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SEASONAL IMPACT OF CASSAVA MILL EFFLUENTS (C M E) ON DUMPSITES PHYSICOCHEMICAL PARAMETERS AND SELECTED ENZYME ACTIVITIES IN ISUIKWUATO AREA, ABIA STATE, NIGERIA

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Abstract: The effects of cassava mill effluent (CME) dumping on soil physicochemical parameters and selected enzymes in two season (Wet: May – September and Dry: November – March) were investigated for four years. A total of eight milling sites were sampled using standard methodologies. Sample collection from each site was from discharge spot of effluent, four and eight meters away from discharge spots along the flow route of effluents. Top soil samples (0-30cm depth) subsoil samples (31-60cm depth) and bottom soil samples (61-90cm depth) were collected from each sampling spot while control samples were from spots within each sampling site uncontaminated with cassava mill effluent (CME). Cassava mill effluent (CME) dumping significantly increased ($P<0.05$) the temperatures of top and subsoils in both seasons. Similarly CME impacted soil samples had higher ($P<0.05$) pH values (alkaline) than corresponding soil samples in both seasons. Both the cation exchange capacities (CEC) and exchangeable acidities (EA) of the dumpsite soil samples remained unchanged ($P>0.05$) from control soil values. There was also an increase in the test soil samples in organic carbon in both seasons. Similarly CME dumpsite soils alkaline phosphatase and urease activities were significantly high ($P<0.05$) relative to control soil samples in contrasts to test soil acid phosphatase, lipase and dehydrogenase which were significantly ($P<0.05$) reduced. However significantly high levels of cyanide ions were obtained from CME dumpsite soils. This suggests a possible cause of low soil enzyme activities obtained in this work and the need to detoxify cassava mill effluents before dumping.
Keywords: CME, Soil, Physicochemical Parameters, Enzyme Activities.

Introduction

The four major types of waste are: agricultural, industrial, municipal and nuclear wastes. Food processing plants produce agricultural wastes which may be either in liquid or solid form. Wastes if not properly handled and disposed may cause pollution of the environment. The processing of cassava (*Manihot esculanta cratez*) has consistently generated so much waste from cassava mills which are usually discharged on land or water indiscriminately. This discharge affects the biota especially in the Southern part of Nigeria where most of the

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mills are located (Olorunfemi *et al.,* 2008). The cassava tuber consist of about 15% peel and 85% flesh for use as human food. The peel is invariably removed and only the flesh is ultilized. Both peel and flesh contain significant amount of hydrocyanic acid which is highly toxic to humans and animals (Maduagwu and Okoro, 1980). This is the reason why cassava tuber usually has to pass through several detoxification processes before it is safe for human and animal consumption (Onwueme and Sinha, 1991). Compounds that are generally toxic to living organisms will also at toxic concentrations prevent germination of seeds (Olorunfemi *et al.,* 2008). After milling the cassava tubers, the resultant pulp is stacked in sacks and pressed using hydraulic press to remove the watery content. The watery content inhabits the cyanide in form of linamarin and lotaustralin. Microbial enzyme activities on these cyanogenic glycoside generate toxic substance inform of hydrogen cyanide (Ogboghodo *et al.,* 2006). Chinyere (2001 and 2003) reported increased soil cyanide concentrations at dumpsites. It has also been reported that the off-odours inherent around areas where these mills are located are as a result of microbial activities leading to the production of ammonia and nitrites (Dimestre *et al.,* 1997). The increased microbial activities at dumpsites has been associated with increased soil alkalinity and reduction in plant growth (Ogboghodo *et al.,* 2006; Hajak *et al.,* 2006; Hajak *et al.,* 1990). This may probably explain in part the high level of plant withering seen at dumpsites. Cyanide is known to be a metabolic inhibitor and may be playing inhibitory roles (at high concentrations) to plant growth and provision of nutrient by micro-organisms. Although a lot of trade and commercial activities are associated with cassava production resulting in increased milling industries in rural/urban areas of Nigeria, literature is scanty on the effects of effluent from these mills on the environment. Industrial effluent and waste not properly treated before discharge into the environment affects the soil microbial biomass (Sial *et al.,* 2006). Since these microbes help in maintaining soil fertility through the provision of plant nutrient, factors that affect them also affect the soil fertility. Organic matter plays an important role in soil productivity and decomposition of these effluents into organic matter is through soil enzymes (Okwute and Isu, 2007). Therefore evaluating the activities of some important soil enzymes associated with soil fertility as affected by cassava mill effluent dumping will give an insight into the potential of the soil to permit the basic biochemical processes necessary for maintaining soil fertility. Seasonal changes in soil moisture, temperature, pH and carbon (C) input can have a large effect on soil microbial biomass and its activities which in turn affect the ability of soil to supply nutrients to plants through soil organic matter turnover. Observation made through this investigation is

used to make a proposal on possible remedial measures to counter the effect of these effluents dumping.

Methodology:

Sample collection: A total of eight (8) sampling sites were used for sample collection. In each site three spots were chosen for the collection thus:

Spot X: Was the discharge point of effluents.

Spot Y: Was a point four (4) meters away from spot X along the route of flow of discharged effluent.

Spot Z: was a point eight (8) meters away from spot X along the route of flow of discharged effluent. At each spot X, Y and Z three samples were collected and designated as X_1, X_2, X_3, Y_1, Y_2, Y_3, and Z_1, Z_2, Z_3. A (X_1+Y_1 +Z_1) = top soil sample from 0 to 30cm depth, B(X_2+Y_2+Z_2) = subsoil sample from 31 to 60cm depth and C(X_3+Y_3+Z_3)= bottom soil samples from 61cm to 90cm depth respectively. Control samples were collected from spots in each site not infiltrated by effluent and designated D_1, D_2 and D_3 respectively. Samples collected were packed separately in marked cellophane bags tightly tied to avoid contamination and stored in refrigerators of temperature 4-6^{o}c before analysis. Samples were sieved (4mm) and sub samples for the determination of physicochemical parameters were air-dried and sieved (2mm) before analysis. The seasons were: WET SEASON (May-September) and DRY SEASON (November-March).

