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MULTIVARIATE STATISTICS OF FERTILITY PARAMETER FLUXES IN CEMENT-DUST-POLLUTED SOILS IN MFAMOSING, NIGERIA: IMPACT ON AGRICULTURE

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ABSTRACT

This study investigated the impact of cement-dust pollution on the fertility status of agricultural soils to ascertain their health and suitability for cropping. Relevant soil nutrients and enzyme activities were determined from 12 control soil, 12 NPK-treated polluted soil and 12 un-amended polluted soil samples, using standard soil analytical and biochemical procedures. Soil microbial biomass-carbon was quantified by chloroform-fumigation-extraction (CFE) method. Cultivable aerobic bacterial count was determined on Tryptic Soy Agar (TSA) while cultivable fungal quantitation was performed on Czapek-Dox agar. Corn (*Zea mays*) yield served to evaluate pollutant effect on tested parameters. Principal component analysis (PCA) extracted two components, PC1 and PC2, from nine studied dependent variables (DVs) which explained 68.33% variability about the data. Number and membership of extracted components were confirmed by two clusters obtained by agglomerative hierarchical cluster analysis (AHCA). Multivariate analysis of covariance revealed significant effect of soil type on the combined DVs when the effect of the covariate (planting period) was controlled. One-way analysis of covariance (one-way ANCOVA) revealed non-significant effect of planting period but a significant main effect of soil type on corn yield when controlling for the effect of the covariate. Relative to control soil, per cent loss in corn yield was 55.69% in cement dust-polluted soil but reduced to 36.07% in polluted soils treated with NPK. The research findings have shown that cement dust pollution significantly reduced corn yield and the stress may persist in agricultural soils amended with fertilizer.

Keywords: Cement-dust pollution; Multivariate statistics; Nutrient dynamics, Enzyme activity fluxes; Microbial biomass; Corn yield

INTRODUCTION

Zea mays L or maize (corn) is a very popular crop plant that thrives excellently in the tropical and warm sub-tropical Africa, Latin America and Asia, where it is primarily used as human food and animal feed owing to its large carbohydrate content (**Olaniyan and Lucas, 2004**). On a global scale, corn ranks third among the cereals in terms of earnings upon investment (**Akongwubel *et al.*, 2012**). On the African continent, Nigeria is only second to South Africa in production but placed eleventh in terms of corn yield (**FAOSTAT, 2014**).

Corn and/or its products have found wide applications in households and industries including food, dairy, brewery and distillery (**Undie *et al.*, 2012**). In Southern Nigeria, corn is mostly consumed directly as snack, in boiled or roasted form, or processed into flour and consumed in a variety of forms (**Akongwubel *et al.*, 2012**).

The dominant factors that influence corn yield have been identified as soil type, climate, soil health and fertility, corn variety and cropping method (**Akongwubel *et al.*, 2012; Undie *et al.*, 2012**). As a hardy plant, corn has the ability to grow on a vast array of soils, but best yield is reported in rich loamy or sandy-loamy soils under salutary climatic conditions (**Undie *et al.*, 2012**). Nigeria has an abundance of these soil types with adequate health and fertility for high yield. However, pH, soil organic carbon, phosphorus, nitrogen, micronutrients and soil moisture levels influence soil fertility owing to changes in their status arising, mostly, from anthropogenic pollution from industries (**Zerrouqi *et al.*, 2008; Gordon *et al.*, 2013**).

Generally, industries are categorized as high-polluting, less-polluting and non-polluting by the ministries of environment and forest, on the basis of their capacities to pollute the environment. Cement industries are regarded as high-polluting, especially with regard to particulate emissions considering their role in pollution hazards and environmental imbalance. Their emissions pollute the environment in the form of dusts as they escape during factory processing (**Kulandaivel *et al.*, 2015**).

Cement manufacturing releases alkaline particulate gaseous pollutants and other particles into the soil (**Hemida, 2005; Bilen, 2010**). Their direct effects have been reported to include ecosystem alkalination and undesirable soil physicochemical composition and biological activity (**Nowak *et al.*, 2003; Ocak *et al.*, 2004**). Documented reports show that cement dust trigger considerable pH changes arising from inputs of oxides like Cao, Fe_2O_3, SiO_2, Al_2O_3, MgO, K_2O, Na_2O and SO_3 and accumulation of toxic metals like hexavalent chromium, nickel, cadmium and lead (**Saddique, 2014**). Mediation of metal toxicity to soil microorganisms and their metabolism by pH occasioned on the availability of suitable ligands has been copiously documented (**Ekpenyong and Antai, 2007a,b; Ekpenyong *et al.*, 2007; Hagmann *et al.*, 2015**). The consequences are reductive changes in microbial quality content and their biochemical and/or physiological activities (**McCarthy *et al.*, 2003; Biyik *et al.*, 2005**). Microbial biomass and soil enzyme activities are therefore reliable soil fertility indices since their responses to variations in soil properties occasioned on pollutant presence are rapid and measureable (**Bilen, 2010; Panettieri *et al.*, 2013**). Their composition and activities also adequately reflect biological and biogeochemical changes brought about by the pollution (**Bayhan *et al.*, 2002; Bacmaga *et al.*, 2015**).

In the ancient city of Calabar, Nigeria, where a cement factory is located near agricultural farmlands, surrounding soils have been reported to yield poorly, even after fertilizer application. This has sparked off youth restiveness in the area with frequent kidnap of Health and Tertiary Institution workers. The present study comparatively investigated the fertility status of farmlands surrounding the cement factory, pristine soils and polluted soils amended with fertilizer. Multivariate statistical tools, namely, correlation analysis (CA), principal component analysis (PCA), agglomerative hierarchical cluster analysis (AHCA) and multivariate analysis of co-variance (MANCOVA) were used. These approaches have earlier been reported as successful and therefore reliable in studies on fertility parameters of humic soil cultivated with coffee (**Silva and Lima, 2012**), trace metal contamination of sediments (**Benson *et al.*, 2016**), soil fertility parameters around nuclear power plant (**Shinde *et al.*, 2016**), determination of soil pedoenvironmental indicators (**Oliveira *et al.*, 2017**) and soil fertility relationships for predicting environmental persistence of pollutants (**Katseanes *et al.*, 2017**).

MATERIALS AND METHODS

Area of study

Calabar soils are characteristically described as sandy-loamy (**Ibanga *et al*., 1989; Akpan-Idiok and Ogbaji, 2013**). The area is a tropical rainforest (**Akpan *et al*., 2017**), with average rainfall almost all year round but with sufficient sunlight that makes corn planting possible three times per year; March to June (early-season), May to September (mid-season) and September to January (late-season corn)(**Undie *et al*., 2012**). The study area was United Cement Company Plant located within Latitude 40°53' and 50°05'N and Longitude 80°15' to 80°27'E of Calabar, Cross River State, at the limestone belt of Mfamosing and surrounding fields in Akamkpa Local Government Area (2 km east and west ends from the factory). Total area of study was 1 km^2 including the factory. The southern end of the factory is bordered by the Akpayafe River while the northern end accommodates roads and residential areas, leaving the western and eastern borders for agricultural practices.

