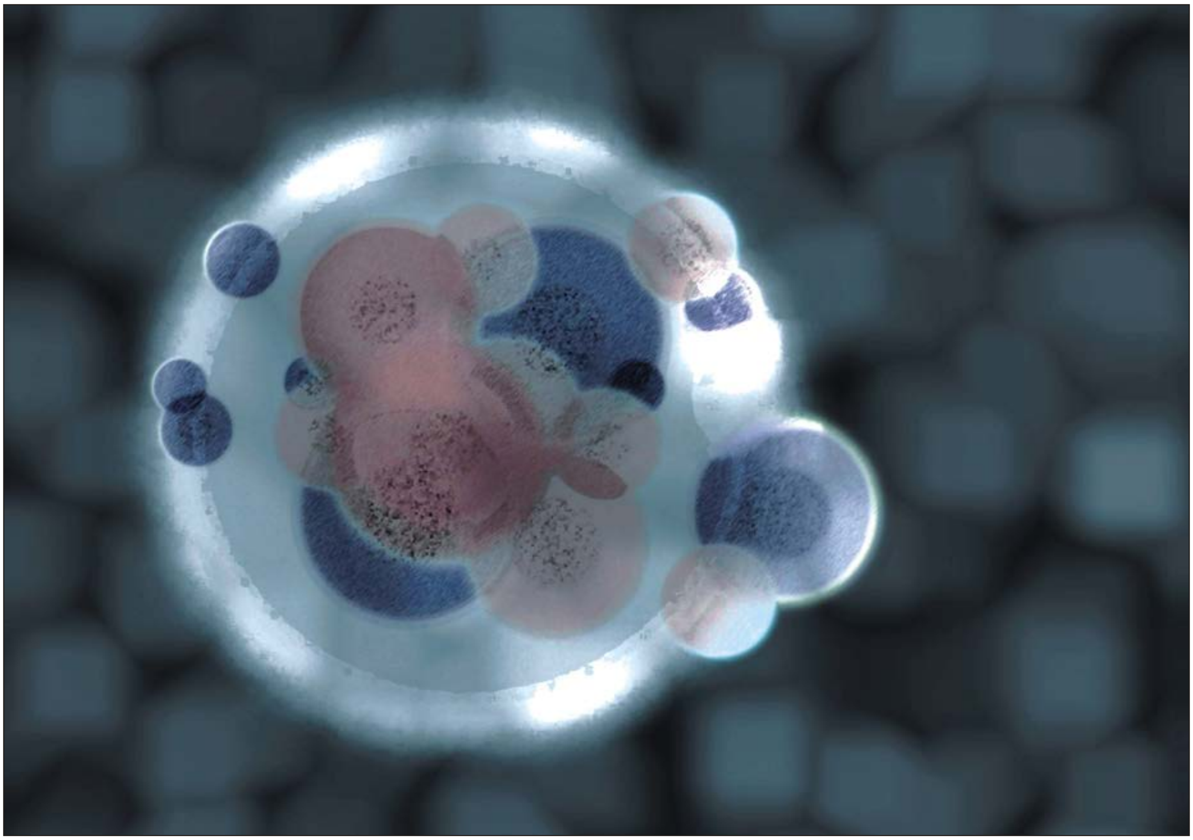

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Trace Metal Content in Relation to Population of Microorganisms in Soils along Some Highways in Nigeria's Guinea Savanna