Statistical Analyses was done between polluted sites and control areas for each parameter. There was also comparison of the two seasons – WET and DRY seasons for all the parameters. All statistical analysis were done using Anova and Duncan Multiple range.

Physicochemical Determinations

Soil temperature was determined insitu at the site of collection of samples using mercury-in-glass thermometer while soil pH was measured ex-situ as described by Bates (1954). Soil moisture was determined as described by APHA (1998) and organic carbon determined by the method of Osuji and Adesiyan (2005) as described by Akubugwo *et al.* (2007).

The cation exchange capacity of the soil samples and their exchangeable acidities were measured as described by Dewis and Freitas (1970).

Soil Enzymes Activities

The activity of the soil extract dehydrogenase was determined by the method of Cassida *et al*., (1964) as modified by Li *et al*., (2005) while the soil samples urease activities were measured as described by Nannipierri *et al.,* (1980) modified by Kandeler and Gerber

(1988). Similarly the soil samples acid and alkaline phosphatases activities were obtained through the methods of Tabatabai and Bremner (1969) and lipase activity was obtained by method of Macedo *et al*., (1997).

Determination of soil cyanide

Soil cyanide was determined by the method of Finkel'shtein (1940). The method is based on the reaction of the cyanide ion (C≡N) with alkaline picrate to produce a light – blue coloration which absorbs at 490nm

Statistical Analysis

Data collected were subjected to statistical analysis using one way Analysis of Variance(ANOVA) procedure and difference in mean were separated using standard t- Test. Values were mean ± standard deviation of triplicate determinations. These were done for values between the seasons plus each season and control. Bars bearing the same letters are not significantly different (P<0.05).

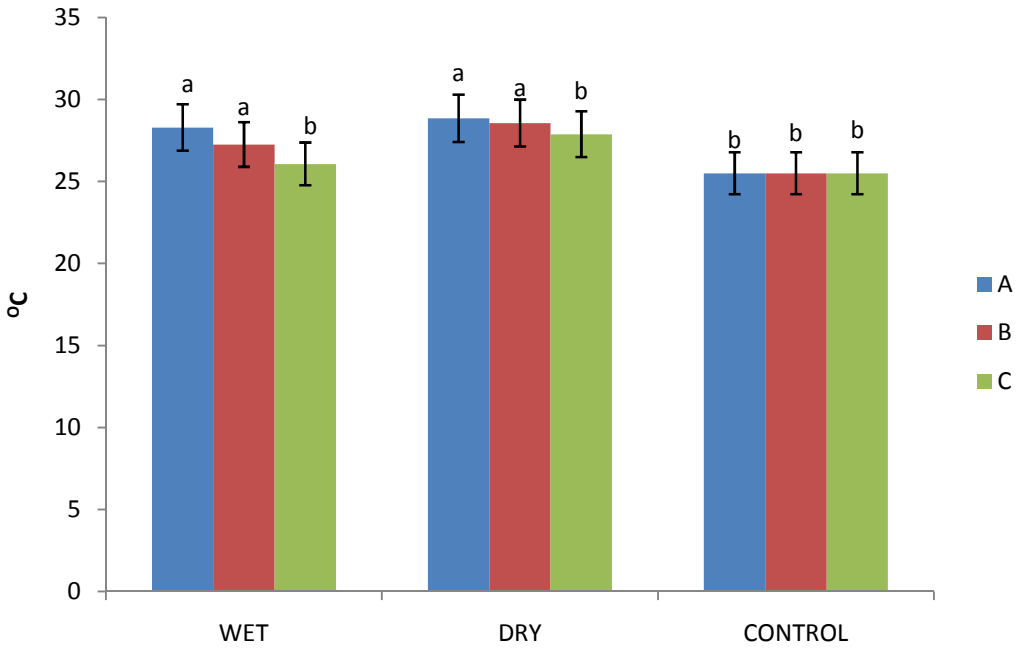

Fig 1 Cassava Mill effluent (CME) infiltrated soil temperature (^{o}C) wet and dry season.

Results are mean of triplicate determinations ± standard deviation (SD)
A = Top soil samples (0 - 30cm depth)
B = Subsoil samples (30 – 60cm depth)
C = Bottom soil samples (60 – 90cm depth)
Bars bearing the same letters are not significantly different (P>0.05)

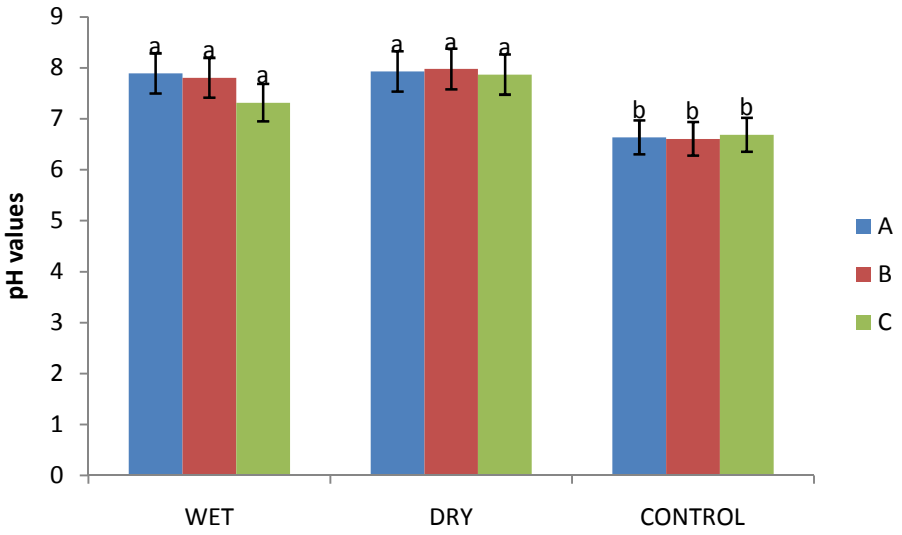

Fig 2: Cassava Mill effluent (CME) infiltrated soil pH values. Wet and dry season.