Study design and sampling

A total of 108 samples were collected from 3 types of soil over 3 planting periods; 36 samples per soil type. The cement-dust polluted area was divided into 24 acres (24 x 4046.86 m^2); 12 acres (6 west and 6 east of factory) were tilled to a depth of 30 cm, fertilized with 2 pounds of NPK/acre (16:16:8) and allowed to fallow for two weeks. This was used as polluted but treated soil (POT). The remaining 12 acres were left un-amended and described as polluted (POL) soil samples. The control soil (CON), located at the Cross River University of Technology research garden on 5º15"N; 8º22"E at approximately 25 kilometers from the factory, was also divided into 12 acres. For each planting period, 4 replicate acres per soil type were sampled by means of a hand auger from the first 20 cm depth of the different soil types after removing vegetation. Three sub-samples were collected from each acre diagonally positioned 500 m one from another into sterile wide-mouth sampling bottles. The bottles were properly labeled, placed in ice-packed coolers and transported to the laboratory within 1 hour of collection for preparation and analyses.

Sample preparation

Broken bottles, sticks, polyethylene substances and other unwanted materials were carefully separated from each of the 3 sub-samples and samples homogenized by means of a laboratory homogenizer (SAMRO-SRH50-80, China). The sub-samples were pooled to obtain 4 composite samples per acre per soil type per planting period. Required amount of samples were weighed, by means of a digital balance (METTLER-TOLEDO), from the composite sample for the various analyses that followed. For analyses that did not require much urgency, samples were preserved in the refrigerator at 4ºC until required. Dilutions of soil samples were prepared for microbial analyses. All analyses were initiated within 16 h following collection of samples.

Determination of soil microbial biomass-carbon

To estimate the amount of carbon trapped in microbial biomass in soil samples, the method of fumigation and subsequent extraction with chloroform, reputed as standard method for microbial biomass determination (**Vance *et al*., 1987; Bailey *et al*., 2002**), was employed. Briefly, 5 g of each of chloroform-exposed and unexposed subsamples were transferred into 70 mL glass (Pyrex) tubes followed by addition of 40 mL potassium sulfate (0.5M K_2SO_4) solution to each sample. Subsequently, 0.5 mL ethanol-free chloroform was added to one sub-sample and tubes covered with chloroform-resistant caps. The tubes were shaken at 150 rpm for 4 h and then left for 10 min. The top 30 mL of the soil extracts were decanted and filtered. The filtrate was collected in 50 mL centrifuge tubes and forcibly injected with air for 30 min (to rid extracts of chloroform) using long spinal tap needles. Potassium sulfate (0.5 M K_2SO_4) served as blank. The control and chloroform-exposed extracts were analyzed for total dissolved C using a persulfate digestion technique. Dissolved C concentration in the K_2SO_4 blanks (no soil added) with and without chloroform were subtracted from the extract concentrations of the chloroform-exposed and the control samples. Amount of biomass carbon extracted was determined as the difference in total dissolved carbon between the chloroform-treated sub-sample and its comparable control sub-sample.
Total Microbial Biomass C (extractable + non-extractable biomass) was determined using equation;

$$Total\ Microbial\ Biomass\ (TMB) = 2.68V - 44.1 \qquad Eqn.1$$

(**Vance *et al*., 1987**)

where V represents net flush of carbon from fumigated and un-fumigated K_2SO_4 (0.5 M, pH 7.0)-extracted soils. Results were expressed as microgram chloroform extractable biomass C/gram of dry soil.

Enumeration of cultivable aerobic bacteria

Three appropriate dilutions from each of a 10-fold serially-diluted sample were plated on tryptic soy agar in triplicates by the pour plating method. Plates were incubated for 36 h at 28 ± 2°C. Bacterial enumerations were performed by means of colony counter (Scan 1200 Colony counter, USA) (**Ekpenyong *et al*., 2007b**). Mean counts presented in standard forms were subsequently transformed to $\log_{10}$ values and used for analysis.

Enumeration of cultivable fungi

Total fungi comprising yeasts and molds were enumerated on Czapek-Dox agar (CDA) and triplicate plates incubated at 30ºC for 48-72 h. Discrete colonies of yeasts and molds were enumerated and expressed as described for bacteria. Attention was paid to the ratio of yeasts to molds per plate and per sample.

Determination of soil enzymatic activities

Dehydrogenase activity evaluation followed the protocol of **Casida *et al*. (1964)** using soil amended with $CaCO_3$. The activity was based on the conversion of 2, 3, 5- triphenyltetrazolium chloride substrate to the reddish-colored water-insoluble formazan products whose intensities were measured spectrophotometrically at 485 nm wavelength with methanol as blank. Results obtained were compared with triphenyl formazan standards.
Alkaline phosphatase activity was evaluated using acetate buffer at pH 9.0. Reactions were stopped using a combination of 0.5 mol/L $CaCl_2$ and 0.5 mol/L NaOH. For one hour, enzyme activity reactions were held in a water bath at 37°C and cleavage product identified at a wavelength of 410 nm (**Alef *et al*., 1998**).
β-glucosidase activity was evaluated by the adding *p*-nitrophenyl-β-D-glucopyranoside to 1 g of soil (**Eivazi and Tabatabai, 1988**). The reaction was stopped by the addition of 0.02 mol/L Tris at pH 12.0. The cleavage product, *p*-nitrophenol glucoside was detected and quantified using UV-Vis spectrophotometer (Perkin-Elmer Lambda 25, MA-USA) at 464 nm wavelength.

Physicochemical analyses of samples

Soil pH was determined by potentiometry using 1 M aqueous solution of potassium chloride (KCl). Total nitrogen (TN) was determined as described by **Janssen (2003).** Available phosphorus (AvP) was determined by the colorimetric ascorbic acid method (**Olsen, 1954; Ichikogu, 2012**) while the procedure of wet oxidation was followed to estimate soil organic carbon (SOC), using chromic acid as oxidant (**McLeod, 1973**).

Validation experiment for determination of corn-crop yield

Maize (*Zea mays* L.), otherwise called corn, was planted on 12 acres (12 x 4046.86 m^2) per planting period on each of polluted soil (POL), treated-polluted soil (POT) and the control soil (CON). Each cultivated acre contained 40 ridges (rows) and each ridge had 500 corn stands. A ridge was separated from the next by a distance of 85 cm while corn stands were separated one from another by a distance of 25 cm. Seeds were planted during the 3 planting periods of October (1), March (2) and July (3), and were harvested 3 months later. Corn yields, expressed as bushels/acre, were determined using the equation below and compared among the three types of soil using one-way analysis of covariance (ANCOVA).

$$Yield\ (bushels\ acre^{-1}) = kernels\ ear^{-1} \times \frac{ears\ acre^{-1}}{kernels\ bushel^{-1}} \qquad Eqn.2$$

Statistical analyses

Data was first subjected to Pearson bivariate correlation to establish data appropriateness for multivariate statistics. Over-correlated data were removed and principal component analysis (PCA) performed on remaining data. Results from PCA were confirmed by a dendrogram obtained by Euclidean distance of Ward linkage method of an agglomerative hierarchical cluster analysis (AHCA). Impact of cement-dust on selected outcome variables was evaluated by multivariate and univariate analyses of covariance (one-way MANCOVA and one-way ANCOVA respectively) of corn yield data. SPSS version 20.0 (IBM, USA) was employed to conduct all the statistical analyses.

