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EFFECT OF LENGTH OF SOIL SAMPLE STORAGE ON SOIL MICROBIOLOGICAL AND BOCHEMICAL PROPERTIES IN UYO, NIGERIA

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ABSTRACT: *In all microbiological and biochemical investigations of soil processes require freshly collected soils from the field. But very often there is some delay between sample collection and experiments or analysis, which require the soil be stored for some period of time. These studies investigate effect of length of soil sample storage at 4^{o}C on soil microbiological and biochemical properties. This experiment was laid out in a completely randomized design (CRD) in triplicates. The experiment was conducted at the Department of Soil Science and Land Resources, Management, University of Uyo, Nigeria, between July 2014 and July 2015. Soil sample was randomly collected from six different points at the old stadium road dumpsite Uyo. The samples were thoroughly mixed to obtain two composite samples. The soil samples were separated into three portions: Soil samples air-dried at room temperature (28^o C) for physico-chemical analysis Samples used for microbiological and enzymatic activities the same day of collection to serve as day 0 or control. Soil samples stored in the refrigerator at 4^{o}C for sequential analysis of microbiological and enzymatic activities on the 7, 14,21,28,35 and 42 days after storage. The results revealed significant (P=.05) reduction in bacterial load from 2.45± 0.5 cfu/g soil in Day o of the experiment or control to 1.2±0.06 cfu/g soil in Day 42 which is 48.51% decrease in 0-15cm depth. In 15-30cm depth, bacterial load decreased from 1.76±0.01 cfu/g soil in the control to 1.11± 0.02cfu/g soil, which is 36.97% decrease. Fungal load follows the same trend with significant (P=.05) decrease from 1.75±0.08 cfu/g soil in Day o to 1.042±0.01cfu/g soil in Day 42 of the experiment at (0 -15cm depth which is 40.57% reduction. And in 15 – 30cm depths, a significant (p = .05) decrease was similarly observed from 1.48±0.06 cfu/g soil in Day o to 0.95±0.05 cfu/g soil in Day 42 of the experiment, which is 35.31% reduction. The result also showed that some bacterial genera especially Bacillus which are gram positive persisted up to the end of the experiment. Some fungal genera especially Aspergillus persisted up to the end of the experiment. Enzymes activities similarly suffered significant (P =.05) reduction in this research. Cellulase decreased significantly (P=.05) which is 80% and 98.75% respectively for (0- 15 and 15 – 30cm depths). The same trend was also followed by dehydrogenase. This study showed that long storage of samples before microbiological or biochemical analysis should be discouraged if optimum result is to be achieved.*

KEYWORD*:* Length of Soil Shortage, Microbiological, Biochemical, Soil Enzymes.

INTRODUCTION

Investigations on soil biogeochemistry often require analytical and experimental procedures that cannot be performed in-situ in the field. For these ex-situ analysis and experiments, it is

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preferable to use freshly collected soil samples. But very commonly, there are some delays between sample collection and experiment or analysis which require the soils to be stored for some period of times. Cold storage at 4^o c is usually recommended for microbiological experiment (Brahom *et al.,* 2001; Gonzalex *et al.,* 2009), because this reduces biomass growth (Anderson 1987; Bloem *et al., 2006)* and substrates depletion as compared to high temperature storage. Cold storage has however, shown to decrease soil microbial enzyme activities (Verchot 1999; Tuner and Romero, 2010). Conversely, samples are often dried to extend storage time, but, this too is known to alter the microbiological and chemical composition of the soils (Bartiett and James, 1980, Shephard and Addison 2007) as well as reduced enzyme activity (Turner and Romero, 2010). One approach to minimize changes in soil metabolism during storage is to impose conditions that slow populations in the native environment (Bloem *et al.,* 2006) found out that storage at the in-situ soil temperature best preserve microbial activities. Following the USDA temperature classification of soil, the optimal storage method may be freezing for frigid soils,40^oC for mesic soils and air drying for thermic soils (soil survey staff 2010). The treatment and handling of samples after collection is a critical aspect of a study design when using microbiological or biochemical methods to compare the composition and diversity of microbial communities from environmental samples. It is widely assumed that these methods must be explored at the process of extraction from the samples immediately after collection or if this not possible, the samples must be frozen, because samples stored at room temperature even for a short period are often considered unfit for down stream analysis, because of changes in microbial community (Rochelle *et al.,* 1994). Although the assumptions are wide spread, but there is dearth of information on the influence of length of sample storage at 4^o c on the microbial community and biochemical properties. The objective of this study was therefore designed to evaluate the effect of length of soil samples storage on soil microbiological and biochemical properties.