Results are mean of triplicate determinations ± standard deviation (SD)
A = Top soil samples (0 - 30cm depth)
B = Subsoil samples (30 – 60cm depth)
C = Bottom soil samples (60 – 90cm depth)
Bars bearing the same letters are not significantly different (P>0.05)

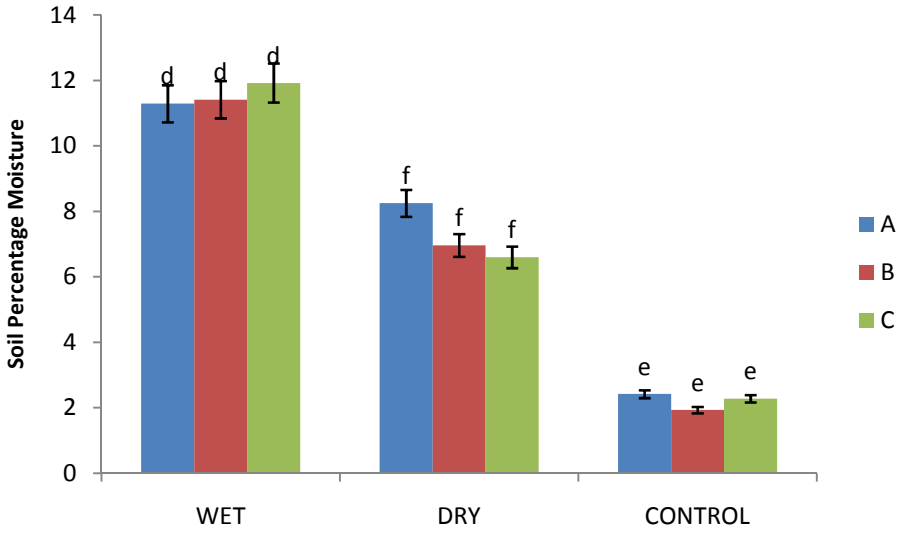

Fig 3: Cassava Mill effluent (CME) infiltrated soil percentage moisture. Wet and dry season

Results are mean of triplicate determinations ± standard deviation (SD)
A = Top soil samples (0 - 30cm depth)
B = Subsoil samples (30 – 60cm depth)
C = Bottom soil samples (60 – 90cm depth)
Bars bearing the same letters are not significantly different (P>0.05)

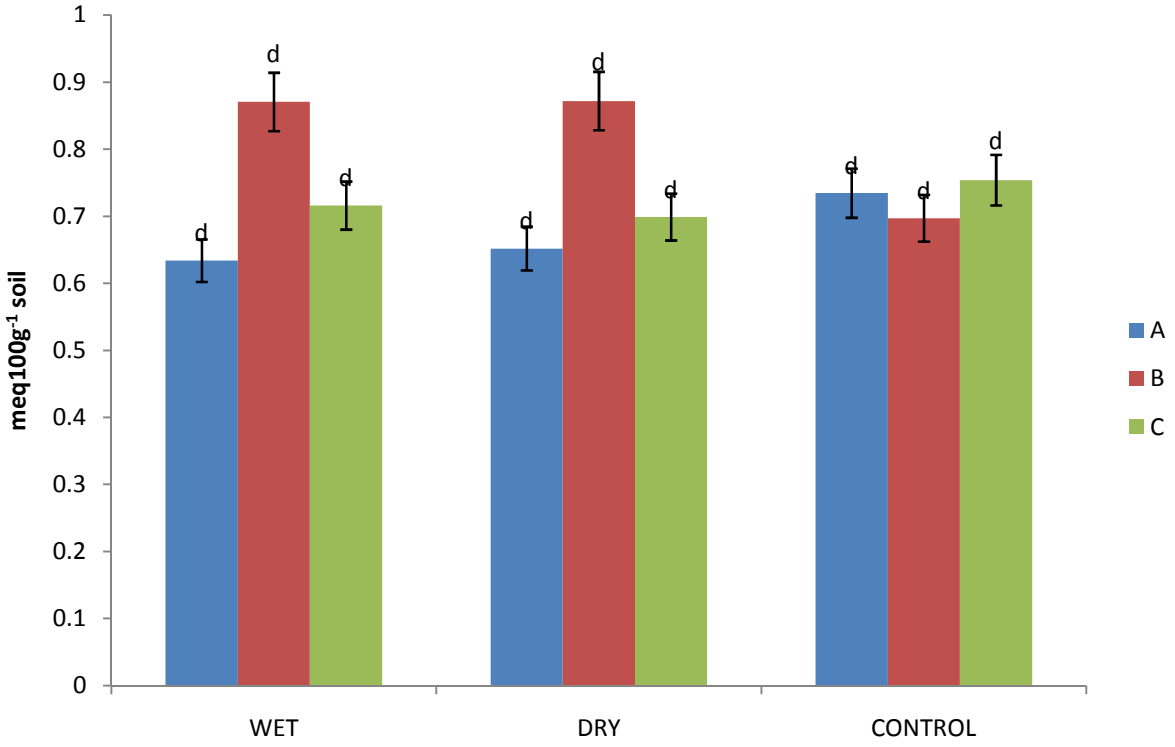

Fig 4: Cassava Mill effluent (CME) infiltrated soil cation exchange capacity (meq100g^{-1} soil). Wet and dry season

Results are mean of triplicate determinations ± standard deviation (SD)

A = Top soil samples (0 - 30cm depth)

B = Subsoil samples (30 – 60cm depth)

C = Bottom soil samples (60 – 90cm depth)

Bars bearing the same letters are not significantly different (P>0.05)