RESULTS

Pearson bivariate correlations of dependent variables

Results of bivariate correlations computed to determine the appropriateness or otherwise of multivariate statistics of data are presented in Table 1. The results showed that soil organic carbon (SOC) had high significant correlation ($p < .01$) with all other 8 dependent variables except a moderately significant negative correlation ($r = -.332$; $p = .048 < .05$) with β-glucosidase activity (BGA). Total

nitrogen (TN) had high significant correlations with other test variables except that its correlations with available phosphorus (AvP: r = .353, p = .034 < .05) and BGA (r = -.332, p = .023 < .05) were moderate. The weak negative correlation of available phosphorus (AvP) with BGA was not significant (r = -.310, p = .066 > .05); however, the variable had moderate correlations (p < .05) with total nitrogen (TN) and fungal count (LOG10FC) but its relationship with the remaining variables were significantly high (p < .01).

Results also showed weak relationships of dehydrogenase activity (DHA) with alkaline phosphatase activity (ALPA: r = .227, p = .101 > .05) and β-glucosidase activity (BGA: r = .328, p = .051 > .050). Alkaline phosphatase activity (ALPA) and β-glucosidase activity (BGA), in turn, showed respectively, weak correlation with fungal count; LOG10FC: r = -.328, p = .051 > .050 and r = -.248, p = .144 > .050. Cultivable aerobic bacterial population (LOG10BC) strongly correlated with LOG10FC and total soil microbial biomass-carbon (SMB-C), however, the relationship with LOG10FC was negative while that with SMB-C was positive.

Principal component analysis (PCA) of interrelationships among soil fertility parameters

Another test of appropriateness of PCA was based on test results of sampling adequacy using Kaiser-Meyer-Olkin (KMO) as well as that of sphericity using Bartlett's test. A KMO of .60 and above with a significant (p < .05) sphericity test result suggest that data was adequate to conduct a PCA. Our results revealed a KMO of .765 and a significant (p < .0005) sphericity test suggesting that the assumption of an identity matrix in the data should be rejected and a PCA accordingly performed to determine the interrelationships between and among fertility parameters in the various soils studied.

The "Total Variance Explained" table of the PCA is presented in Table 2 to show the importance of each of the 9 principal components. The table revealed that only two components had initial eigenvalues ≥1.0 suggesting that the PCA constructed two principal components from the survey items. The table showed a cumulative explained variance of 68.33% with principal component 1 (PC1) contributing 57.12% and PC2 only 11.21%. The number of extracted components is also presented as a Scree plot in Figure 1 to show the elbow (break) point of the plot indicating two extracted principal components.

The two components were subjected to matrix rotation using Varimax with Kaiser Normalization. The resultant rotated matrix revealed that LOG10BC (r = .766), SMB-C (r = .754) and ALPA (r = .923) all had high positive loadings on PC1 and respectively low negative loadings (r = .355), (r = -.344), (r = -.107) on PC2. Fungal count (LOG10FC) had a high positive loading (r = .863) on PC2 but low negative loading (r = -.185) on PC1 while DHA had a high negative loading (r = -.830) on PC2 but a low positive one (r = .230) on PC1. The factor loadings in rotated space are presented as Figure 2. The angles of rotation were determined by treating the correlation coefficients, r of the two principal components as cosines of angles. Since the correlation coefficient for PC1 was .824, the angle of rotation was 34.51º and that for PC2 (r = .566) was 55.53º.

Cluster analysis of soil fertility parameters

The results of principal component analysis (PCA) were confirmed with hierarchical cluster analysis (HCA) using Euclidean distance of a Ward linkage of an agglomerative schedule. The proximity matrix, just like the correlation matrix in principal component analysis (PCA) showed that Euclidean distance between soil organic carbon and total nitrogen (SOC-TN), soil organic carbon and available phosphorus (SOC-AvP), soil organic carbon and fungal count (SOC-LOG10FC) were respectively 4.549, 4.460 and 5.991. These results, presented as a dendrogram in Figure 3, therefore revealed two hierarchical clusters of variables. Cluster 1 comprised SOC, TN, AvP and LOG10FC with low (4.00 - 6.00) Euclidean distance between any pair. Cluster 2 consisted of alkaline phosphatase activity (ALPA), β-glucosidase activity (BGA), dehydrogenase activity (DHA), bacterial population (LOG10BC) and soil microbial biomass-carbon (SMB-C) with high Euclidean distance between any pair (6.00 - 12.00).

Table 1 Pearson bivariate correlations of dependent variables

		SOC	TN	AvP	DHA	ALPA	BGA	LOG10 BC	LOG10 FC	SMB-C
SOC	Correlation	1								
	Sig.							.		
TN	Correlation	.704**	1							
	Sig.	.000		.				.		
AvP	Correlation	.716**	.353*	1					.	
	Sig.	.000	.034							
DHA	Correlation	-.517**	-.500**	-.486**	1					
	Sig.	.001	.002	.003						
ALPA	Correlation	-.733**	-.596**	-.685**	.277	1				
	Sig.	.000	.000	.000	.101					
BGA	Correlation	-.332*	-.378*	-.310	.328	.486**	1			
	Sig.	.048	.023	.066	.051	.003				
LOG10 BC	Correlation	-.589**	-.665**	-.569**	.440**	.691**	.436**	1		
	Sig.	.000	.000	.000	.007	.000	.008			
LOG10 FC	Correlation	.487**	.573**	.377*	-.584**	-.328	-.248	-.461**	1	
	Sig.	.003	.000	.023	.000	.051	.144	.005		
SMB-C	Correlation	-.546**	-.748**	-.528**	.450**	.663**	.403*	.714**	-.385*	1
	Sig.	.001	.000	.001	.006	.000	.015	.000	.020	

Legend: ** Significance level, p = 0.01, * Significance level, p = 0.05, SOC-Soil organic carbon, TN-Total nitrogen, AvP-Available phosphorus, DHA-Dehydrogenase activity, ALPA-Alkaline phosphatase activity, BGA-β-glucosidase activity, LOG10BC-LOG 10 Bacterial count, LOG10FC-LOG 10 Fungal count, SMB-C-Soil microbial biomass-carbon

Table 2 Total explainable variance of a principal component analysis showing two extracted components

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.141	57.117	57.117	5.141	57.117	57.117	3.817	42.408	42.408
2	1.009	11.214	68.331	1.009	11.214	68.331	2.333	25.923	68.331
3	.794	8.826	77.156						
4	.706	7.846	85.002						
5	.465	5.164	90.166						
6	.397	4.412	94.578						
7	.261	2.897	97.475						
8	.155	1.719	99.195						
9	.072	.805	100.000						

Extraction Method: Principal Component Analysis.

