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ASSESSMENT OF MICROBIAL AND SOIL ENZYME ACTIVITIES AS POTENTIAL INDICATORS OF SOIL QUALITYIN ULTISOLS, UYO, NIGERIA

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ABSTRACT
This study was conducted to investigate microbial biomass and soil enzyme activities as potential indicators of soil quality inultisols, Uyo, Nigeria. Soil samples for experiments were collected from old stadium Road (OSR), Old Ring Road (ORR) and Ukana Offot (UKO) at the depth of 0-20cm. soil samples for microbial and enzymatic analyses were stored in a cooler of iced block, while the samples for the determination of soil physical and chemical properties were stored in polythene bags and conveyed to the laboratory for analysis. The enzymes determined were; phosphatase, dehydrogenase, cellulase, catatase urease and invertase. The models for biological and enzyme indicator were used to determine soil quality. All the data obtained were subjected to descriptive statistics. The results showed that total heterotrophic bacteria (THB) in the study sites were 5.45 (±1.5)x10^{6}cfu/g, 3.30 (±1.0)x10^{6}cfu/g and 1.30 (0.2)x10^{6}cfu/g soil for OSR, ORR and UKO respectively. Total heterotrophic fungi were: 3.05 (±0.5) x10^{5}cfu/g, 2.00 (±0.02)x10^{6}cfu/g and 3.00 (±0.2) x10^{5}cfu/g soil for OSR, ORR and OKU respectively. The enzymological analyses showed that all the enzymes determined were present in all the samples, differences were obvious in the intensity. Potential dehydrogenase activity the only indicator of the possible sources of pollution implicated the presence of either chemical or biological pollution. Based on theoretical values of bacteria indicator (BISQ) and enzymatic activity, enzymatic indicator of soil quality (EISQ) showed low values. The low values of both BISQ and EISQ showed high anthropogenic influence and possible pollution.
Key Words: Enzyme Activities, Microbial, Potential Indicators, Soil Quality, Ultisols.

INTRODUCTION
Soil quality has been defined in several ways including fitness for use and dependent upon the extent to which soil fulfilled its destined role. Soil is one of the most dynamic environments of biological interactions in nature. It is also the receiver of plenty organic and inorganic substances resulting from human deliberate or accidental activities such as xenobiotic treatment used in agriculture (Filimon etal, 2012). These chemical substances might affect the growth and the dynamics of soil micro-organisms. The presence of different chemical substances in the soil has negative influence on the enzymatic activities. Bacterial enzymatic activities in the soil provide the decomposition of organic residue of plants or animals and so they allow the biogeochemical cycle of medimchemical elements: C, N, S, P, Fe (Filimon etal 2012). The decomposition of organic waste is caused by intracellular and extracellular enzymatic components produced by microorganisms or

vegetal sources (Filimon etal., 2012). Enzymes can be used to measure the effect of disturbance factor in soils (Taylor etal., 2002). Enzymatic activities are often proposed as indicators of environmental stress when pollutants are found in soil ecosystems. Some groups of micro-organisms are able to use different kinds of pollutants as minerals (C, N, P) and energy required for growth and development (Gimsing etal, 2004; Merini etal., 2007; Zabaloy et al.,2010). Enzymes acts to catalyze a series of biochemical and recycling of soil nutrients (Dick 1996). Some have substantial involvement in the process related to soil quality as is through them that soil microorganisms will degrade complex organic molecules be assimilated. Therefore, the objectives of this study were to examine microbial and enzyme activities as potential indicators of soil quality.

MATERIALS AND METHODS

The study was conducted in Uyo, located between latitude 4°30′and 05° 30N and Longitude 7° 30′ and 08°30′E. Three locations were chosen forthe study which includes Old Stadium Road (OSR), Old Ring Road (ORR), and UkanaOffot (UKO). The locations consistsof vast area of lands which have been put to various uses such as planting of vegetable and refuse dump.

SOIL SAMPLING

Three composite soil samples from each site of 0-20 cm depth in May 2013. For making one composite sample, five sampling points were selected using soil auger. Some samples meant for soil physical and chemical properties were properly air-dried and stored in labeled polythene bags. The field moist soil samples were stored in refrigerator at 4°c for preserving the enzymes and microbiological activities till the analysis were over. All chemical and biochemical results are results of triplicates analysis.