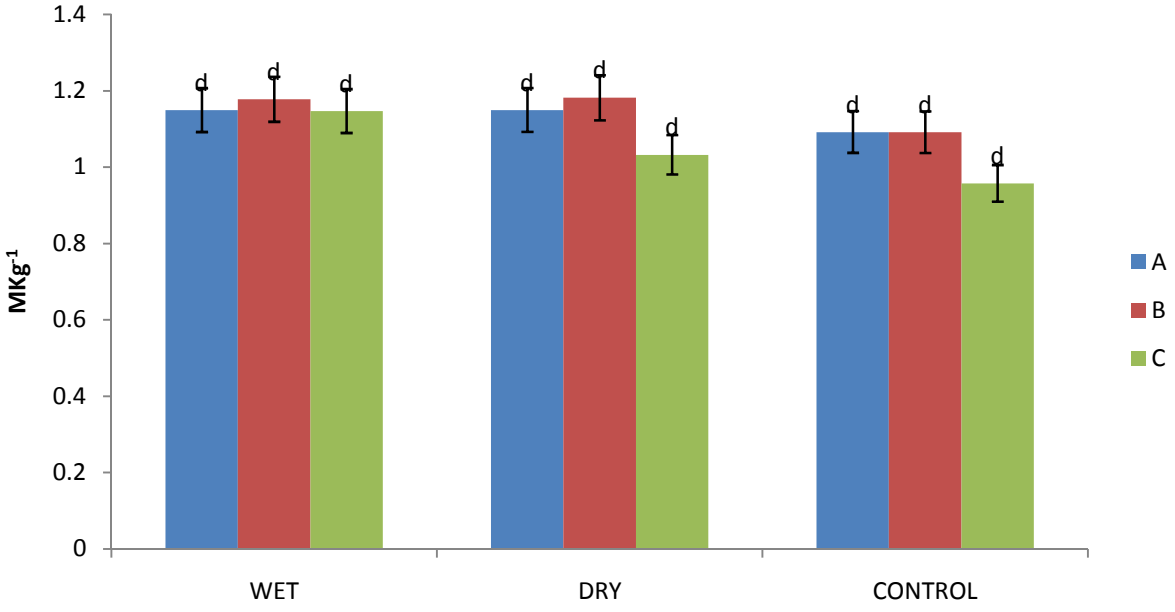

Fig 5: Cassava Mill effluent (CME) infiltrated soil exchangeable acidity (MKg^{-1}). Wet and dry season

Results are mean of triplicate determinations ± standard deviation (SD)
A = Top soil samples (0 - 30cm depth)
B = Subsoil samples (30 – 60cm depth)
C = Bottom soil samples (60 – 90cm depth)
Bars bearing the same letters are not significantly different (P>0.05)

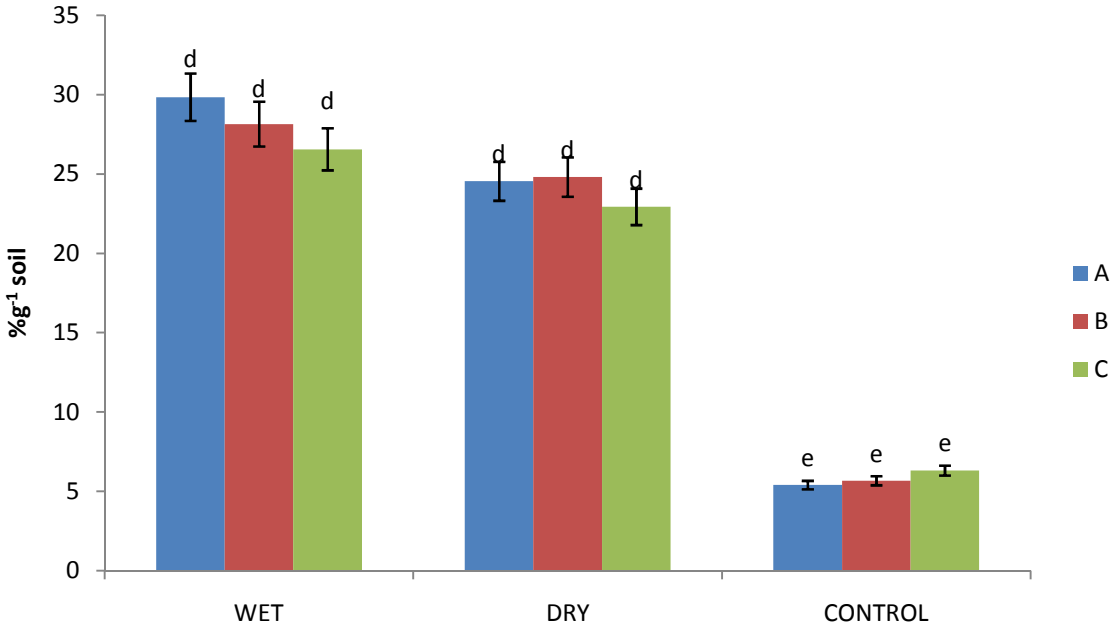

Fig 6: Cassava Mill (CME) infiltrated soil organic carbon ($\%g^{-1}$ soil). Wet and dry season

Results are mean of triplicate determinations ± standard deviation (SD)
A = Top soil samples (0 - 30cm depth)
B = Subsoil samples (30 – 60cm depth)
C = Bottom soil samples (60 – 90cm depth)
Bars bearing the same letters are not significantly different (P>0.05)

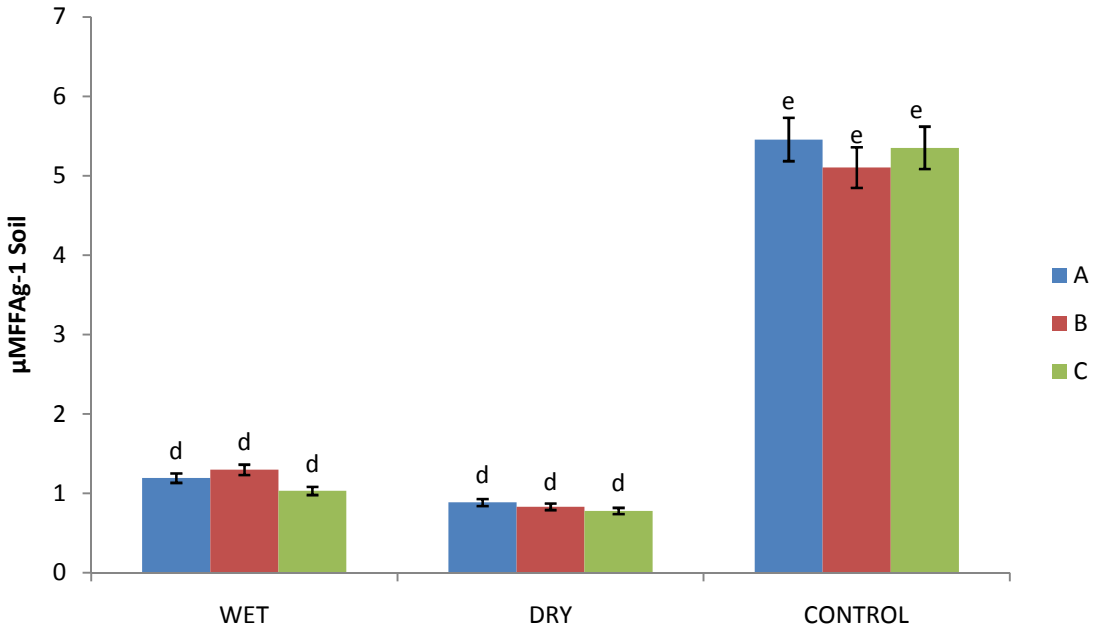

Fig 7: Cassava Mill effluent (CME) infiltrated soil lipase activities (µMFFAg-1 Soil). Wet and dry season