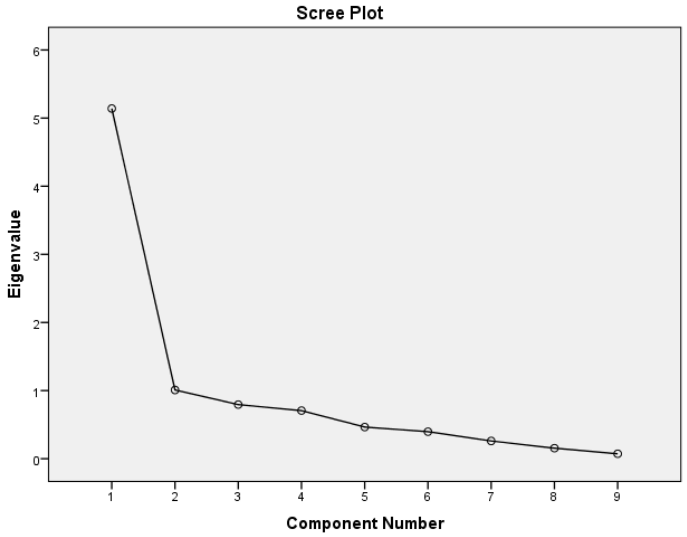

Figure 1 Scree plot of Eigenvalues against component number showing the elbow point for two principal components

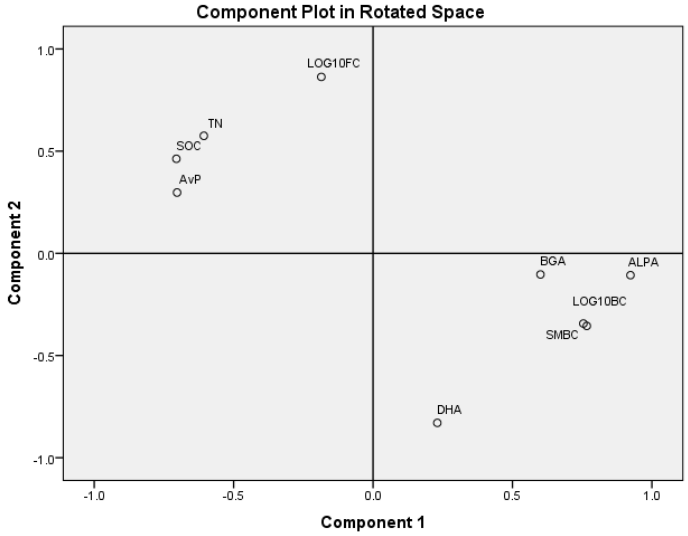

Figure 2 Extracted principal components of dependent variables in rotated space

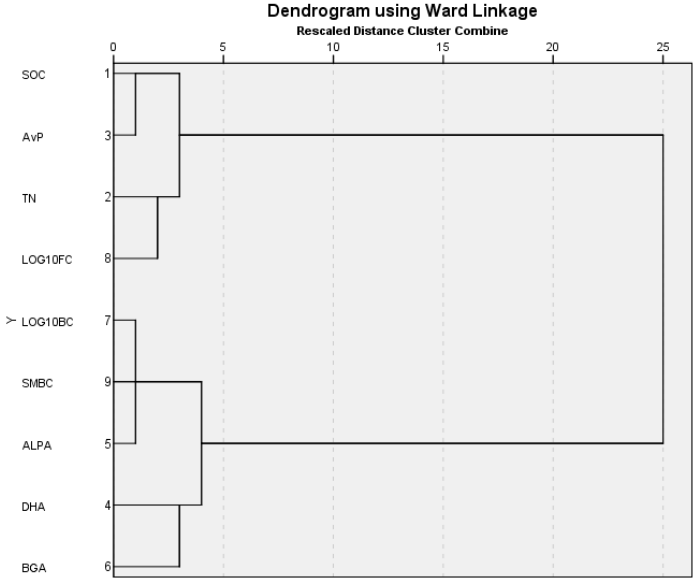

Figure 3 Dendrogram of a Ward linkage of an agglomeration schedule of a hierarchical cluster analysis (HCA) showing two variable clusters

Multivariate analysis of effect of soil type and planting period on fertility variables

Multivariate analysis of covariance (one-way MANCOVA) was adopted to ascertain the main effect of soil type on the nine moderately correlated dependent variables (DVs) when controlling for planting period as covariate. The homogeneity assumption given by the Box's test was significant, $F = 1.692$, $p < .0005$, indicating violation of the assumption. However, the assumption of homogeneity test for equality of error variance was met (Table 3) by the non-significant ($p > .05$) Levene's test for all 9 DVs.

Table 4 presents the four different multivariate tests conducted to explain the effect of soil type on the response variables when controlling for the effect of the covariate (planting period). The table showed that all four multivariate tests for the covariate were significant ($p < .0005$). Because the Box M test of homogeneity assumption was violated, we reported the result from the very robust Pillai's Trace test. The results showed a statistically significant effect of the covariate on the DVs in combination: planting period, F (9, 24) = 48.36, $p < .0005$; Pillai's Trace = .948, partial $\eta^2 = .948$ and a statistically significant main effect of the categorical predictor variable on the DVs in combination when controlling for the covariate: soil type, F (18, 50) = 31.12, $p < .0005$; Pillai's Trace = 1.84, partial $\eta^2 = .918$.

An extract of the tests of between-subjects effect (Table 5) results revealed that the covariate effects were not significant ($p > .05$) for available phosphorus (Avp), dehydrogenase activity (DHA), β-glucosidase activity (BGA) and fungal count (LOG10FC). However, for soil organic carbon (SOC), total nitrogen (TN), alkaline phosphatase activity (ALPA), bacterial count (LOG10BC) and soil microbial biomass-carbon (SMB-C), there were significant main effects among the independent levels for the covariate. Furthermore, Bonferoni's post hoc test was employed to separate significant means. The test revealed that significant difference in mean SOC levels existed between control soil (CON) and untreated polluted soil (POL) as well as between NKP-treated polluted soil (POT) and POL but not between CON and POT ($p = .448 > .05$) as can be seen in Figure 4a. Mean total nitrogen (TN) was only significantly different between CON and POL ($p = .02 < .05$). The mean difference in TN between CON and POT ($p = .761 > .05$) and POT and POL ($p = .276 > .05$) was not statistically significant (Figure 4b). Mean alkaline phosphatase activity (ALPA) was significantly different between all soil type pairs (Figure 5a). Bonferroni multiple comparison result also showed that mean bacterial population (LOG10BC) (Figure 6b) differed significantly between CON and POL and between CON and POT but not between POT and POL. There was significant nean difference in soil microbial biomass-carbon (SMB-C) between CON and POL ($p = .011 < .05$) (Figure 6a). However, no significant difference existed between CON and POT ($p = .062 > .05$) or between POT and POL ($p = 1.000 > .05$).

The tests of between-subjects effect for the main effect revealed that only dehydrogenase activity (DHA) (Figure 5c) and fungal count (LOG10FC) (Figure 6c) were not significantly affected ($p > .05$) by soil type. Bonferroni multiple comparisons showed non-significant mean difference in available phosphorus (AvP) between CON and POT but significant mean difference existed between POT and POL and between CON and POL (Figure 4c). Mean β-glucosidase activity (BGA) (Figure 5b) differed significantly between CON and POL and between CON and POT but not between POT and POL ($p > .05$).

