.48	2.85	1.35	ND
J_3	9.26	0.37	6.28	0.75	ND
K_3	8.45	1.21	1.88	2.54	ND
L_3	10.6	3.18	7.28	1.29	ND
Mean value	16.2	22.2	4.42	1.24	0.01

Legend: Zn: zinc, Pb: lead, Cu: copper, Co: cobalt, Hg: mercury, ND: not detected

A-L = Soil samples; 3 = 100m sampling point to the roadside

The amount of lead from the soil samples by the road side ranged from 76.5-174.2 mg.kg^{-1} with a mean value of 104.3 mg.kg^{-1}, whereas the lead values of the soil sample into the street ranged 0.25-8.21 mg.kg^{-1}; with a mean value of 22.2 mg.kg^{-1}. According to the report of **Ali (2003)**, the median value of lead concentrations on road side soil of Iran highway was 85.01 mg.kg^{-1} at road side points and about 64.48 mg.kg^{-1} at 100m distance from the road points. Normal range for lead concentration ranged from 2-300 mg.kg^{-1} **(Alloway, 1995)**. McGrath reported 75 mg.kg^{-1} as mean value for lead in urban top soils of Wales. Considering

the general range of total lead content in 80% road side soils and this research study, it can be deduced that only few soils from road side were heavily contaminated. In the last decades, much attention has been directed towards lead in road side environments as a result of its wide spread use as antiknock agent in gasoline.

The amount of copper ranged between the values 11.3 $mg.kg^{-1}$ and 40.3 $mg.kg^{-1}$ with a mean value of 22.6 $mg.kg^{-1}$, and the value at 100m distance from the road ranged 1.56-7.50 $mg.kg^{-1}$ with a mean value of 4.42 $mg.kg^{-1}$. **Park *et al.*, (2006)** reported a value range of 21.5-28.2 $mg.kg^{-1}$ for copper in the soil of Ginkobiloba in Korea city. In France, **Dachaufour (1998)** reported copper value as 100 $mg.kg^{-1}$. In India, **Singh and Saha (1997)** reported the range value of copper in India soil as 1.18- 70.2 $mg.kg^{-1}$. The normal range falls between 0-250 $mg.kg^{-1}$ **(Alloway, 1995; Sparks, 2003)**. **Khalid (2006)** reported copper values ranged as 15- 240 $mg.kg^{-1}$ in England roadside soils. Comparing recent studies with the values of this research study, there is a decrease in the values obtained in this research from the background studies. It can be deduced that road side soils in Ado-Ekiti metropolis are not heavily contaminated with copper and still falls within range allowable for copper in soils 2-250 $mg.kg^{-1}$ **(Sparks, 2003)**. Copper, like other metals showed higher concentration at the road points with a significant decrease 100metres away from the road points.

Cobalt amount in the soil samples ranged between 28.54 $mg.kg^{-1}$ and 140.03 $mg.kg^{-1}$ with a mean value of 80.42 $mg.kg^{-1}$ whereas in the soil samples 100m away from the road, the values ranged between 0.37 $mg.kg^{-1}$ and 2.54 $mg.kg^{-1}$ with a mean value of 1.24 $mg.kg^{-1}$. Mercury had a range of 1.38-4.52 $mg.kg^{-1}$ with a mean value of 0.69 $mg.kg^{-1}$. There was a range of 0.01-0.10 $mg.kg^{-1}$ in soil samples 100m away from the road, with a mean value of 0.01 $mg.kg^{-1}$. Like other heavy metals, there was significant decrease in cobalt and mercury content with increased distance from the road side. This is also attributable to the factor of road construction activities and heavy traffic at the main road, as compared to less traffic in the streets.

Comparing the bacterial counts and heavy metal analysis results, it can be deduced that the content of these metals in the road side soils affected the microbial load of these soils. Microorganisms isolated are far more sensitive to heavy metal stress than other soil organisms **(Ghorbani *et al.*, 2002)**. Increasing urbanization will increase the content of these metals over the years. With proximity of houses to roads, these metals could be washed into wells through percolation or via cracks in wells. Consumption of this contaminated well water could in turn affect/pose a risk to the health of the public.

CONCLUSION

It can be concluded that the amount of these heavy metals in Ado-Ekiti road side soils had a significant negative effect on the microorganisms in the soil due to bioaccumulation and biomagnifications. If left to over accumulate, becomes a serious problem that poses risk to the health of Ado-Ekiti residents and the microbial load in the soils.