Results are mean of triplicate determinations ± standard deviation (SD)

A = Top soil samples (0 - 30cm depth)

B = Subsoil samples (30 – 60cm depth)

C = Bottom soil samples (60 – 90cm depth)

Bars bearing the same letters are not significantly different (P>0.05)

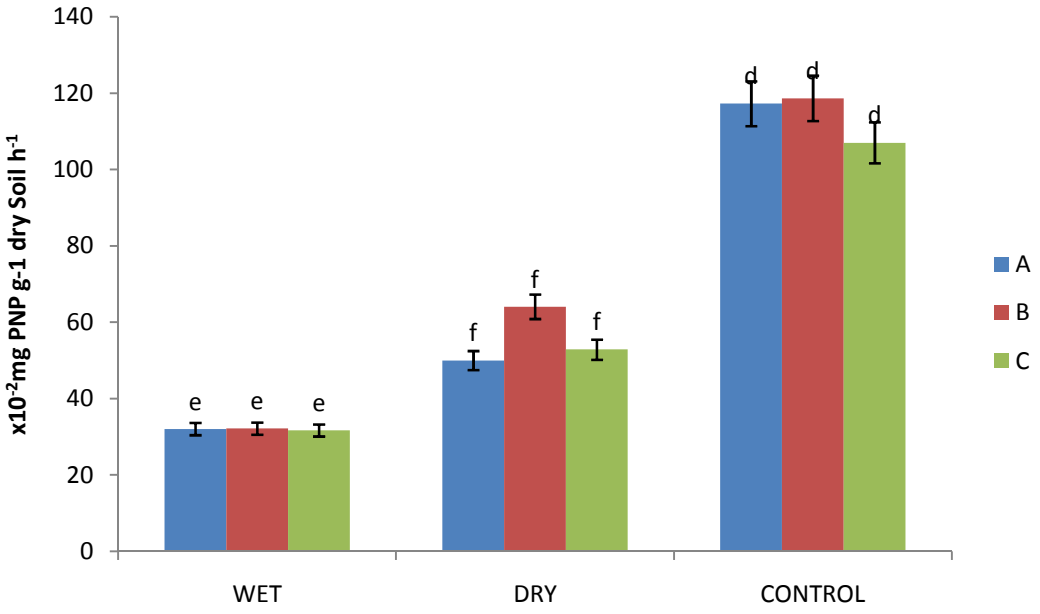

Fig 8: Cassava Mill effluent (CME) infiltrated soil acid phosphatase activities(x10^{-2}mg PNP g-1 dry Soil h^{-1}). Wet and dry season.

Results are mean of triplicate determinations ± standard deviation (SD)

A = Top soil samples (0 - 30cm depth)
B = Subsoil samples (30 – 60cm depth)
C = Bottom soil samples (60 – 90cm depth)
Bars bearing the same letters are not significantly different (P>0.05)

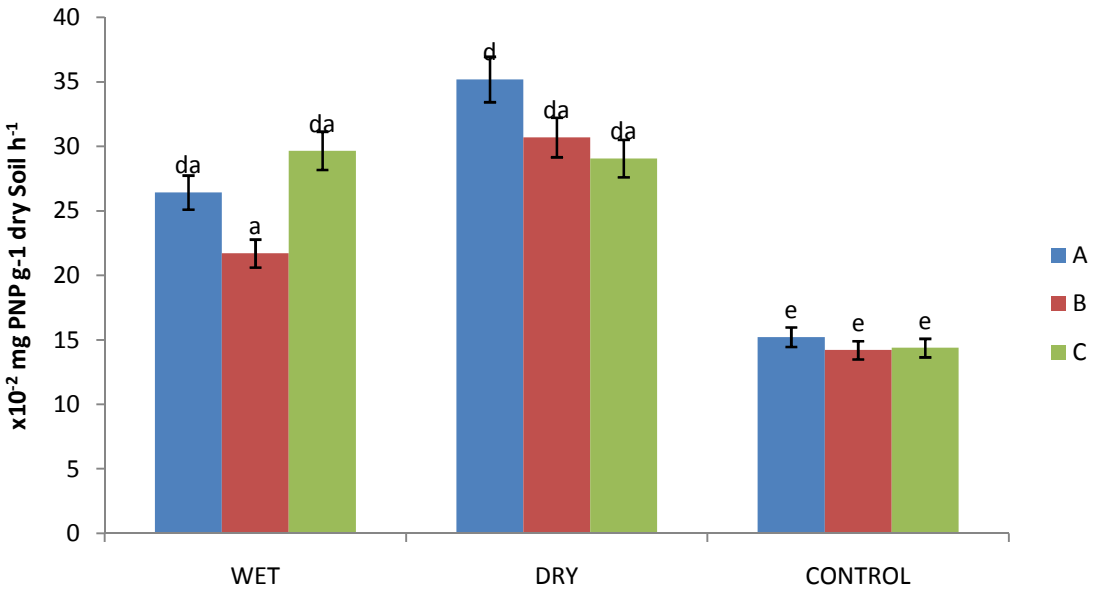

Fig 9: Cassava Mill effluent (CME) infiltrated soil alkaline phosphatase activities(x10^{-2} mg PNP g-1 dry Soil h^{-1}). Wet and dry season.
Results are mean of triplicate determinations ± standard deviation (SD)
A = Top soil samples (0 - 30cm depth)
B = Subsoil samples (30 – 60cm depth)
C = Bottom soil samples (60 – 90cm depth)
Bars bearing the same letters are not significantly different (P>0.05)