Evaluation of cement dust pollution effect on corn yield

Analysis of covariance (one-way ANCOVA) was used to analyze corn yield data in cement dust polluted (POL), NPK-amended (POT) and control (CON) agricultural soils. Homogeneity assumption of the model was met by non-significant Levene's test result. The ANCOVA result, presented as Table 6 revealed that corn yield did not differ significantly with the covariate (planting period) but with soil type, F (2, 32) = 21.54, $p < .0005$; model adjusted $r^2 = .534$. The control soil (CON) had a mean corn yield of 7.89 bushels/acre which was higher than those from NPK-amended polluted soil (POT-5.04 bushels/acre) and the un-amended polluted soil (POL-3.50 bushels/acre) as illustrated in Figure 7. Bonferroni multiple comparisons showed that mean difference in corn yield between CON and POL soils as well as that between CON and POT soils were significant. However, mean difference between POT and POL was not statistically significant ($p = .089 > .05$). We calculated loss in corn yield in relation to control soil using the equation: $Yield\ loss\ (\%) = Y_{con} - \frac{Y_x}{Y_{con}} \times 100$ $Eqn. 3$

where Y_{con} is corn yield in control soil (CON) and Y_x is corn yield from NPK-treated polluted soil (POT) or un-amended cement dust polluted soil (POL). Relative to control soil, per cent loss in corn yield was 55.69% in un-amended cement dust-polluted soil and 36.07% in NPK-treated polluted soil.

DISCUSSION

Soil fertility parameters are all interrelated and interact with the biological component of the soil in diverse ways. Multivariate statistics; a robust statistical tool with potential to identify, classify, quantify and interpret these relationships; was employed in this study. Multivariate statistics are used to account for

confounding effects, account for more variance in an outcome, and predict for outcomes. There are different multivariate approaches in frequent use (**Benson *et al*., 2016**). These include correlation analysis; Principal component analysis or factor analysis, agglomerative hierarchical cluster analysis (**Francl, 1993; Jia *et al*., 2010; Benson *et al*., 2016**) and multivariate analysis of variance or covariance (**Samec *et al*., 2007**). The present study employed the techniques of correlation analysis (CA), principal component analysis (PCA), agglomerative hierarchical cluster analysis (AHCA) and multivariate analysis of covariance (MANCOVA) to investigate the impact of cement dust pollution on nine fertility parameters of an agricultural soil and the consequence of that impact on crop yield.

Soil fertility studies suggest a large number of parameters to indicate soil health and fertility, particularly for agricultural purposes (**Dawes and Goonetilleke, 2006; Samec *et al*., 2007; Shinde *et al*., 2016**). Our study originally evaluated 12 fertility parameters including soil organic carbon (SOC), total nitrogen (TN), available phosphorus (AvP), pH (pH), soil moisture content (SMC), dehydrogenase activity (DHA), acid phosphatase activity (ACPA), alkaline phosphatase activity (ALPA), β-glucosidase activity (BGA), bacterial count (LOG10BC), fungal count (LOG10FC) and soil microbial biomass-carbon (SMB-C). However, Pearson bivariate correlation removed pH, SMC and ACPA on the basis of violation of the multicolinearity assumption for a multivariate analysis. It is important to comment here that, although multivariate statistics simulates real life situations, its nine assumptions are often not met in real life. Attempts at meeting multivariate statistics assumptions, often attained by removal of 'over-correlated' variables, frequently result in loss of very useful information (**Juhos *et al*., 2015**). In this study, pH, SMC and ACPA were removed before PCA, AHCA and MANCOVA were conducted.

The high significant but moderate inter-correlation among SOC, TN and AvP was not surprising because these nutrient elements are the major building blocks of cellular organisms including plants and microorganisms. Carbon is required for the synthesis of the carbon skeleton of all life forms while nitrogen remains a major limiting nutrient for several life processes and/or functions. The synthesis of the genetic material (DNA) and energy currency of cellular organisms, ATP, leans heavily on the availability of phosphorus in the environment without which biochemical reactions will not occur (**Ekpenyong *et al*., 2017a**). Availability of these nutrients in adequate amounts is the major driver of numerous soil biological activities required to establish and sustain soil fertility. Fertility limits/ranges of these elements for agricultural practices are detailed in **Landon (1991)** and **FDALR (1990).**

Table 3 Levene's test for homogeneity assumption

	F	df1	df2	Sig.
Soil organic carbon	1.859	2	33	.172
Total nitrogen	1.953	2	33	.158
Available phosphorus	.820	2	33	.449
Dehydrogenase activity	.576	2	33	.567
Alkaline phosphatase activity	1.492	2	33	.240
β-glucosidase activity	.194	2	33	.825
LOG10 Bacterial count	.346	2	33	.710
LOG10 Fungal count	1.302	2	33	.286
Soil microbial biomass-carbon	.291	2	33	.749

Significance level, $p = .05$

Table 4 Multivariate tests results of a one-way multivariate analysis of covariance (MANCOVA)

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
Intercept	Pillai's Trace	.990	253.153	9.000	24.000	.000	.990	2278.379	1.000
	Wilks' Lambda	.010	253.153	9.000	24.000	.000	.990	2278.379	1.000
	Hotelling's Trace	94.932	253.153	9.000	24.000	.000	.990	2278.379	1.000
	Roy's Largest Root	94.932	253.153	9.000	24.000	.000	.990	2278.379	1.000
Period	Pillai's Trace	.948	48.357	9.000	24.000	.000	.948	435.213	1.000
	Wilks' Lambda	.052	48.357	9.000	24.000	.000	.948	435.213	1.000
	Hotelling's Trace	18.134	48.357	9.000	24.000	.000	.948	435.213	1.000
	Roy's Largest Root	18.134	48.357	9.000	24.000	.000	.948	435.213	1.000
Soiltype	Pillai's Trace	1.836	31.122	18.000	50.000	.000	.918	560.195	1.000
	Wilks' Lambda	.006	32.133	18.000	48.000	.000	.923	578.398	1.000
	Hotelling's Trace	25.909	33.106	18.000	46.000	.000	.928	595.916	1.000
	Roy's Largest Root	17.898	49.715	9.000	25.000	.000	.947	447.439	1.000

Table 5 Extract of Tests of between-subjects effects of MANCOVA showing only covariate and the categorical variable