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REFERENCES

ABOU, R. – SHANAB, A. – GHANEM, K. – KALAIBE, A. D. 2008. The role of bacteria on heavy metal by plants growing on multi – metal contaminated soils. In *World J. Microbial, Biotechnol.,* vol. 24, 2008, p. 253-262.

AKPOR, O. B. – OKOH, A. I. – BABALOLA, G. O. 2006. Cultural microbial population dynamic during decomposition of *Theobroma cacao* leaf litters in a tropical soil setting. In *J. Bio. Sci.*, vol. 6, 2006, no. 4, p. 768-774.

ALI, A. S. S. 2003. Lead contamination and impacts on microorganisms in the vicinal soils of Razan-Hamadan highway, Iran. Proceedings of the fourth International Iran and Russian conference, 2003,
p. 463-469.

ALLOWAY, B. J. 1995. Heavy metals in soils 2nd Ed. Blackie Academic and Professional, London, 1995.

AMIR, H. – PINEAU, R. 1998. Influence of plants and cropping on microbiological characteristics of some new Caledonian Ultramafic soils. In *Australian Journal & soil Resource,* vol. 36, 1998, no. 3,
p. 457-470.

ANDERSON, J. P. E – DOMSCH, K. H. 2005. A physiological method for the quantitative measurement of microbial biomass in soils. In *Soil Biology and Biochemistry Journal*, vol. 10, 2005,
p. 214–221.

BIANCIOTTO, V. – ANDREOTTI, S. – BALESTRINI, R. – BOFANTE, P. – PEROTTO, S. 2001. Mucoid mutants of the biocontro strain *Pseudomonas fluorescens* CHAO show increased ability in biofilm formation on mycorrhizal and non mycorrhizal carrot roots. In *Molecular Plant-Microbe Interraction*, vol. 14, 2001, p. 255-260.

CHRISTIAN, S. – RIESENFELD, R. – GOODMAN, M. – HANDELSMAN, J. O. 2004. Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. In *Environmental microbiology*, vol. 6, 2004, no. 9, p. 981–989.

CLSI. 2005. Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement, Clinical and Laboratory Standard Institute Wayne, Pa. M100-S15, vol. 25, 2005, no.1.

DAS, P. – SAMANTARARY, S. – ROUT, G. R. 1997. Studies on Cadmium Toxicity in Plant. In *A Review. Environ. Pollut.*, vol. 98, 1997, p. 29 - 36.

DE JAGER, E. S. – WEHNER, F. C. – KORSTEN, L. 2001. Microbiolecology of the mango phylloplane. In *Microbiology and Ecology*, vol. 42, 2001, p. 201-20.

DEVRIESE, L. A. – COLLINS, M. D. – WIRTH, R. 1992. The genus *Enterococcus*. In The Prokaryotes: a Handbook on the Biology of Bacteria, Ecophysiology, Isolation, Identification, Applications, 2nd edn, vol. 2, 1992, p. 1465-1481.

DUCHAUFOUR, P. 1998. Handbook of Pedology: Soils, Vegetation, Environment. Oxford and IBH Publishing Co. Private. Ltd., New Delhi,1998, 264 pp.

EJA, M. E. – OGRI, O. R. – ARIKPO, O. E. 2003. Bioconcentration of Heavy Metals in surface sediments from the Great Kwa River Estuary, Calabar, Southeastern Nigeria. In *J. Nig. Environ. Soc.*, vol. 1, 2003, p. 247 - 256.

FAKAYODE, S. O. – OWOLABI, B. I. 2003. Heavy metal contamination of roadside topsoil in Osogbo, Nigeria: its relationship to traffic density and proximity to highways. In *Environmental Geology*, vol. 44, 2003, p. 150-157

FERANDO, H. C. – AMANDA, V. – WRIGHT, J. S. 1994. Tropical forest litter decomposition under seasonal drought nutrient release, fungi and bacteria. In *Oikos,* vol. 70, 1994, p. 183 – 190.

GARCIA, R. – MAIZ, I. – MILLAN, E. 1996. Heavy metal contamination analysis of road side soils and grasses from Guipuzcoa Spain. In *Science of Total Environmental*, vol. 17, 1996, no. 7, p. 763-770.

GHORBANI, N. R. – SALEHRASTIN, N. – MOEINI, A. 2002. Heavy metals affect the microbial populations and their activities.17th WCSS, Thailand, In *Symposium 54*, 2234:1-11.