MATERIALS AND METHODS

Study Site

This research was conducted in Uyo, Akwa Ibom State, Nigeria. Uyo is located within (N5.3"o", E7.55^o)

Soil Sampling and Treatment

Soils were collected from a single location at the old Stadium road dumpsite. This dumpsite has been receiving solid waste for the past 20years. Samples were collected at the surface and substance depths of 0-15 and 15-30cm. we focused our sampling on these horizons because large portion of microbial activity occurs in these horizons, and the effects of plant roots are mostly within these horizons. Soil sampling were randomly collected from six different points. The samples were thoroughly mixed to obtain two composite samples. Following removal of plant remains, broken bottles and other solid waste, each composite sample was passed through a 2-mm mesh and separated into three portions.

1. Soil samples air-dried at room temperature (28^o ^{C}) for physico-chemical analysis.
2. Samples used for microbiological and enzymatic activities the same day of sample collection to serve as control or Day o.

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3. Soil samples stored in the refrigerator at 4° c for sequential analysis of microbiological and enzymatic activities on the 7, 14,21,28,35 and 42 days after storage.

Determination of Physicochemical Properties

Soil Routine Analysis was carried out based on standard laboratory procedures. For instance, particle size distribution was analyzed based on the method described by day (1965).

Soil reaction (pH) was determined using glass electrode pH meter in water (1:2.5 soil: water ratio IITA, 1979)

Organic carbon was determined following Walkley and Black wet oxidation method elaborated by Allison (1965).

Available phosphorus was determined by extracting the soil with Bray P -1 extractant and phosphorus in solution determined by method of Murphy and Riley (1962).

Total nitrogen was determined by the Micro-Kjeldahl digestion method (Bremner, 1965).

Exchangeable acidity, exchangeable cations (Ca,Mg,K and Na), percentage base saturation were performed according to methods described by Udo and Ogunwale (1986).

Soil reaction (pH).

MICROBIOLOGICAL ANALYSIS

Serial Dilution

Ten-fold serial dilution of the soil samples as described by (inoculation and incubation) one milliliter of appropriate ten-fold serial dilution of the soil samples were inoculated into nutrient agar and sarbourand Dextrose Agar plates in triplicates using pour plate technique. Inoculated plates were incubated at 37°C for 18 -24 hours and 48 – 72 hours for enumeration of total heterotrophic bacteria and fungi respectively. Visible discrete colonies in incubated plates were counted and expressed as colony forming unit per gram soil C cfu/g of soil.

Maintenance of Pure Culture

Discrete colonies were purified by repeated sub-culture unto appropriate agar media. Pure cultures were preserved on nutrient agar slants and stored in the refrigerator at a temperature of 4° C.

Characterization and Identification of Microbial Isolates.

Pure cultures of microbial isolates were identified based on cultural parameters, microscopic techniques, and biochemical test, which included the following: Grain's staining, motility, indole, methyl red test, citrate utilization, H_2S production, spore formation and starch hydrolysis (suckshank *et al.*, 1976). Identification of the bacterial isolates was accomplished by comparing the characteristics of the culture with that of known taxa as in (Holt *et al.*, 1994). Characterization and identification of fungal isolates was carried out as outlined by (Domsch *et al.*, 1980; Barnet and Hunters, 1987).

ISSN 2053-5805(Print), ISSN 2053-5813(Online)

Enzymatic Analysis

Urease activity was determined as described by Pancholy and Rice (1973). Briefly, 1ml toluene was thoroughly mixed with 10g moist soil sample in a 100ml Erlenmerger Flask. After 15 mins, 20ml phosphate buffer (PH, 6,7) and 20ml of 10% Urea solution was added to the flask. The reactants were incubated at 30^{o} C for 24 hours followed by shaking for 15 mins. With 30ml of NKCl solution. The content were filtered and the filtrate made up to 100ml with deionized water. Aliquot (5ml) were analyzed for the NH_4 –N content. The optical density reading was taken with the calorimeter at 587nm and expresses as mg/NH_4 - N/g soil.

Cellulase Activity

One milliliter toluene was thoroughly mixed with 10g moist soil samples in a 100ml – Erlenmerger flask, and 20ml – 0.5M acetate buffer (PH 5.9), 20ml of freshly prepared 2% carboxymethyl cellulose (CMC) were mixed appropriately. The soil mixture hours followed by centrifugation at 4000 rpm for 20 mins. The supernatant was filtered through a wharman No 41 filter paper and aliquots analysis for reducing sugar content using colorimeter and the absorbance measured at 578nm. Cellulose activity was expressed as mg reducing sugar per gram soil.

Catalase Activity

Catalase activity was determined according to the method of Dragan – Bularda (2000).