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
Period	SOC	36.482	1	36.482	22.777	.000	.416	22.777	.996
	TN	.446	1	.446	64.055	.000	.667	64.055	1.000
	AvP	3.627	1	3.627	.700	.409	.021	.700	.128
	DHA	107.823	1	107.823	3.783	.061	.106	3.783	.471
	ALPA	67.402	1	67.402	28.624	.000	.472	28.624	.999
	BGA	.290	1	.290	.010	.920	.000	.010	.051
	LOG10BC	12.980	1	12.980	10.966	.002	.255	10.966	.894
	LOG10FC	3.534	1	3.534	2.445	.128	.071	2.445	.329
	SMB-C	394632.954	1	394632.954	98.578	.000	.755	98.578	1.000
Soil type	SOC	203.300	2	101.650	63.464	.000	.799	126.928	1.000
	TN	.172	2	.086	12.381	.000	.436	24.763	.993
	AvP	200.356	2	100.178	19.345	.000	.547	38.690	1.000
	DHA	92.454	2	46.227	1.622	.213	.092	3.243	.317
	ALPA	572.569	2	286.284	121.576	.000	.884	243.152	1.000
	BGA	463.433	2	231.716	8.235	.001	.340	16.470	.943
	LOG10BC	49.306	2	24.653	20.829	.000	.566	41.657	1.000
	LOG10FC	4.246	2	2.123	1.469	.245	.084	2.937	.290
	SMB-C	170761.907	2	85380.954	21.328	.000	.571	42.656	1.000

Legend: SOC-Soil organic carbon; TN-Total nitrogen; AvP-Available phosphorus; DHA-Dehydrogenase activity; ALPA-Alkaline phosphatase activity; BGA-β-glucosidase activity; LOG10BC-LOG 10 Bacterial count; LOG10FC-LOG 10 Fungal count; SMB-C-Soil microbial biomass-carbon

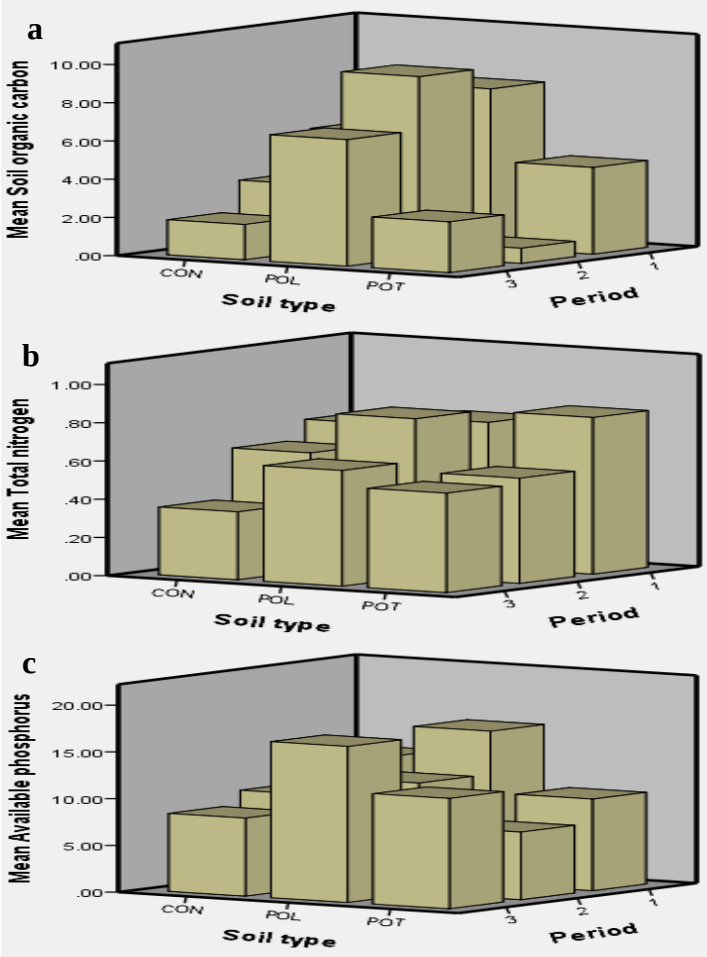

Figure 4 3-D bar representation of nutrient parameter fluxes in studied soils. a-Soil organic carbon; b-Total nitrogen; c-Available phosphorus

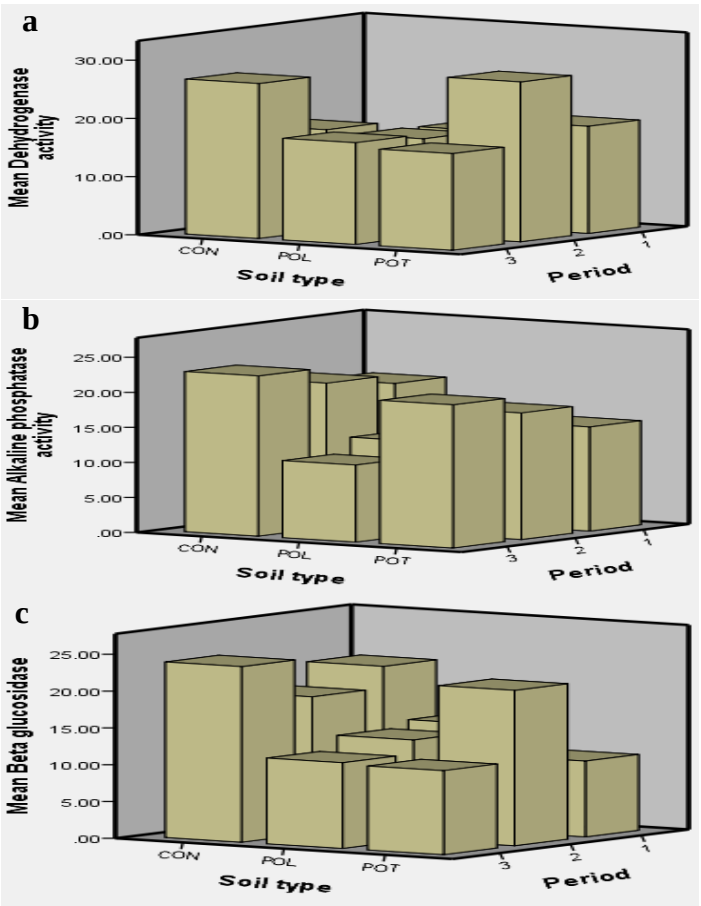

Figure 5 3-D bar representation of enzyme activity fluxes in studied soils. **a**-Dehydrogenase activity; **b**- Alkaline phosphatase activity; **c**- β-glucosidase activity