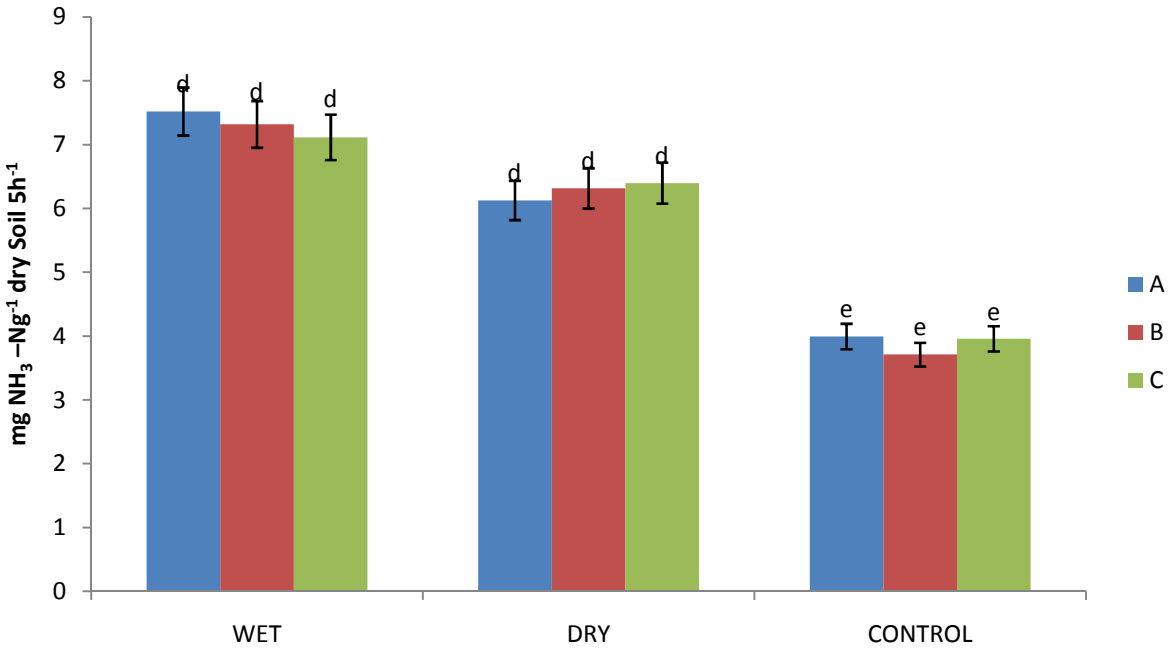

Fig 10: Cassava Mill effluent (CME) infiltrated soil urease activities(mg NH_3 –Ng^{-1} dry Soil $5h^{-1}$). Wet and dry season.

Results are mean of triplicate determinations ± standard deviation (SD)

A = Top soil samples (0 - 30cm depth)

B = Subsoil samples (30 – 60cm depth)

C = Bottom soil samples (60 – 90cm depth)

Bars bearing the same letters are not significantly different (P>0.05).

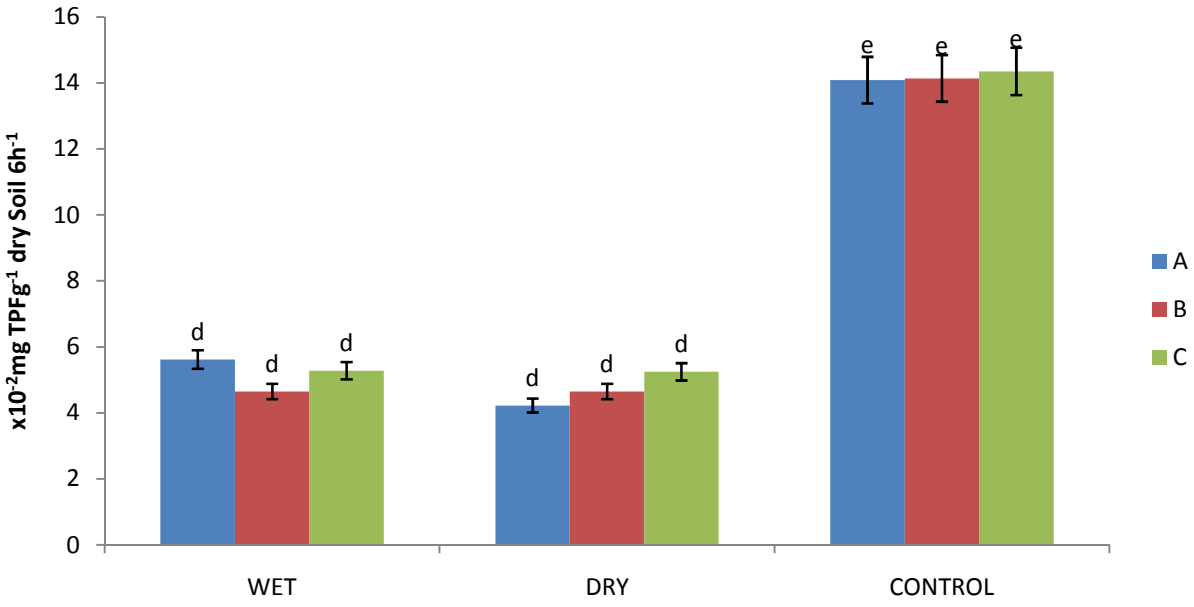

Fig 11: Cassava Mill effluent (CME) infiltrated soil dehydrogenase activities(x10^{-2}mg TPFg^{-1} dry Soil $6h^{-1}$). Wet and dry season.