Briefly, 3g moist soil samples was added to 250ml flask, then 40ml distilled water and 10ml phosphates buffer (pH 6.8), 3% H_2O_2, were poured into the flask and shaken for 20 minutes. After shaking 10ml 3N H_2SO_4 was added to terminate the reaction. The wavelength was measured colorimetrically at 480nm and expressed as mg H_2O_2 g^{-1} soil.

Dehydrogenase Activity

Dehydrogenase activity was determined using the method described by Dragan – Bularda (2000). The reaction mixtures included the following, 3g field moist soil, 1ml 3% 2,3,5 – triphenyl tetrazolium chloride (TTC), 2.5ml distilled water or 2.5ml 3% glucose solutions for actual and potential dehydrogenase respectively. The mixture was incubated for 24hours at 37^{o} C. after incubation 10ml methanols was added and shaken for 30mins. The mixture was then filtered through Whatman no 42 filter paper. Dehydrogenase activity was measured calorimetrically at 485nm and expressed as mg formazan g^{-1} soil .

Statistical Analysis

Data were subjected to statistical analysis using mean. Standard deviation and analysis of variance (ANOVA) Analysis of variance was used to compare treatment.

RESULT AND DISCUSSION

The results in table 1 showed the soil properties of the experiment at site. The soil of this area is described as Ultisols or typic paleudult (Kang et al., 1999). The soil is known to have low structural stability, making it susceptible to soil compaction and erosion. Ultisol have low nutrient reserve and consequently nutrient levels in the soil solution are usually inadequate to

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provide for the nutrient needs of crops. In addition toxic aluminum and manganese, levels, low calcium magnesium and potassium levels and multi-nutrient deficiencies severely limit crop growth and yields.

PHYSICO-CHIMICAL PROTERIES OF THE SOIL

Table 1: Physico-chemical properties of experimental soil (0-20cmdelpth)

Particulars	Value
pH (1:2.5; soil : water)	6,.1 ± 0.08
Electrical Conductivity ((dS/m)	0.16 ± 0.12
Organic Carbon (%)	4.31 ± 1.70
Total Nitrogen (%)	0.10 ± 0.04
Available phosphorus (mg/kg)	38.40 ± 7.29
Calcium (cmol/kg)	11.62±0.63
Sodium (cmol/kg)	0.10 ± 0.01
Magnesium (cmol/kg)	3.92 ± 0.27
Potassium (cmol/kg)	0.34 ± 0.03
Exchangeable acidity (cmol/kg)	2.08 ± 0.04
Base saturation (%)	88.42 ± 0.22
Sand (%)	80.53 ± 1.63
Silt (%)	7.40 ± 0.7
Clay (%)	12.07 ± 0.18

EFFECT ON MICROBIAL LOAD

The result in tables 2 and 3 showed both bacteria and fungal loads. Microbial loads decreased significantly (P =.05) as the length of sample storage increases. Bacteria loads decreased from 2.45±0.5 cfu/g soil in Day o or control to 1.2±0.06 cfu/g in Day 42 of the experiment which is 48.57% decrease and from 1.76±0.01 cfu/g soil in Day o to 1.11±0.02 cfu/g soil in day 42 of the experiment which is 36.97% decrease, fungal population with significant (P =.05) decrease from 1.75±0.08 cfu/g soil in Day o to 1.042±0.01 cfu/g soil in Day 42 of the experiment in 0.15cm depth which is 40.57% reduction. And 15-30cm depth a significant decrease was similarly observed from 1.48±0.06 cfu/g soil in Day o to 0.95±0.05 cfu/g soil in Day 42 of the experiment, which is 35.31% decrease. The reason for the decrease in microbial loads with increase in length of sample storage and with increase in depth of sampling may probably be due to decrease in available substrate in the soil samples. The decrease in microbial load with depth was expected as the A horizon of the soil profile contains more organic matter than the B and C surfaces which is the main source of nutrients for microbes. This result was inconsistent with other studies. For instance, Ross (1991) found a 41% reduction of behaviour after 14 months of storage at 4^{o} c as well as a similar reduction in basal respiration rate.

ISSN 2053-5805(Print), ISSN 2053-5813(Online)

TABLE 2: Total Heterotrophic Bacterial counts from the Dumpsite, (cfu/g)

	STORAGE PERIOD (DAYS)						
Sampling Depth	0	7	14	21	28	35	42
Θ -15	2.45±0.5	2.32±0.4	1.8±0.04	1.7±0.01	1.61±0.08	1.58±0.04	1.26±0.09
15 - 30	1.76±0.011	1.53±0.09	1.55±0.09	1.5±0.07	1.39±0.12	1.27±0.07	1.11±.12

TABLE 3: Total heterotrophic Fungal counts from the Dumpsite, (cfu/g)