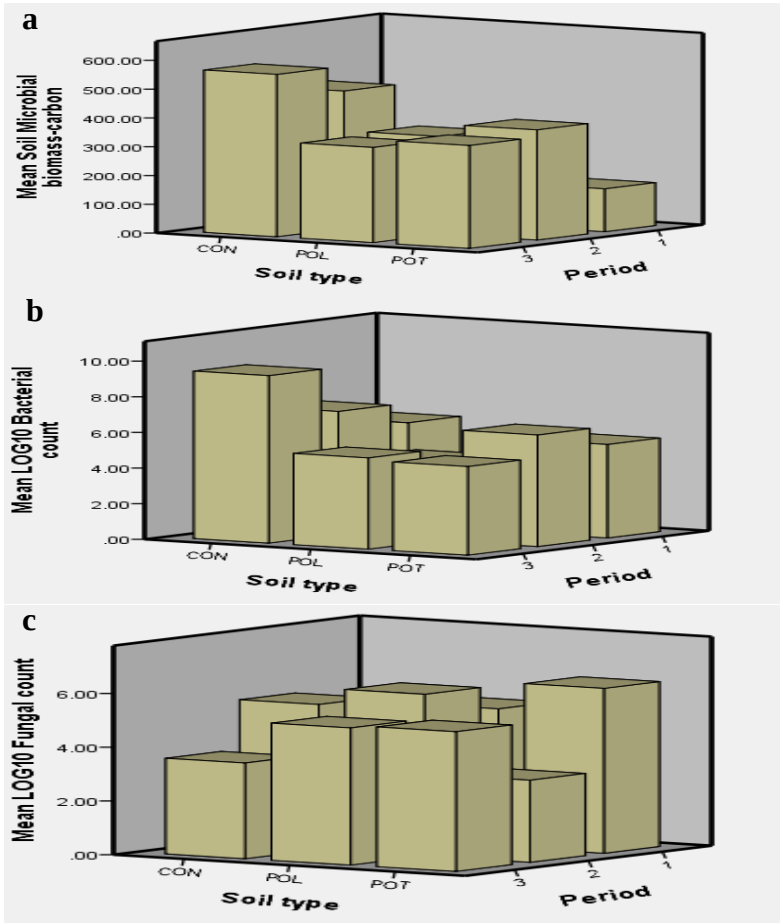

Figure 6 3-D bar representation of microbial biota fluxes in soils: **a**- Soil microbial biomass-carbon; **b**- LOG 10 Bacterial count; **c**- LOG 10 Fungal count

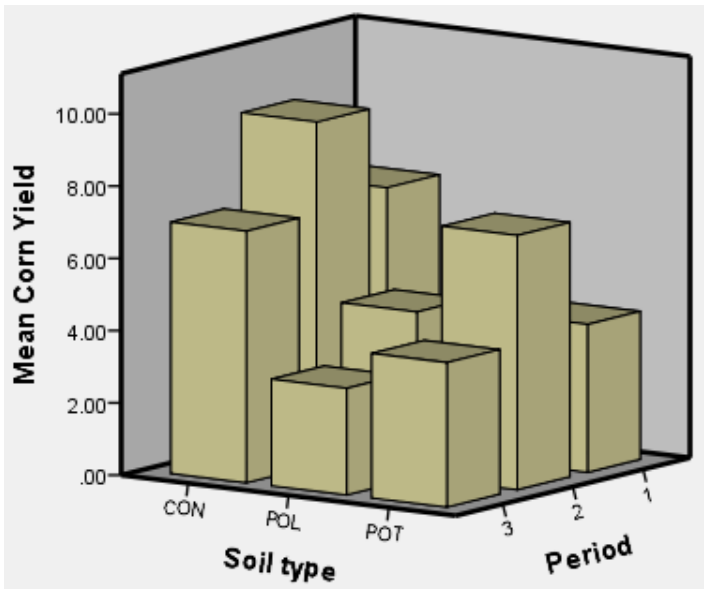

Figure 7 3-D bar representation of mean corn yield from studied soils at different planting period

Results from this study showed that these three elements correlated positively and significantly with each other. A fourth parameter with which they shared positive correlation was fungal population (LOG10FC). Understandably, the nutrient elements showed negative significant correlation with soil microbial biomass-C (SMB-C). This indicates that when soil microbial biomass-carbon increases in the soil, the organic carbon content (non-microbial) decreases owing to increased metabolism and conversion to microbial biomass carbon (**Kaur *et al.*, 2000**). This study showed that fungal count, which forms a part of the

microbial community, does not share the negative correlation with nutrient elements, but bacterial count (LOG10BC) does. We hypothesize that bacteria with their high specific growth rate outcompetes fungi in the presence of high organic carbon, thereby constituting the bulk of SMB-C, hence their significant positive correlation. Therefore as LOG10BC rises along with SMB-C, soil concentrations of LOG10FC, SOC, TN and AvP decrease. Spore-forming bacteria, particularly from *Bacillus*, and molds of the genera *Aspergillus*, *Penicillium* and *Cladosporium*, dominated the first surface layer of cement dust polluted soils but their populations were grossly reduced in control soil (CON) but less so in NPK-treated soils (POT) which were overtaken by yeast genera and more diverse bacterial species and genera.

Table 6 Tests of between-subjects effects of ANCOVA for corn yield data

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
Corrected Model	119.302	3	39.767	14.368	.000	.574	43.104	1.000
Intercept	160.593	1	160.593	58.022	.000	.645	58.022	1.000
Period	.074	1	.074	.027	.871	.001	.027	.053
Soil type	119.228	2	59.614	21.538	.000	.574	43.077	1.000
Error	88.569	32	2.768					
Total	1287.869	36						
Corrected Total	207.871	35						

Pearson bivariate correlation also showed that dehydrogenase activity (DHA), alkaline phosphatase (ALPA) and β-glucosidase had negative correlations with the nutrient elements but positive correlation with SMB-C and bacterial count. It has been reported that microbial biomass represents very little source of nutrients relative to soil organic matter and standing tree biomass, however, it remains a repository of plant nutrients and a channel for soil organic matter incorporation (**Templera *et al*., 2003**). Biological oxidations of soil organic compounds are mostly, if not entirely, dehydrogenase-mediated reactions (**Wolinska and Stepniewska, 2012**). Since DHA measures microbial respiration, it very readily serves as a reliable index of soil respiration which correlates strongly and positively with SMB-C (**Salazar *et al*., 2011**). β-glucosidase is an important soil enzyme because of its involvement in the final steps of biodegradation of the most abundant carbon substrate, cellulose, in the soil (**Vinhal-Freitas *et al*., 2010; Adetunji *et al*., 2017**). Its activity (BGA) is therefore significant in carbon cycling and hence an important indicator of biological activity and soil fertility (**Stege *et al*., 2010**). Alkaline phosphatase is essential in phosphate metabolism and is a useful index for determining agricultural soil quality (**Nalini *et al*., 2014**). As soil microbial biomass increased, the activities of these three enzymes increased. Another poorly understood relationship was the negative correlation between AvP and ALPA. It was expected that an increase in alkaline phosphatase with increased bacterial population will lead to increased available phosphorus but the reverse was the case. This suggests that other parameters; especially trace metals (**Ekpenyong *et al*., 2017b**) or micronutrients (**Fernandez-Moya *et al*., 2014**) might be involved in the adjustment of the relationship between this enzyme and other parameters because relationships in natural environments are hardly ever bivariate.

Principal component analysis (PCA) is a dimension-reduction analytical process that involves the transformation of a large number of weakly to moderately correlated variables into a smaller number of uncorrelated variables. A correlation matrix that shows only a few correlations above .30 suggests discontinuation of the analysis (**Tabachnick and Fidell, 1996**). Our correlation analysis results showed that all but one of the correlations was above .30 suggesting that PCA could be conducted on the data. Our results revealed a KMO of .765 and a significant ($p < .0005$) sphericity test suggesting that the assumed identity data matrix of the null hypothesis should be rejected and a PCA accordingly performed. Only two principal components with initial eigenvalues ≥1.0 were extracted suggesting that the PCA constructed two principal components from the survey items based on eigenvalues. An eigenvalue is a ratio of the shared variance to the unique variance accounted for in the construct of interest by each factor obtained from the extraction by principal components. Eigenvalues of 1.0 or greater are an arbitrary criterion accepted to help decide if a factor should be further interpreted or not. Extraction of the two components with cumulative explained variance of 68.33% suggests that a two factor solution would be adequate for the study.