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Abstract: Soil samples from three different locations in the Nigerian Guinea Savanna were analyzed in a study of the relationship between soil trace metal levels and soil microbes with respect to distance from the roadside and season. The three different sites were selected based on their vehicular traffic density. Soil microbes were analyzed by pathological techniques while trace metal levels (Pb, As, Zn, Cu, Co, Br and Ni) were analyzed using the energy dispersive X-ray fluorescence. Populations of soil microbes increased with increasing distance from the roadway, while metal contents decreased with increasing distance from the roadway for both dry and wet seasons. The soil microbes and trace metal content were higher for the wet season than for the dry season. Interactions between soil microbe populations and trace metal contents were statistically highly significant ($p=0.05$ and $p=0.01$) for season, location and interaction of distance, season and location. The mean increase of soil microbes between the dry and wet season were also highly significant. Five species of fungi were identified. *Aspergillus* sp., *Monilia* sp., *Penicillium* sp., *Helminthosporium* sp. and *Rhizoctonia* sp. while the bacteria were mainly *Bacillus* sp. These species responded to the presence of trace metals in the soil and may thus serve as microbial indicator species for metal pollution levels in the Nigerian Savanna.

Key words: Trace metals, soil microbes, distance from roadside, seasons

INTRODUCTION

Soil microorganisms play significant roles in nature and are critical to ecosystem function and the well being of plants, animals and humans. They also function as biogeochemical agents for the maintenance of life on earth. Trace metals are naturally present in the biological world in acceptable quantities, but increase of these through anthropogenic contributions, has since the last century, been known to affect microbial growth, numbers, survival, biomass and abundance[1-4]. Extensive literature is available on the effect of heavy metals on microbial population and microbial processes[5-7]. However, tolerance and adaptation of microorganisms to trace metals are common phenomena and the presence of tolerant fungi and bacteria in polluted environments has frequently been observed[8,9]. One of the main sources of environmental trace metal load is vehicular emission[10] from internal combustion engines of automobiles. In many urban areas, analyses of roadside soils have shown that the concentration of heavy metals decreases with increasing distance from the roadway and is related to traffic volume[11,12]. In Nigeria, not much work has been done especially with reference to microbial population and trace metal content/levels on roadside soils.

The aim of this study was to determine whether the metals contained in the roadside soils had effect on the microbial population with respect to increasing distance from the roadway. This will serve as a database for future monitoring programs.

MATERIALS AND METHODS

Three sampling or study locations were selected based on their vehicular traffic densities. Soil samples were collected at vertical depths of 15 cm at distances of 0-20, 20- 40, 40-60, 60-70 and 70-80 m from the edge of the roads at three locations namely, Zaria- Funtua/Sokoto road-(L_1)(10,720 vehicles/day) and Zaria-Kano road-(L_2)(12,440 vehicles/day) representing the high traffic sites and the road to the Ahmadu Bello University Dam (L_3)(296 vehicles/day) representing the low traffic site. Soil samples were collected in three replicates during the dry (February-March) and wet (August-September) season, 2003. Triplicate soil samples for the wet season were oven-dried and ground. The ground soil samples were taken to Centre for Energy Research and Training (CERT) at Ahmadu Bello University, Zaria for analyses of Pb, As, Cu, Co, Zn, Ni and Br levels, using the energy dispersive X-ray fluorescence. In this procedure,

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samples were ground to fine powder in a micro-blender. Subsequently, about 40 g sub-sample from each sample was weighed out on a Mettler balance and placed in a Pyrex glass beaker. These were covered and put in a Muffle furnace at 550°C for ashing. Ashed materials (0.1-0.9 g) were then pelleted at 10 tons pressure and subjected to elemental analyses. This system consisted of a 925 MBq ^{109}Cd annular isotopic source, which emits Ag-K X-rays (22.1 keV). The X-ray spectra were acquired with a computer based MCA card; Sensitivity calibration of the system was performed using thick pure metal foils (Ti, Fe, Co, Ni, Cu, Zn, Nb, Mo, Sn, Ta and Pb) and stable chemical compounds (K_2CO_3, $CaCO_3$, Ce_2O_3, WO_3, ThO_2 and U_3O_8). The measurement time of 5000s was used for each sample and spectral analysis was performed with an AXIL program[13] from a QXAS software package. The International Atomic Energy Agency distributed this package. Other details of the measurement are as described by Funtua[14].