	STORAGE PERIODS (DAYS)						
Sampling Depth	0	7	14	21	28	35	43
Θ -15	1.75±0.8	1.59±0.04	1.40±0.14	1.22±0.04	1.26±0.03	1.21±0.02	1.04±0.01
15 – 30	1.48±0.026	1.47±0.16	1.34±0.10	1.13±0.06	1.05±0.04	1.02±0.01	0.95±0.05

THE EFFECT ON MICROBIAL ISOLATES

The result in tables 4 and 5 showed the bacterial and fungal species isolated from the soil samples respectively. It was observed that most of the bacterial species isolated from the samples in the control, that is, day 0 of the experiment were not detected after 14 days of storage (table 4). For example, Aerococcus vircolans was no longer detected after 14 days of storage. It was also observed that some bacterial species persisted up to the end of the experiment. Those that persisted up to the end of the research were mainly the Gram positive and spore forming organisms Bacillus polymyxa, Bacillus substilis micrococcus. The reasons for the persistence of these organisms may probably be due to their endowed ability to resist stressed environment.

Also, Gram positive bacteria are organisms that possess thick cell wall that is stacked with 26 layers of peptidoglycan which gives the organisms the strength to resist hashed environments (Madigan *et al.*, 2009). The reasons for the early disappearance of some of the bacterial species may probably be due to decrease in available substrate in the soil sample or their organisms in ability to resist harsh environment.

This finding was in consistent with (Breitenback and Bremner 1987, Stenberg *et al*., 1993, Ross *et al*., 1980) who reported that storage of soil sample may change the size of bacterial populations as well as their activities due to depletion in nutrient.

ISSN 2053-5805(Print), ISSN 2053-5813(Online)

Table 4: Occurrence of Bacterial Isolated after Storage

	STORAGE PERIODS DAYS						
BACTERIAL ISOLATES	0	7	14	21	28	35	43
Aerococcus viridans	D	D	D	ND	ND	ND	ND
Alcaligene faecalis	D	D	D	D	D	ND	ND
Bacillus subtilus	D	D	D	D	D	D	D
Bacillus polymyxa	D	D	D	D	D	D	D
Corynebacterium murium	D	D	D	D	D	ND	ND
E.coli	D	D	D	D	ND	ND	ND
Entrerococcus faecalis	D	D	D	D	ND	ND	ND
Clostridium sporogenes	D	D	D	D	D	D	D
Listeria morrocytogenes	D	D	D	D	ND	ND	ND
Pseudomonas Varians	D	D	D	D	D	D	D
Pseudomonas dimiuta	D	D	D	D	D	D	D
Staphylococcus aureus	D	D	D	ND	ND	ND	ND
Pseudomonas aeruginosa	D	D	D	D	D	D	D

D = Detected, ND = Not detected

Table 4: Occurrence of Fungi and Yeast after Storage

	STORAGE PERIODS (DAYS)						
Fungal Species	0	7	14	21	28	35	42
Asperigillus clavatus	D	D	D	D			
Aspergillus flavus	D	D	D	D	D	D	D
Aspergillus fumigatus	D	D	D	ND	D	D	D
Aspergillus glaucaucus	D	D	D	D	ND	ND	D
Aspergillus niger	D	D	D	D	D	ND	D
Aspergillus ochraceus	D	D	D	D	D	ND	D
Aspergillus porasiticus	D	D	D	D	D	D	ND
Aspergillus terreus	D	D	D	D	D	ND	D
Aspergillus virsicolor	D	D	D	D	D	ND	ND
Cladoporium herbarum	D	D	D	b	D	ND	D
Microsporium aenigmaticum	D	D	D	D	D	ND	ND
Pericillium expansum	D	D	D	D	D	D	D
Yeast	D		ND	ND	ND	ND	ND
Canida krusei	D	D	ND	ND	ND	ND	ND
Candida albicans	D	D	ND	ND	ND	ND	D
Candida tropicalis	D	b	ND	ND	ND	ND	ND
Candida utilis	D	D	ND	ND	ND	ND	ND
Saccharomyces cerevisie	D	D	ND	ND	ND	ND	

D = Detected ND = Not detected

Others found out that the microorganisms that are neither Gram positive or spore formers that could not adapt to the stress factor and therefore less resistant to the length of storage period. The unadapted and most vulnerable microorganisms are killed and utilized as energy sources by the survivors, increasing the overall enzymatic activity of extra cellular enzymes (Zelles *et al*., 1991). Fungi were also affected by length of storage as well as the temperature of storage (4^{o}C). Some fungal species persisted up to the end of the research such as, *Aspergillus*

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fumigatus, Aspergillus terreus, Aspergillus niger and *Aspergillus ochraceus, Cladosporium herbarum, Saccharoniyces cereviseae, Candida utilis and Candida tropicalis.* The following fungal genera could not persist after 21 days of storage *Absidia confubifera, Aspergilus claratus, Aspergilus virsicolor* and *Candida albicans.*

The reason for the persistent of some of the fungi may be due to their ability to resist harsh environment. Decreased in some of the fungi and yeast was expected because they are always susceptible to cold temperature and prolong storage in cold temperature will result in the dead of most of them and subsequently serve as substrate for surviving organisms. This result corroborated with AL – Jasser (2010) who reported a significant decrease in yeast and mold counts in samples stored at 19^{o} C for 6 month, although he recorded decrease in the yeast and mold stored at 26^{o} and 4^{o} C but the decreases were not statistically different.