Results are mean of triplicate determinations ± standard deviation (SD)

A = Top soil samples (0 - 30cm depth)

B = Subsoil samples (30 – 60cm depth)

C = Bottom soil samples (60 – 90cm depth)

Bars bearing the same letters are not significantly different (P>0.05)

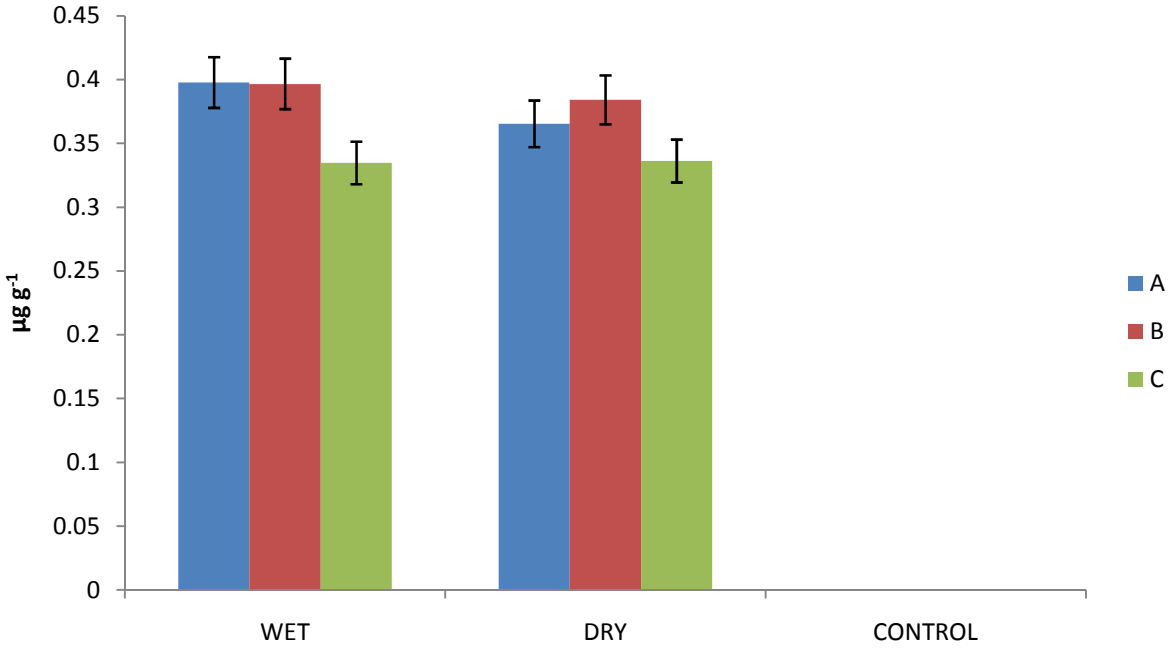

Fig 12: Cassava mill effluent (CME) infiltrated soil cyanide (µg g^{-1}). Wet and dry season

Results are mean of triplicate determinations ± standard deviation (SD)

A = Top soil samples (0 - 30cm depth)
B = Subsoil samples (30 – 60cm depth)
C = Bottom soil samples (60 – 90cm depth)
Bars bearing the same letters are not significantly different (P>0.05)

Results and Discussion

In this study it was observed that the discharge of cassava mill effluent (C M E) led to rises in the temperature of these dumpsites (P<0.05) in both seasons (fig I). The top soil samples had the highest temperatures flowed by the sub soil while the least temperature was that obtained for the bottom soil samples. These rises in top and subsoil temperatures of the cassava mill effluent dumpsites were significantly higher (P<0.05) than those of cassava mill effluent bottom soil samples and control samples. Similar results on soil temperature changes at discharge points of CME effluents were obtained by Nwaugo *et al.,*(2008). These rises in temperature of CME dumpsite soils is attributed to increased microbial activities associated with decaying of CME. Soil temperature can strongly influence root growth, nutrient uptake by plants, hinder shoot development and mineral nutrient accumulation of plants (Hogue and Nelson, 1986; Tagliavini *et al.,* 1991; McMicheal and Burk, 1998). Low and high soil temperatures have been shown to influence water viscosity and metabolic activities in plant roots through changes in membrane lipids or enzyme activities associated with nutrient uptake e.g H^+-ATPase (Ryppo *et al.,* 1994; Shufu *et al.,* 2001). The CME dumpsite soil pH increased significantly (P<0.05) in both seasons (wet and dry) when compared with control. However this increase was more marked during the dry season and inversely correlated with depth of sample collection in the wet season. Similar pH results for CME dumpsites was reported by Ogboholo *et al.,*(2003, 2006); Olorunfemi *et al.,*(2007) and Nwaugo *et al.,*(2008b).

Soil pH is said to increase with increasing soil depth (Skyllberg, 1993). In this study, soil samples were collected from 0-90cm depth which contained already degrading CME. Since effluent dumping is a continuous process, it is associated with the observed pH changes. Most crops grow at pH of 6.4 to 7.0 (Hajek *et al.,* 1990) and soil acidity is one of the principal factors affecting nutrient availability to plants. Therefore the availability of plant nutrients (e.g nitrogen, potassium and phosphorous) in soils is affected by the impacted soil pH changes. If the soil pH is not correct, crop germination and yield are affected and at pH values less than or approximately 5.5 or higher than 7.5 toxic levels of these nutrients may become present in the soil (Okwute and Isu, 2007a). The moisture content of CME soil samples changed with depth and distance of sample collection from discharge point. This

suggests that CME increased the water holding capacity of soils (Akubugwo *et al.,* 2009). The CME dumpsite soil sample had higher percentage moisture content (P<0.05) than control in both seasons (fig 3). Excess soil water reduces soil oxygen available to plants and microbes thus altering microbial activities. The cation exchange capacity (CEC) usually expressed in milliequivalent/100grams of soil is a measure of the quality of readily exchangeable cations neutralizing negative charges in the soil (Rhoades, 1982). Both the cation exchange capacity (CEC) and exchangeable acidities (EA) of the CME impacted soil were not affected (P> 0.05) by the effluent dumping compared to control soil samples. Also there was no seasonal variations of both CEC and EA of CME impacted soil (fig 4 and 5). The soil organic carbon was significantly high (P<0.05) in CME soils compared with the control. Higher organic carbon was obtained in wet season than dry season soils for CME soil samples, though non-significantly (P>0.05) (fig 6). The high total organic carbon (TOC) obtained from CME soil samples was similar to those of Shanhinirokhsar *et al.,* (2008) who associated it with increased stimulated urease activity and Cookson and Lepiece,(1996) to high nitrogen production by CME impacted soils. The increased metabolism of TOC has been observed to result in the release of high amount of ammonium ions which evolved the characteristic urine odour around cassava effluent dumpsites (Nwaugo *et al.,* 2008b). Akani *et al.,* (2006) observed high level of fermentation associated with CME dumping. In the CME dumpsites, organic carbon decreased with depth and distance from discharge point following the level of impaction.