The accuracy and precision of the measurements were confirmed though an analysis of IAEA-V10 (hay powder) and IAEA-359 (cabbage) certified reference material, distributed by International Atomic Energy Agency (IAEA). There was a general good agreement between measured and certified values (Table 1). Uncertainties in the measurement included errors from counting statistics, calibration error and uncertainty of the absorption correction factor. Results obtained were subjected to statistical analyses according to Parker[15].

The portion of soil retained for the isolation and enumeration of soil microorganisms was analyzed using standard pathological techniques. Bacteria were plated out on nutrient agar in duplicates while fungi were plated on Potato Dextrose Agar (PDA) using chloramphenicol as a bacteriostatic agent. Soil samples were screened and homogenized. Sub-samples of one gram were weighed and transferred to sterile distilled water blanks, shaken and allowed to settle before serial dilution (1:1000000) was made. Dilutions were mixed and poured into sterile petri plates containing the appropriate media. Plates were rotated carefully to ensure uniform distribution, allowed to solidify and then incubated at room temperature. Counts were made between 3 to 7 days using a colony counter[16,17].

Table 1: Precision of measurement ranges for elements (ppm) in IAEA-V-10 (hay powder) and IAEA-359 (Cabbage) compared with certified values

Metal	IAEA-V10 (Measured)	IAEA-V10 (Certified)	IAEA-359 (Measured)	IAEA-359 (Certified)
Cr	LOD	6.5±1.5	LOD	1.3±0.120
Mn	43±12.0	47.0±7	30±3	31.9±1.200
Fe	152±14.0	186.0±13	136±8	148.0±7.800
Ni	LOD	4.2±1.1	LOD	1.05±0.100
Cu	11±04.0	9.4±0.9	6±2	5.67±0.360
Zn	22±04.0	24.0±2	39±3	38.6±1.400
As	LOD	-	LOD	0.1±0.008
Cd	LOD	-	LOD	0.12±0.022
Sb	LOD	0.009±0.002	LOD	-
Pb	LOD	1.6±1.100	LOD	-

LOD= Limit of Detection

RESULTS AND DISCUSSION

Trace metal contents of roadside soils: Trace metal (Pb, As, Cu, Co, Ni, Zn and Br) contents of the soil decreased, as distance from the roadway increased during both dry and wet season (Table 2). For all the three sites, the wet season samples had the higher trace metal contents than the dry season samples. For both seasons Co and Br, were quantitatively the highest and lowest elements, respectively among the 7 elements studied (Table 3 and 4). There was significant variation (p=0.05) for interaction of season and distance for L_1 only. For all the 3 locations during the dry and wet season the general trend in metal concentration was in the order Co >Ni> Cu > Pb> Zn > As> Br. The low traffic location (L_3) had higher trace metal contents than both high traffic locations (L_1 and L_2) (Table 2). The high trace metal content on L_3 as compared to L_1 and L_2 may have been as a result of the texture and high organic matter content in L_3 due to the re-afforestation measures carried out on the site. High organic matter content affects the mobility of the metals in soils[18-20]. It may also be due to meteorological condition since the site is located in an open area that is subjected to windy condition. Turbulence and re-suspension leads to significant fractions of heavy metals being deposited on the roadside soils and this may have been so in the case of L_3[18,21].

Table 2: Trace metal content of soils from low traffic (L_3) and high traffic (L_1 and L_2) sites as related to distance from the roadway during the dry and wet seasons

	Metal contents (ppm)					
	L_1		L_2		L_3	
Distance (m)	Dry	Wet	Dry	Wet	Dry	Wet
0-20	907.10	859.70	652.80	750.80	1101.2	1005.90
20-40	792.30	809.90	602.10	947.70	860.3	1137.20
40-60	814.80	916.10	671.50	746.30	858.3	1133.80
60-70	80.90	906.50	628.60	721.80	938.4	916.30
70-80	776.10	929.90	630.80	665.90	832.3	1027.40
Mean	819.20	884.40	637.20	766.50	918.1	1044.10
SE±	22.92	22.05	11.76	47.75	49.0	41.70