EFFECT ON ENZYME ACTIVITY

Enzymes play a fundamental role in the cycles of important elements such as nitrogen (Ureases and proteases), phosphorus (phosphotases) and carbon (B – glucosidases) (Ross *et al.*, 2004) carry out hydrolysis reactions involved the transformation of complex organic compounds into simpler compounds (Bastida *et al.*, 2007). Several research works have studied soil enzymatic activities with the aim to assses soil quality since soil degradative processes strongly influence soil enzymes (Ceccantic and Garcia 1994, Bastida *et al.*, 2007).

In this study reduction in the activity of soil enzymes was observed from the day 0 (which is the control) to the end of the experiment (Day 42). Urease decreased from 3.8mgNH_4 – Ng^{-1} soil (in the control, Day 0) to 0.6mg/0.6Mg NH_4 – Ng^{-1} at the end of the research (day 42) which was 84.21% decrease (0-15cm depth) and 1.8 mg/ NH_4 - Ng^{-1} in the control to 0.2mg NH_4 - Ng^{-1} in Day 42 of the experiment (in 15 – 30cm depth) which is 88% decrease our results showed general reduction in enzymes activities throughout the period of the experiment.

Catalase is an iron propilyrin enzyme which catalyses very rapid decomposition of hydrogen peroxide to water and oxygen (Nelson and Cox, 2000). The enzyme is widely present in nature, which accounts for its diverse activities in soil such as its data use as simple toxicity test (Roger and Li 1985).

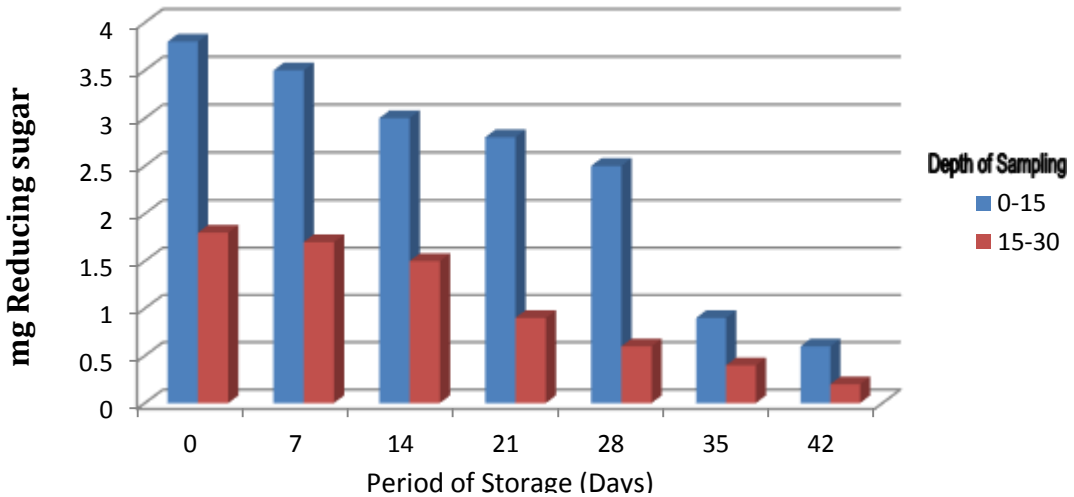

Fig 1: Effect of Length of Sample Storage on Urease Activity.

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In this study catalase activity exhibited a decrease significantly at (P=0 .5) from 3.6 $mgH_2O_2g^{-1}$ soil control to 0.6 in the 42 days of storage. The decrease in catalase activity was as the length of soil storage period increases. The decrease in catalase activity was 88.33% at 0 – 15cm depth and in 15 – 30cm depth it decreased from 2.7 $mgH_2O_2g^{-1}$ soil in the control (day 0) to 0.5 $mgH_2O_2g^{-1}$ which was significant at (P=0.5) and the rate of decrease was 81.48%. The decrease could be because catalase being an enzyme its activity is altered by unfavourable conditions, such as hypoxia, unavailability of nutrient, temperature and changes in pH. Turner Romero (2010) also observed decrease in enzyme activities due to effect of cold temperature at 4ºC and period of storage on the hydrolytic enzyme activity of tropical soils.