HO, Y.B - TAI, K.M. 1988. Elevated levels of lead and other metals in roadside soils and grass and their use to monitor aerial metal depositions in Hong Kong. In *Environ. Pollut. J.*, vol. 49, 1988, p. 37-51.

KHALID, F. A. – WILLIAM, H. G. – HALE, A. D. H – MOHAMMAD, A. 2006. Heavy Metal Contamination of Roadside Soils of Northern England. In *Soil and Water Res.*, vol. 1, 2006, no. 4, p. 158–163.

MCGRATH, S. P. – LOVELAND, P. J. 1992. The Soil Geochemical Atlas of England and Wales. Blackie Academic and Professional, London, 1992.

MOHAN, R. A. J. – RAVICHADRAN, K. 2010. Study of soil microflora indicating pesticide contamination of Cauvery River belt in India. In *Indian Journal of Science and Technology*, vol. 3, 2010, p. 80-82.

MONGE-NAJERA, J. – GONZALEZ, M. I. – RIVAS, R. – MENDEZ-ESTRADA, V. H. 2002. Twenty years of lichen cover change in a tropical habitat (Costa Rica) and its relation with air pollution. In *Revised Biological and Tropical Resource*, vol. 50, 2002, p. 309-319.

NRIAGU, J. O. 1990. Global metal pollution poisoning the atmosphere. In *Environ.*, vol.7, 1990, p. 87 - 93.

ODEYEMI, A. T. – DADA, A. C. – OGUNBANJO, O. R. – OJO, M. A. 2010. Bacteriological, physicochemical and mineral studies on Awedele spring water and soil samples in Ado Ekiti, Nigeria. In *African Journal of Environmental Science and Technology,* vol. 4, 2010, no. 6, p. 319-327.

OGUNMWONYI, I. N. – IGBINOSA, O. E. – AIYEGORO, O. A. – ODJADJARE, E. E. 2008. "Microbial analysis of different top soil samples of selected site in Obafemi Awolowo Univ. Nigeria." Academic Journals, 3, 2008, 3, 120-124.

OLUTIOLA, P. O. – FAMUREWA, O. – SONNTAG, H. S. 2000. An introduction to General Microbiology (A practical Approach). In *Measurement of microbial growth*, 2000, p. 101-111.

PARK, C. – LEE, M. – LEE, B. – KIM, S. W. – CHASE, H. A. – LEE. J. – KIM, S. 2006. Biodegradation and biosorption for declolrization of synthetic dyes by *Funalia trogii*. In *Biochem. Eng. J.*, 2006, doi:10.1016/j.bej.

PRESCOTT, L. M. – HURLEY, P. J. – KELVIN A. D. 2008. Microbiology. 7th edn. 2008, 4, pp.876-877.

RAMAN, N. – SAMBANDAN, S. 1998. Distribution of VAM fungi in tannery effluent polluted soils Tamil Nadu, India. Environmental Contamination & Toxicology, vol. 60, 2008, p. 142-150.

RAMAN, N. – NAGARAJAN, N. – GOPINATHAN, S. – SAMBANDAN, K. 1993. Mycorrhizal status of plant speciescolonizing a magnesite mine spoil in India. In *Biology and Soil Fertility*, vol. 16, 1993, p. 76-78.

SAWYER, R. 1998. Attachment A: summary of a workshop on metal based fuel additives. University of California, Berkely, 1998.

SINGH, M. V. – SAHA, J. K. 1997. 27th Annual report of the All India coordinated research project of micro and secondary nutrients and pollutant elements in soils and plants. Institute of Soil Science, Bhopal, 1997, 108 pp.

SPARKS, L. D. 2003. Ion exchange processes. Environmental soil chemistry. Academic press London.

Weckwerth, G. 2001. Verification of traffic emitted aerosol components in the ambient air of Cologne In *Germany.Atmos. Environ.*, vol. 35, 2003, p. 5525-5536.

ZAUYAH, S. – JULIANA, B. – NOORHAFIZAH, R. – FAUZAH, C. I. – ROSENAMI, A. B. 2004. Concentration and Speciation of Heavy Metals in some Cultivated and Uncultivated Ultisols and Inceptisols in Peninsular Malaysia, Super-Soil 3rd Austrlian New Zealand Soils Conference, University of Syndney Australia, 2004.