Analysis of Selected Physical and Chemical Properties of Soils of the Experimented Sites

- Soil particle size distribution was done by hydrometer method (Bouyoucos, 1962)
- Soil reaction (pH) in 1:25/water suspension using pH meter (Rowel, 1984).
- Electrical conductivity (EC) in 1:5 Soil/Water suspension using an electrical conductivity meter (Rhodes, 1982)
- Organic Carbon: was determined using walkley-Black wet oxidation method as described by Allison (1965)
- Total nitrogen was determined using Micro-Kjeldah digestion method (Bremner, 1965).

Microbiological Analysis

The serial dilution technique was used for all soil plate counts. Seven sterile test tubes were set up in the test tube rack and 9ml of sterile distilled water dispensed into them as diluents or dilution blank. One (1) gramme of the soil sample was added to the first test tube containing 9ml of diluents to give a dilution factor of (10^{-1}). 1ml of the aliquot was passed through logarithmetric dilutions to the fourth factor. 1ml of the sample diluents in the fourth factor was plated out on a different sterile commercial growth medium appropriate for the organism in question. Total heterotrophic bacteria was placed on Nutrient agar, total heterotrophic fungi was placed on potatoe dextrose agar. The different cultures were incubated at different temperatures and time regimes required for optimum growth of the microbial organisms (Bacteria incubated at 37°c for 24 hours and fungi 72 hours at 28°c). After incubation, visible colonies were carefully counted, studied, characterized and identified to species levels (Collins and Lyne. 1976).

DETERMINATION OF ENZYMATIC ACTIVITIES

The activities of six enzymes were evaluated. These included dehydrogenase, invertase, produced by all microorganisms and indirectly related to organic carbon content, urease, phosphatase(acid and alkaline) important in nitrogen and phosphorous recycling respectively. Dehydrogenase was analyzedaccording toCasida et al (1964) involving the use of triphenyltetrazolum chloride (TTC) amended soil with formation of triphenylformanzan (TPF) absorbance of the soil 485nm. Urease activity was determined by the method described by Gu and Kang (2009) with urea as substrate, alkaline and acid phosphase were investigated according to (Tabatabai and Bremner, 1969) at pH of 11 and 6.5 respectively using P-nitrophenyl phosphate as substrate, and formation of p-nitro-phenol. The yellow colour intensity was measured colorimetrically at the wavelength of 400-420 nm. Invertase activity was measured according to the method of (GUand Kang 2009) with sucrose as substrate and the wave length was measure at 508 nm and the invertase activity expressed as Mg NH_4-Ng^{-1} soil. Cellulase activity was determined as described by pancholy and Rice (1973). Toluene-treated soil samples (as described for urease) were mixed with 20ml 0.5m acetate buffer (pH 5.9) and 20ml fresh prepared 2% carboxymethyl cellulose (CMC). The soil mixture was incubated at 30oC for 24hrs followed by shaking the supernatant, was filtered through whatmem No. 41 filter paper and aliquots analyse for the reducing sugars content cellulose activity was expressed as mg reducing sugars produced g^{-1} soil.

Soil Quality Indicator Models: Bacterial indicator of soil quality (BISQ)

This was evaluated according to the ecophysiological bacterial group. It was based on the model proposed by Mutean et al. (1995).

$$= \frac{1}{n} \times \sum \log N$$

BISQ = Bacterial Indicator of Soil Quality
N = Number of ecophysiological group
n = Number of bacteria which belongs to each ecophysiological group.

Enzymatic indicator of soil quality (EISQ)

The calculation of enzymatic indicator model was based on the absolute values of the enzymatic activities from every sample analysed. In order to do this, the model proposed by Munteen et al. (1995) was used.

$$EISQ = \frac{1}{N} \times \frac{\sum Vr\,(i)}{Vmax(ii)}$$

Where: EISQ = enzymatic indicator of soil quality
n = number of activities
Vr(i) = Real individual number
Vmax(ii) = maximal theoretical individual value.

STATISTICAL ANALYSIS

All the data obtained were subjected to descriptive statistics such as mean, standard deviation, standard error.

RESULTS AND DISCUSSION

Soil properties

The results in Table 1 showed the soil properties of the study areas. These are known as chemical indicators which include pH, Salinity (electrical conductivity), organic matters content, phosphorus availability, cation exchange capacity. These indicators determine the presence of soil-plant-related organisms, nutrient availability, water for plants and other organisms and mobility of contaminants. The soil reactions for the three study sites were slightly acid with the pH of 6.45±0.29, 5.65±0.08 and 6.35±0.2 respectively for OSR, ORR and UKO, with Electrical conductivity are generally low in all the study sites with 0.13±0.000, 0.16±0.02 and 0.19±0.05dsm^{-1}for OSR, ORR and UKO respectively, Organic carbons in the study sites were generally high with 3.44±0.63, 2.48±0.3 and 2.35±0.2% for OSR, ORR and UKO respectively. Total nitrogen in the areas are generally very low when compared with the lower critical level of 0.1 – 0.2% proposed by Esu et al, (2009).