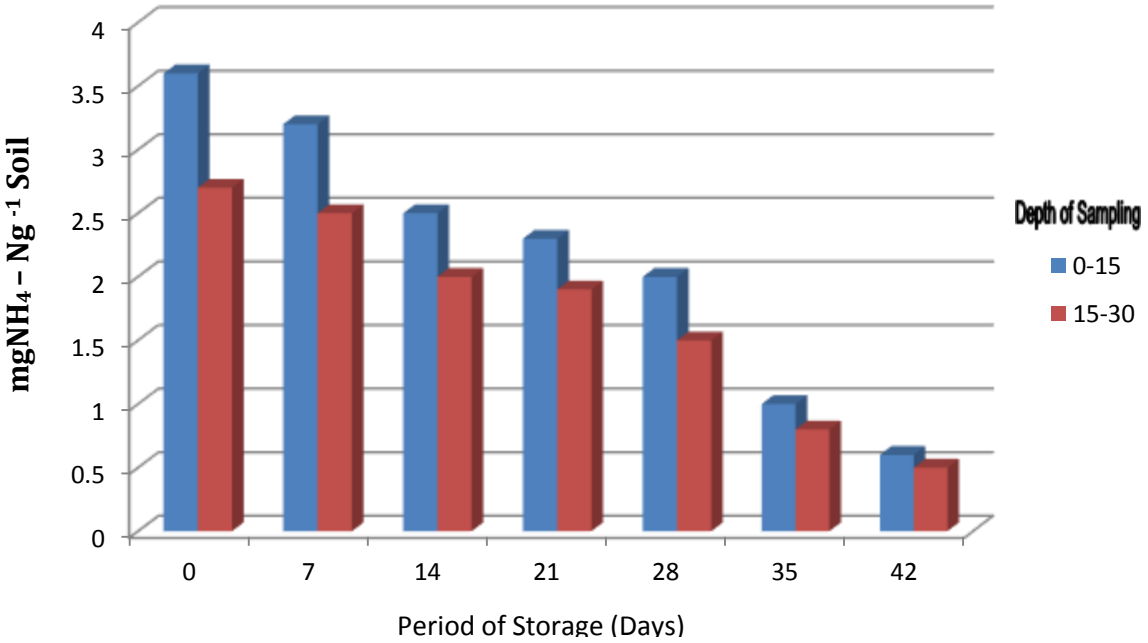

Fig 2: Effect of Length of Sample Storage on Catalase Activity.

Cellulase enzyme is restricted to the organisms capable of using cellulose as a carbon source and mostly restricted to fungi (Gascorgne and Gascogne 1968). This is an extracellular enzyme and converts cellulose into glucose, cellubiose and higher molecular weight oligosaccharides (Deng and Tabatabai, 1994). In this present study, we observed gradual decrease in cellulase activity in response to length of soil storage at 4ºC for a period of 42 days. Cellulase activity decreased from 2.5 mg Reducing sugar g^{-}soil in Day 0 to 0.5 mg Reducing sugar g^{-1} soil in Day 42 of the experiment which is 80% decrease and significantly at (P=.05), at (0.15cm depth). The same trend was observed in the 15 – 30cm depth. Cellulase activity decreased from 0.8 mg Reducing sugar g^{-1} in day o to 0.01 mg Reducing sugar g^{-1} in Day 42 of storage which is 98.75% decrease and (was significantly at (P=.05) (Fig.3).