Soil Enzymes.

The CME soil lipase activity is shown in fig 7. The activity of this enzyme negatively correlated with CME impaction. In fact the activity of this enzyme decreased in CME soil samples (P<0.05) compared with control soil samples. The decrease in activity of this enzyme in soil samples infiltrated with CME in the two seasons understudied may have arisen from the presence of cyanide ions in the soil samples. Cyanide is a metabolic poison and may have reduced the biomass of microorganisms within the impacted soil. Wyszkowka *et al.,* (2006) observed that contamination of soil alters the succession of microorganism which is directly associated with the activities of soil enzymes. Ehiagbonare *et al.,* (2009) reported that soil samples from cassava processing area in Okada contaminated by CME deposits had their fungi population completely decimated. However there was no seasonal variation in the activities of this enzyme for the CME infiltrated soils. The activities of acid and alkaline phosphatases in CME soils opposed each other in the two test seasons. In the

CME infiltrated soil low acid ($P<0.05$) and high alkaline phosphatase activities ($P<0.05$) were obtained compared with the control. However the alkaline phosphatase activity was higher ($P<0.05$) in the dry season than wet season soil samples. Various factors have been adduced to account for the activities of acid and alkaline phosphatases in soils. One of such factors is the soil pH. While the optimum pH of acid phosphatase activities is between 5-6.8, alkaline phosphatase acts best at pH 10.5 -11.5 (Nwaugo *et al.*, 2008b). This correlates with alkaline pH of the CME soil samples. The CME soil sample urease activitiy was found to be significantly high ($P<0.05$) against the control soil sample in this study (fig 10). Increased soil moisture content and solubilization of nutrient have been shown to increase soil enzyme activities (Ladd and Paul, 1973). Soil samples urease activities also decrease with depth of sample collections. This is probably partly due to the inhibitory effect of cyanide ions of CME on subsoil samples microorganisms. Cyanide is volatile and will impact its inhibitory effect on microorganisms maximally when the concentration is high. Ogboghodo *et al.,* (2001) reported such inhibitory effect of CME in relation to soil microorganism population. In their report increased soil pollution with CME reduced the population of soil microorganisms especially E.coli. However, as mineralization of organic materials in the soil continued, multiplication of responsible organisms also increased and was also related to increases in some soil properties (pH, organic carbon and total nitrogen) of CME impacted soils (Ogboghodo *et al.,* 2006). Inhibition of serratia macrosence by CME had earlier been reported with the appearance of previously non-existent bacteria (staphylococcus) and fungus genera, (*chlamydosporum*)(Ogboghodo *et al.,* 2001). The activity of this enzyme was higher in the wet season than dry season pointing to the fact that perhaps the high moisture content of soil sample during wet season may have reduced the toxic effect of CME. Cyanogenic glycoside on the soil microbes thus enhancing enzyme production in the wet season. However CME soil samples showed a significant ($P<0.05$) decreased dehydrogenase enzyme activity during the two seasons(fig 19). The inhibitory effect of cyanide on cytochrome oxidase of the electron transport chain is not disputable and may explain this observation. Dehydrogenase are found intact bacterial cells and their activities are positively correlated with bacteria populations in soils (Ramamukharachchi and Doi, 2009). It therefore follows that the more the bacteria cells, the higher the production and consequently activities of the enzyme. Nwaugo *et al.,*(2008b) had reported a similar observation with microbial population/ dehydrogenase activities. Soil dehydrogenase activities are good indicators of overall microbial activities in soil and can serve as good indicators of soil condition (Dick, 1997;

Smith *et al.,* 1993). Soil dehydrogenase activity has been shown to respond to change in soil moisture (Roa *et al.,* 2003), previously land usage and pollution with heavy metals (Hinojosa *et al.,* 2004) or herbicide (Wingfield *et al.,* 1977). Various workers (Chinyere,2001 and 2003; Nwaugo *et al.,* 2008; Ehiagbonare *et al.,* 2009) have reported CME impacted soil cyanide levels up to 1.05ugg^{-1}.Similarly many factors have been adduced to explain the distribution of cyanide ion concentration in CME infiltrated soils. These included the cyanogenic glycoside content of the cassava varieties (Siller and Winter,1998), the volume of cassava processed in each mill (Chinyere, 2001) and the rate of effluent flow from discharge point to distant areas occasioned by the topography of the processing site (Chinyere,2003).Soil texture has also been cited as influential to effluent retention (Okwute and Isu,2007) and in agreement clayey soil samples were observed in this study to retain more effluents atop though a variety of methods have been employed for the degradation of cyanide (Wateribe *et al.,* 1998), complete degradation is difficult and biological processes involving cyanide resistant microorganisms (e.g Bacillus sp, Pseudomonas and Klebsiella oxytoca) are being used (ijzan *et al*., 2000, Roa *et al.,* 2003). These microorganisms were reported to degrade cyanide to nontoxic end products and use the cyanide nitrogen as a sole nitrogen source under aerobic/anaerobic environment (Ebb, 2004).

Conclusion

In this study, efforts have been made to establish various factors contributing to pollution problems posed by CME on the environment. These included increased soil temperature, moisture and pH (alkaline) of the CME infiltrated soils. Similarly soil enzyme activities were low except urease activity which was enhanced. Low soil enzyme activities are synonymous to reduced soil microbial populations. This is associated with low fertility as plant nutrients are affected. The determination of soil dehydrogenase activity is used as an index of measurement of overall soil microbial activity. The low soil dehydrogenase enzyme activity is associated with the presence of cyanide ion from CME which invariably will affect oxidation-reduction reactions in non-resistant microorganisms to counter the pollution problems associated with CME. Chinyere *et al.,*(2013) proposed altering of CME pH before dumping as an alternative measure

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