Table 1: Distribution of Physico-chemical Soil Properties at Three Different Sites.

Soil Property	OSR	ORR	UKO
pH	6.45±0.29	5.65±0.08	6.35±0.2
EC (dSm-1)	0.13±0.006	0.16±0.02	0.19±0.05
OC (%)	3.44±0.63	2.48±0.3	2.35±0.2
OM (%)	5.95±1.70	4.07±1.2	4.3±1.2
TN (%)	0.071±0.001	0.073±0.07	0.084±0.04
AV.P (mgkg-1)	15.73±2.16	21.54±2.3	16.48±2.2
Ca (cmolkg-1)	7.85±0.08	8.75±2.5	5.9±0.5
Mg (cmolkg-1)	2.8±0.26	3.24±0.27	2.7±0.2
Na (cmolkg-1)	0.15±0.007	0.16±0.01	0.13±0.01
K (cmolkg-1)	1.06±0.03	1.09±0.12	0.87±0.02
EA (cmolkg-1)	2.09±0.14	1.42±0.11	1.82±0.2
ECEC (cmolkg-1)	13.94±2.6	14.65±2.4	11.41±2.8
BS (%)	77.72±3.20	83.11±4.0	76.62±5.2
Sand%	86.53±1.6380.53±1.6385.34±1.62		
Silt %	5.80±1.406.75±0.7 7.40±0.71		
Clay%	7.70.±2.1 8.50±0.18 9.65.±1.50		

Microbiological Activities

Microorganisms are widely used as soil quality indicators. Soil contains a large variety of microbial taxa with a wide diversity of metabolic activities (Parkinson and Colema, 1991). Soil microbial biomass compared with that of superior organisms is a more sensitive indicator and is influenced by different ecological factor like plant diversity, soil organic matter content, moisture, and climate changes. The ecophysiological groups of microorganisms anlaysed were total heterotrophic bacteria (THB), total heterotrophic fungi (THF), Total salmonella, shigella count (TSSC), Eschericia coli (E. coli) and total coliform bacteria (TCB). The microbial counts of microorganisms isolated from the study sites are as shown on the. Total bacterial counts were 5.45(±1.5) x106cfu/g, 3.30(±1.0)x 10^6cfu/g and 1.50(0.2)x10^6cfu/g respectively for OSR, ORR and OKU. Total Eschericia coli (TEC) were 5.15(±1.5) x106cfu/g, 1.20(±0.1) x106cfu/g and 2.250(±0.1) x106cfu/g for OSR, ORR and UKO respectively. Total heterotrophic fungi were 3.05(±0.5) x106cfu/g, 2.00(±0.02) x106cfu/g and 3.00(±0.2) x106cfu/grespectively for OSR, ORR and UKO study sites. The results revealed that bacterial counts were significantly (P<0.05) higher in the study sites particularly in the samples from OSR (Old Stadium Road). The reasons for the high bacteria counts may be attributed to the availability of favourable growth factors such as organic matter and oxygen. Fungal counts were the next abundant organisms.

Table 2: Distribution of Microbial Density at the Three Different Locations.

Microbe	OSR	ORR	UKO
	← CFU/g →		
THB (NA)	$5.45(\pm 1.5)x10^6$	$3.30(\pm 1.0)x10^6$	$1.30(\pm 0.2)x10^6$
TEC (EMBA)	$5.15(\pm 1.5)x10^6$	$1.20(\pm 0.1)x10^6$	$2.50(\pm 0.1)x10^5$
TSSC (SSA)	$4.35(\pm 1.2)x10^6$	$3.80(\pm 0.9)x10^6$	$2.55(\pm 0.6)x10^6$
THF (PDA)	$3.05(\pm 0.5)x10^6$	$2.00(\pm 0.02)x10^5$	$3.00(\pm 0.2)x10^5$
TCB (MAC)	$5.00(\pm 1.5)x10^4$	$5.00(\pm 1.5)x10^4$	Nil