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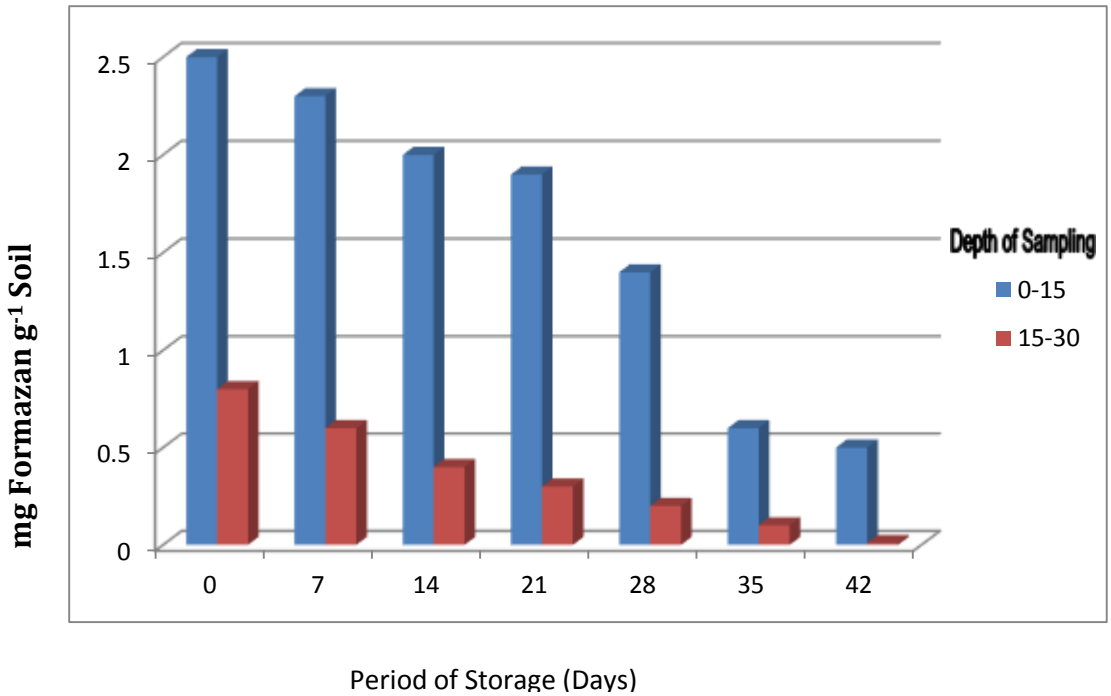

Fig 3: Effect of Length of Sample Storage on Cellulase Activity

Dehydrogeriase is a universal enzyme that belongs to the group of oxidoreductase enzymes produced by all organisms and linked with respiratory process (Botton *et al., 1986).* It transfers the hydrogen from hydrogen-containing compounds to another hydrogen carrier. Dehydrogenase activity is a measure of the intensity of microbial metabolism in soil and thus the microbial activity in soil (Tabatabai, 1982). Result in (Figs 4 and 5) showed decrease in dehydrogenase activities. The level of decrease in both actual and potential dehydogenase was gradual. In actual dehydrogenase at the depth of 0 -15cm, it decreased from 3.5 mgg^{-1} in soil day 42 storage soil and the decrease was significantly at (P=.05) which was 65.71% decrease. At the depth of 15 – 30cm decrease in actual dehydrogenase was gradual, from 1.6mgg^{-1} soil in day 0 to 0.5 mgg^{-1} soil in day 42 of the experiment. The decrease in actual dehydrogenase was significantly (P=.05) and the rate of decrease was 68.75% 4 similar observation was made in potential dehydrogenase. The decrease was gradual as in actual dehydrogenase at the depth of 0 – 15cm, the decrease from 4.8mgg^{-1} soil in day 0 to 1.2mgg^{-1} soil in day 42 of the experiment.

The decrease was significant (P=.05) with the rate of 75% decrease. At the depth of 15 -30 cm decreases in actual dedydrogenase decrease was also observed. In day 0 of the experiment with the value of 4.4mgg^{-1} soil in day 42 of the experiment, the decrease was significant (P=.05) and 71.42% decrease.

The decrease in soil dehydrogenase may probably be due to the fact that dehydrogenase are enzymes and their activities can be altered by unfavourable environment conditions such as high or low temperature, and decline in substrate. This result was similar to the report by Coxson and Parkinson (1987) who reported decrease in enzymes activity due to depletions of available substrates in soil samples refrigerated at 4^{o}C. Also in their studies Brain *et al., (2014)* concluded when storage time was increased to six and 12 month, both microflora and enzymatic activities were significantly reduced.