Table 3: Individual trace metal content (ppm) in soils from low traffic (DL_3) and high traffic (DL_1 and DL_2) sites during the dry season

	Metal contents						
Traffic sites	Pb	As	Zn	Cu	Co	Ni	Br
DL_1	11.5	7.0	9.5	16.0	34.0	19.2	3.0
DL_2	11.2	7.0	9.5	15.7	34.5	19.6	2.9
DL_3	11.0	7.0	9.5	16.2	33.5	20.2	3.0

Table 4: Individual trace metal content (ppm) in soils from low traffic (WL_3) and high traffic (WL_1 and WL_2) sites during the wet season

Traffic sites	Metal contents Pb	As	Zn	Cu	Co	Ni	Br
WL_1	10.8	5.0	10.4	17.0	31.8	20.1	5.0
WL_2	10.0	4.5	10.0	23.5	28.6	18.8	4.6
WL_3	10.8	4.9	9.0	17.0	33.0	19.5	4. 7

Table 5: Population counts (No. of organisms/100 g of soil x 10^7) of soil microbes in relation to increasing distance from the roadway for low traffic (DL_3) and high traffic (DL_1 and DL_2) during the dry season

Traffic sites	Distance (m) 20	40	60	70	80
DL_1	25.0	22.0	29.0	34.5	66.0
DL_2	32.0	26.0	44.0	48.0	98.0
DL_3	16.0	13.0	68.0	24.0	11.0

Table 6: Population counts (No. of organisms/100 g of soil x 10^7) of soil microbes in relation to increasing distance from the roadway for low traffic (WL_3) and high traffic (WL_1 and WL_2) during wet season

Traffic sites	Distance (m) 20	40	60	70	80
WL_1	62.0	70.0	80.0	70.0	120.0
WL_2	44.0	50.0	96.0	100.0	120.0
WL_3	84.0	110.0	140.0	160.0	170.0

Low metal contents on L_2 may be attributed to agricultural activities that result in upturning of the soil and subsequent removal of the metals from the reachable layers. For L_1 the lower trace metal contents may be due to the slope of the site. This may have encouraged washout of the metals down the slope.

The high trace metal contents of wet season samples were found to be in agreement with Sansalone and Buchberger[22] who observed that the levels of trace metals often exceeded surface quality discharge standards during rainfall events especially for metals like Zn, Cu and Cd. This is because they are mostly in dissolved forms. Furthermore, soil moisture binds and holds the soil together thereby retaining the water-soluble metals in the topsoil[19,23].

Microbial community of roadside soils: For both seasons the population counts of bacteria and fungi increased with increasing distance from the roadway (Table 5 and 6). The bacterial community was made up of mainly *Bacillus* sp., while the fungal community included *Aspergillus* sp., *Rhizoctonia* sp., *Monilia* sp., *Helminthosporium* sp. and *Penicillium* sp. There was a highly significant variation in population of the bacterial community due to seasonal effects (p= 0.05) for L_1 and L_3. This was significant (p= 0.05) for fungi on L_3 only. The student's t-test revealed that the mean increase of soil microbes between the dry and wet season was highly significant (p=0.05 and p=0.01) Microbial populations were higher for the wet season than for the dry season. This maybe as a result of favorable environmental condition such as moisture, moderate temperature etc. L_3 had the highest microbial population despite the high trace metal contents during the wet season and this may be due to the interaction of several factors such as Cation Exchange Capacity (CEC), microbial biomass, stability of aggregates and organic matter content which increase the resistance of soil microbes to such stresses and disturbances[24-26]. It may also be due to the ability of the organic matter to adsorb the toxic metals thus reducing their effects on the soil microbes. Furthermore, this maybe as a result of the tolerance of the micro-flora (bacteria and fungi) to the contaminating metals. Some soil microorganisms are genetically resistant to many of these metals. The species of microorganisms that responded to the presence of trace metals in the soil, may thus serve as microbial indicator species for metal pollution in the Nigerian Savanna.

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