Microbial Isolates from the Study Sites

The bacterial isolates from the study sites include the following species: *Staphylococcus aureus, Staphyloccusepidermidis, Pseudomonas aeroginosa, Escherichia coli,Salmonella typhi, Proteus vulgaris* and *Aerobacteraerogens*. The fungal isolates were mostly, *Aspergillusfumigatus, Aspergillusniger, Aureobasidium, spp, Microsporiumgypseum, Batrytisspp,* and *Pemicilliumnotatum*. The bacterial species isolated from the study sites are mainly from the family *Enterobacteriaceae*. The occurrence of these organisms may suggest that the soil has more anthropogenic activities because of the microorganisms are those that play a key role in nutrient cycling and energy flow (Li and chen 2004), microbial communities respond to environmental stress or ecosystem disturbance, affecting the availability of energetic compounds that support microbial population (Marinari et al. 2007).

Enzymes Activities

Enzymes activities have been association with indicators of biogeochemical cycle, degradation of organic matter and soil remediation processes, so they can determine, together with other physical or chemical properties, the quality of a soil (Gelsomino et al. 2006). Also Dick (1996); Nelsen and Winding (2001); Eldor (2009), reported enzymes as good indicators because, they are closely related to organic matter, physical characteristics, microbial activity and biomass in the soil and provide early information about changes in quality. In this study the enzymes activity we determined were: Dehydrogenase (Actual and potential), phosphatases (Acidand alkaline), Catalase and Urease. Actual dehydrogenase activity in the study site were 2.10, 2.0 and 0.2 mgformazan/g soil with the mean of 1.43mg formazan per gram soil for OSR, ORR and UKO respectively, while, potential dehydrogenease activity were 5.3, 6.3 and 6.0 mg formanzan per gram soil respectively for OSR, ORR and UKO. Potential dehydrogeneaseactivity were significantly ($P<0.05$) higher than

actual dehydrogenase and well represented, being the only enzymes which gives us indication regarding the possible source of pollution. The actual and potential dehydrogenase activities are the ones which reflect the numerical density of the microorganisms existing in soil.The more these types of activities recorded higher values the highest the number of microorganisms is in the soil so pollution is reduced (Fig. 1).

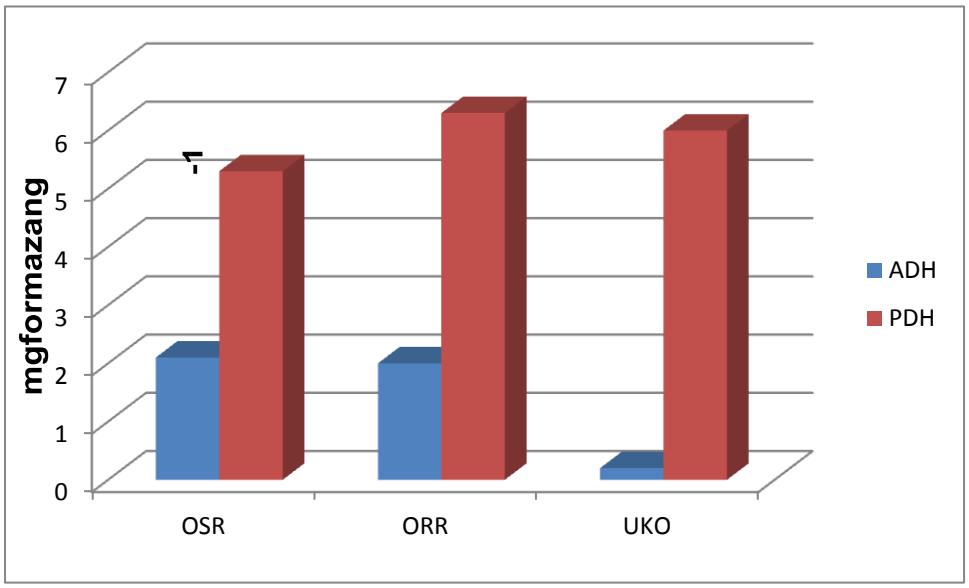

Fig 1: Actual and Potential Dehydrogenease Activities in the Study Sites

Enzyme is often used as a measure of any disruption caused by pesticide, trace elements or management practices to the soil (Paddy and Paza, 1989)as well as direct measure of soil microbial activity (Skujims 1978), it can also indicates the type and significance of pollution in soils. Actual dehydrogenaze was significantly ($P<0.05$) lower than potential Dehydrogenaze in the study sites. Phosphomoesterases (acid and alkaline Phosphotases). The result showed that acid phosphotases was significantly ($P<0.05$) higher in soils (7.2 phenylg/soil obtained from OSR compared to the soil samples from ORR (4.5 phenol/g soil) and UKO (3.3) Phenol/g soil). Acid phosphateaseis one of the enzymes regulating phosphorous availably in soil and plant roots are the major producer of acid phosphatase (Spier and Cowling 1991). Acidic nature of the studied soil is the contributing factor for the higher values of acid phosphatase as compared to alkaline phosphates. Higher values of acid photophase in acid soils were also reported by (Dick et al 2000; Wang et al., 2012). Soils from the three study sites showed lower values of alkaline phosphates (2.8, 3.0 and 2.9 phenol/g soil). The lower values of alkaline phosphatase may probably