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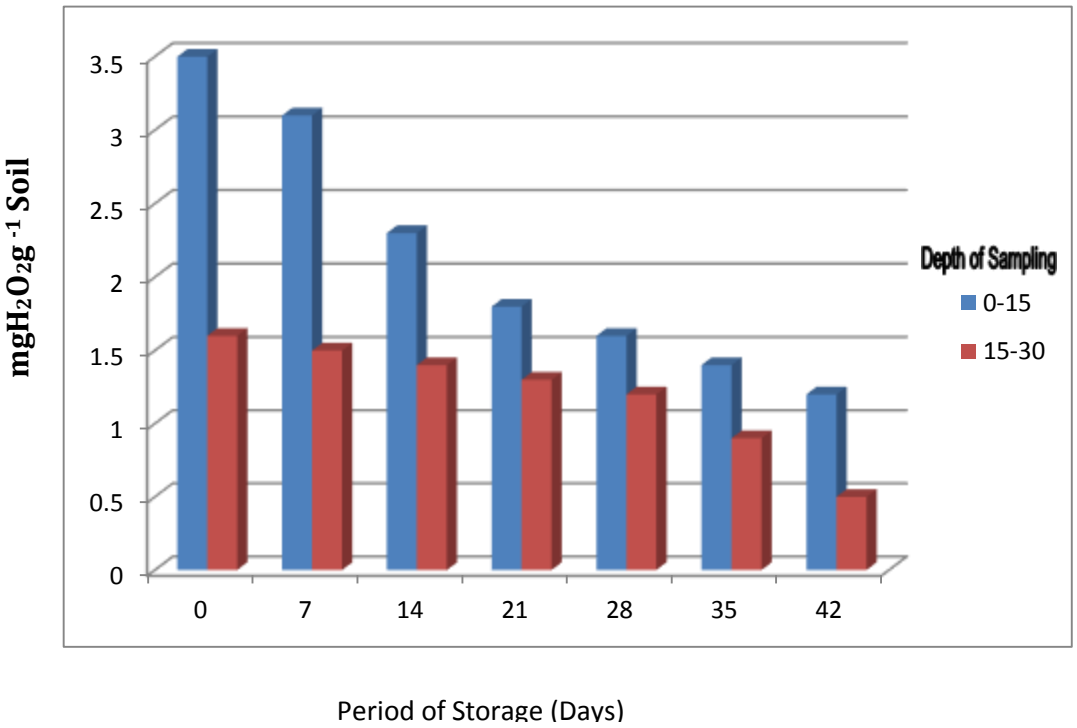

Fig 4: Effect of Length of Sample Storage on potential dehydrogenase activity

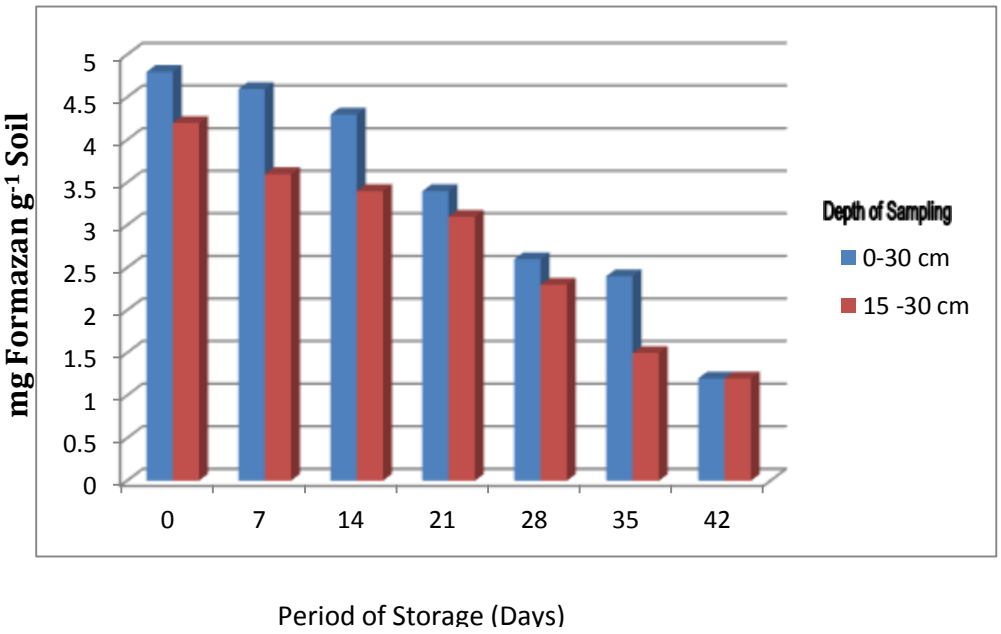

Fig 5: Effect of Length of Sample Storage on actual dehydrogenase activity

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CONCLUSION

The effect of length of soil samples storage on soil microbiological and biochemical properties was determined. The results showed increased in microbial load in all the treatment. For example, viable bacterial counts decreased from 2.45± 0.5cfu/g in the control or day 0 of the treatment to 1.26± 0.05 cfu/g soil in day 42 storage periods in o -15cm soil sample depth. And from 1.76±0.01cfu/g soil in day 0 of the treatment to 1.11±.12cfu/g soil in 15 – 30cm depth. Fungal viable count also decreased from 1.75±0.08cfu/g in the control or day 0 to 1.04±0.01cfuu/g soil in day 42 of the experiment in 0- 15cm depth and in 15 – 30cm depth, the decrease was from 1.48±0.26cfu/g soil in the control or day 0 to 0.95±0.05cfu/g. it was observed that some bacteria persisted in the samples up to the end of the experiment, most prominent were the Gram positive bacteria and the spore formers, whereas the Gram negative bacteria were unable to survive up to the end of the experiment. Some fungal species also persisted up to the end of the experiment. All the enzymes were significantly affected by length of soil sample storage. To achieve optimum result in biogeochemical study; soil samples should not be stored more than seven days.

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