The un-rotated factor loadings of the PCA (data not shown) showed that most of the dependent variables had high positive or negative loadings ($r > .70$) on PC1. Apart from dehydrogenase activity (DHA) and fungal count (LOG10FC) which had moderate negative and positive loadings respectively on PC2, other variables had low positive and low negative loadings on PC2. Our reproduced correlations (data not shown) revealed small residuals indicating that there was very little difference between the reproduced correlations from the two extracted components and the actual correlations observed between the variables. This suggests that the 2 factor solution could provide an accurate summary of the relationships in the data.

The two component matrices rotated by Varimax with Kaiser Normalization through angles of 34.51º (PC1) and 55.53º (PC2) revealed that only LOG10BC, SMB-C and ALPA had high positive loadings on PC1 and low loadings on PC2 while LOG10FC had a high positive loading on PC2 but low loading on PC1. The effect of rotation is to spread the importance or contribution more or less equally between the two extracted principal components. A close observation of the right side of Table 2 reveals that the eigenvalues of the rotated factors had been spread between the two components as 3.817 and 2.333 for PC1 and PC2 respectively, compared to 5.141 and 1.009 in the initial solution. The 68.33% cumulative variability was accordingly distributed; 42.41% for PC1 and 25.92% for PC2 compared respectively to 57.11% and 11.21% in the initial solution. Variable membership of PC1 included SOC, TN, AvP and LOG10FC while PC2 contained DHA, ALPA, BGA, LOG10BC and SMB-C. This result agrees with those of **Jia *et al*. (2010)** where they obtained three principal components with available phosphorus and organic matter extracted into one component. Our results showed that all members within a principal component were positively correlated among each other but correlated negatively with every other variable from another component.

The study confirmed the two factor solution of PCA with hierarchical cluster analysis (HCA) using Euclidean distance. The dendrogram showed that all the nine tested variables could be classified into two clusters with similar membership to that of PCA; a confirmation of the reliability of the PCA results. **Firdous *et al*. (2016)** reported excellent results using similar approaches in their study on soil quality parameters in Rawal Lake watershed. Our observations show that both component extraction and variable clustering were performed on the basis of variable relationship to soil microbial biomass-carbon. Variables in PC 1/cluster 1 were all negatively correlated to SMB-C but the variables that shared PC 2/cluster 2 with SMB-C were all positively correlated.

A one-way multivariate analysis of covariance (MANCOVA) was conducted on data to ascertain the main effect of soil type on the nine moderately correlated dependent variables (DVs). The homogeneity of covariance assumption was violated; however, that of homogeneity of error variance was met. This enabled the interpretation of the multivariate test by Pillai's Trace instead of the frequently reported Wilks' λ test. This test is reported as the most robust of all multivariate test because it could accommodate MANCOVA analysis when the homogeneity test of equality of covariance; the Box M test, is violated. The statistically significant effect of the covariate on the combined DVs suggests that the covariate adjusted values of the outcome, however, the main effect of soil type on the combined DVs was still statistically significant when controlling for the covariate. These results simply imply that the different soil types affected the response variables and that planting/sampling period could also adjust whatever influence soil type had on those responses.

The tests of between-subjects effect showed that sampling/planting period did not adjust the effects of soil type for Avp, DHA, BGA and LOG10FC indicating that whatever outcome was observed was purely due to differences in soil type. Bonferoni's test; the most robust and discriminating post hoc multiple comparison tests was used to separate significant means. The test revealed that no significant difference ($p = .448 > .05$) in mean SOC levels existed between control soil (CON) and NPK-treated polluted soil (POT) indicating that fertilizer application could improve SOC content of the cement dust polluted soil. The non-significant difference in TN between CON and POT ($p = .761 > .05$) and POT and POL ($p = .276 > .05$) indicate improvement of mean TN content by fertilizer application on cement dust polluted soil. Mean difference in available phosphorus (AvP) between CON and POT was not significant but significant difference existed between POT and POL and between CON and POL indicating respectively the effect of fertilizer application and the impact of cement dust pollution. Previous study by **Ibanga *et al*. (2008)** showed that cement dust pollution does significantly influence the chemical properties of Calabar soils. Mean alkaline phosphatase activity (ALPA) was significantly different between all soil type pairs suggesting that fertilizer treatment had little or no effect on this important soil activity. Bonferroni multiple comparison result also showed that mean β-glucosidase activity (BGA) and mean bacterial population (LOG10BC) differed significantly between CON and POL and between CON and POT but not between POT and POL suggesting that the fertilizer treatment only slightly improved these variables in the cement polluted soil but the improvements were still a long way off those in control soil. Lastly, mean difference in soil microbial

biomass-carbon (SMB-C) was significant between CON and POL underlining the impact of cement dust on the variable. However, no significant difference existed between CON and POT (p = .062 > .05) or between POT and POL (p = 1.000 > .05) indicating the effectiveness of fertilizer treatment in improving total soil microbial biomass-carbon (SMB-C) content.

The ANCOVA analysis of corn yield revealed that yield did not differ significantly with planting period but with soil type, F (2, 32) = 21.54, p < .0005; model adjusted r^2 = .534. This model could only explain 53.4% of the variations in the data suggesting that a lot of variations have been left unexplained. This is clearly due to the attempts made to meet most of the assumptions of this important statistical tool. It is actually because the planting periods of corn in Southern Nigeria do not significantly influence yield, that the region is known for an almost all-year round supply of the product. The significant mean difference in corn yield between CON and POL as well as that between CON and POT presented by Bonferroni multiple comparisons indicated that cement dust pollution significantly reduced corn yield and that fertilizer amendment of the polluted soil could not significantly reduce the impact of cement dust on the agricultural soil. Relative to the control soil, per cent loss in corn yield was 55.69% in un-amended cement dust-polluted soil but the loss reduced to 36.07% in NPK-treated polluted soil.

CONCLUSION

Principal component analysis (PCA) extracted two principal components, PC1 and PC2, from the nine dependent variables (DVs) which explained 68.33% variability about the data. Members within each component correlated positively with one another but negatively with members of the second component. The number and membership of extracted components were confirmed by the dendrogram of hierarchical cluster analysis (HCA) which presented two clusters. Significantly, component extraction and variable clustering were performed on the basis of variable relationship to soil microbial biomass-carbon (SMB-C). A one-way multivariate analysis of covariance (one-way MANCOVA) established significant main effect of soil type on the DVs after controlling for sampling period as covariate. The test of between-subjects effect showed that mean values of fungal population and dehydrogenase activity were not significantly affected by cement dust pollution or fertilizer treatment of the agricultural soils. Relative to control soil, per cent loss in corn yield was 55.69% in untreated cement dust-polluted soil but reduced to 36.07% in fertilizer-treated-polluted soil. The analysis has shown that cement dust pollution significantly reduced corn yield and that fertilizer amendment of polluted soil has little potential to reduce the impact and restore the fertility of cement dust polluted soil for high productivity.

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