be due to lower microbial activity in the studied sites. Our results for alkaline phosphatase were in agreement with Aseri et al (2009) who suggested that lesser microbial activity in the soil could be directly related to lower alkaline phosphatase activity in the soil and microorganism are major source of alkaline phosphatase.

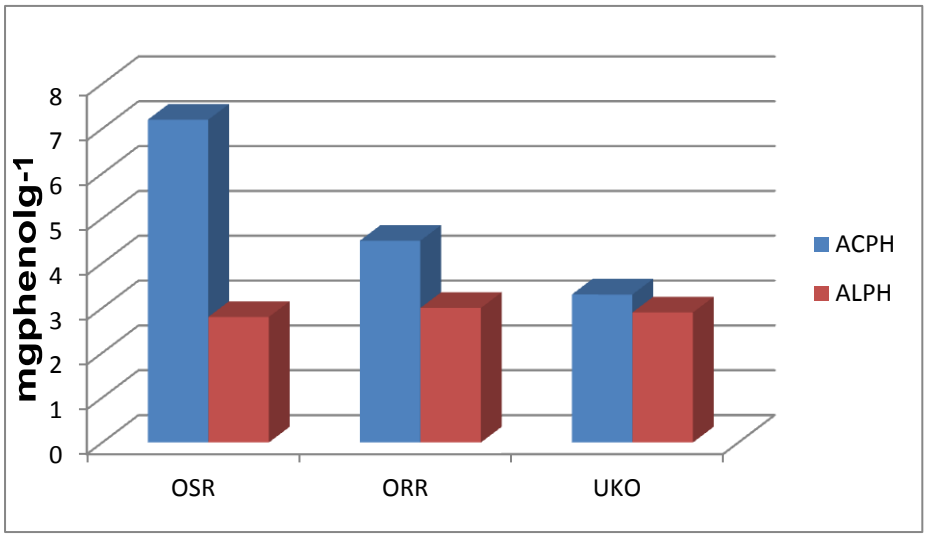

Fig 2: Acid and Alkaline Phosphatase Activities in the Study Sites

The values for Catalase activity in the studied soil were 4.5, 7.6 and 5.8 mgH_2O_2/g in the soil in OSR, ORR and OU respectively (fig. 3). The value was significantly ($P>0.05$) higher in samples from ORR. Catalase is an intracellular enzyme and involved in microbial oxidoreductase metabolism (Garcia-Gil et al., 2009)(fig 4). These enzymes are involved in urea hydrolysis into O_2 and NH_4 and consequently with soil pH increase and N loses by NH_3 volatilization. Urease has been widely used to evaluate changes on soil quality related to management, since its activities increases with organic fertilization and decreases with soil tillage (Saviozi et al., 2001). Urease activity is used as a soil quality indicator because it is influenced by soil factors such as croppinghistory, organic matter content, soil depth, management practices, heavy metals and environmental factors like temperature and pH (Yang et al., 2006).

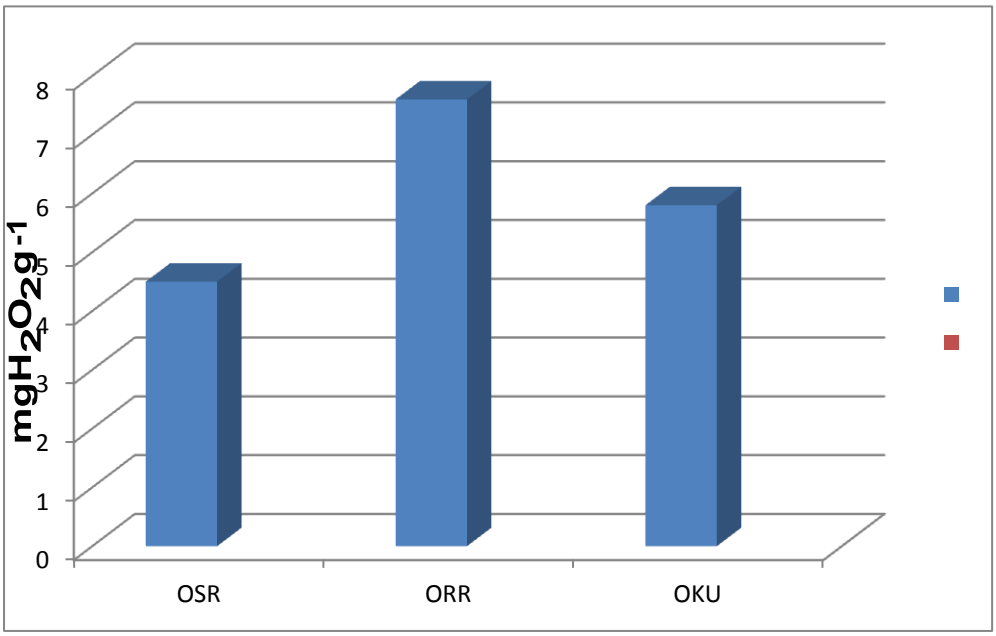

Fig 3: Catalease Activity in the Study Sites

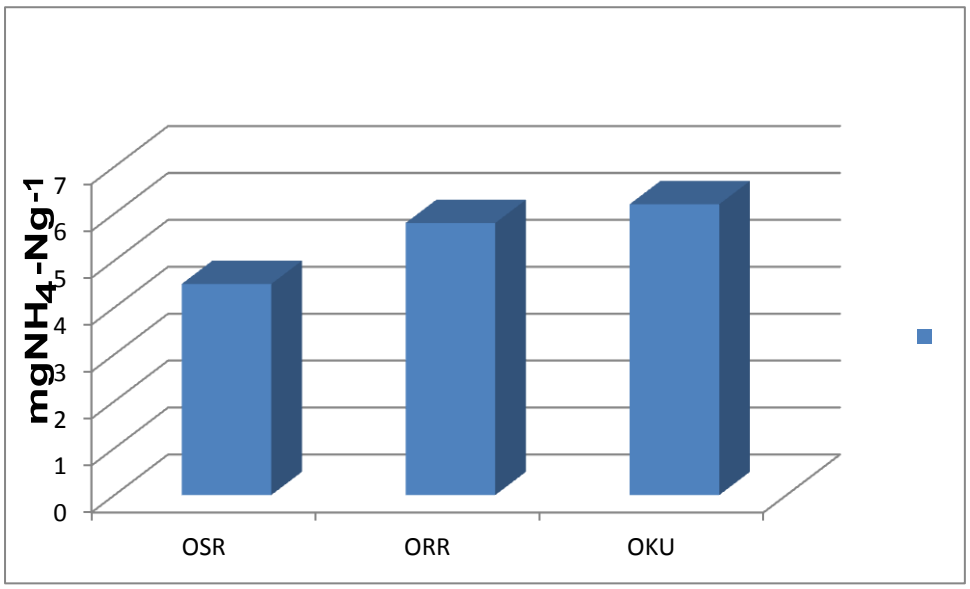

Fig 4: Urease Activity in the Study Sites

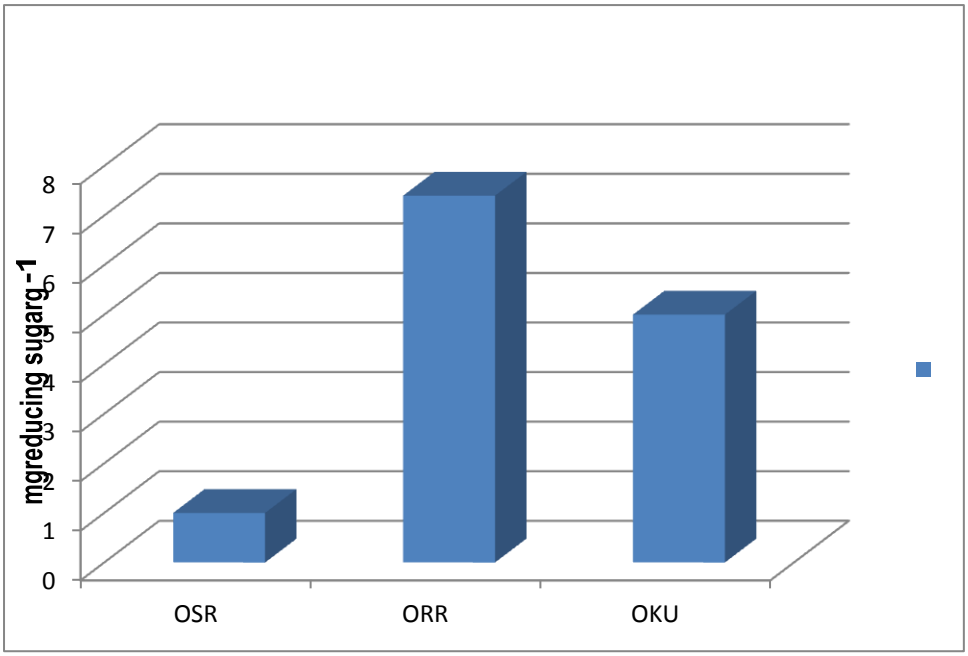

Fig 5: Cellulase Activity in the Study Sites

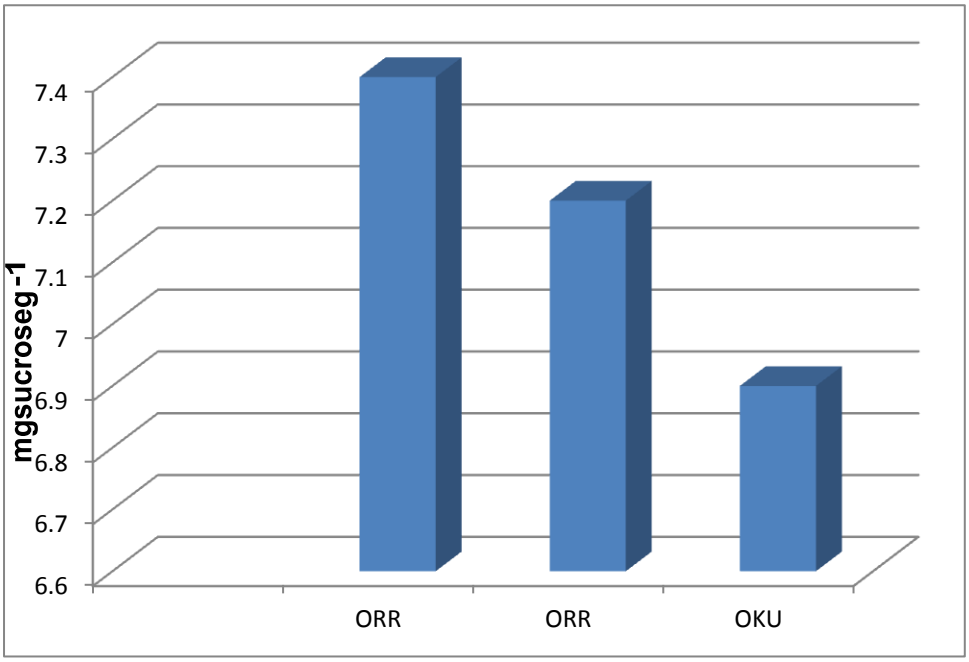

Fig 6: Invertase Activity in the Study Sites

Bacterial Indicator of Soil Quality

Ecophysiological groups of bacteria that have been isolated are those involved in human diseases and environmental pollution and were represented by bacteria found in the environment, They can cause diarrhea, urinary tract infections, respiratory illness and pneumonia, Salmonella typhi they are bacteria that cause typhoid fever, *Aerobacter, aerogene,*Proteus vulgaris,Proteus mirabilis and *Pseudomonas aeroginosa*. Based on the model proposed by Munteem, et al. (1996), it was observed that these bacteria that belong to the same ecophysiological group.ORR has the highest bacterial indicator followed by UKO while OSR have the least indicator.

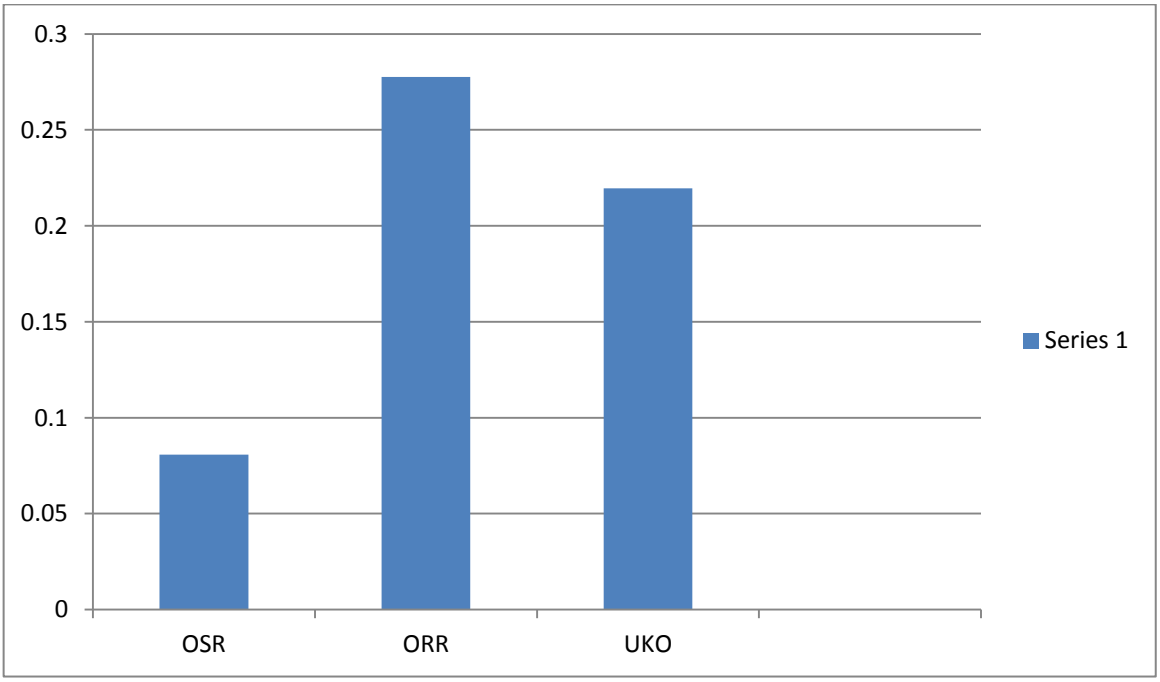

FIG 7: Distribution of bacteria quality indicator across the different location.

Based on absolute values each enzyme activity studied, the enzymatic indicator of soil quality (EISQ) was calculated according to the model (Munteem et al., 1996). Theoretically, the enzymatic indicator may exhibit values between 0 (where no activity exist in the studied samples) and 1 (where the real individual values are equal to the maximal theoretical individual values of all activities).The results showed that OSR had an enzymatic indicator of soil quality value of 0.029, ORR had the value of 0.023 and UKU had the value of 0.028. The value of EISQ in all the studied soils showed low enzymatic activity with high anthropogenic influence. These may most probably be due changed in the soil properties due to external factors such as human and

pollutants. The low EISQ could however be expected because the studied sites were for more them a decade used as refuse dump sites (Fig.8).

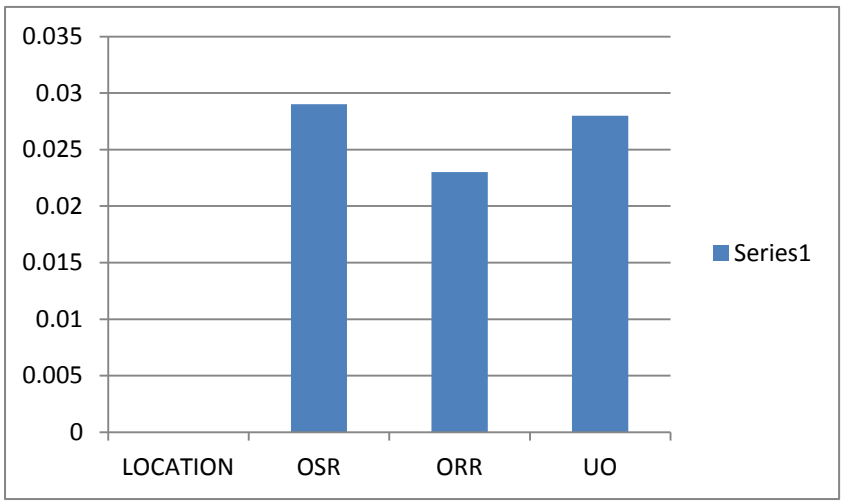

FIG 8: Distribution of enzymatic quality indicator across the different location.

CONCLUSION

The analysed soil samples showed the presence of the bacterial species from the family *Enterobacteriaceae*such as Escherichia coli, *Staphylococcus, Salmonella* and *Aerobacter*. All the enzymes tested were present an all the soil sample. The enzymatic and biological indicators of soil quality had low values representing a poof of anthropic activity and pollution

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