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Impact of Cassava Mill Effluent (CME) on Soil Physicochemical and Microbial Community Structure and Functions

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Abstract

Impact of Cassava Mill Effluent (CME) on soil physicochemical parameters microbial diversity and enzyme activities were analyzed in Akaeze. an agrarian community of Ebonyi State Nigeria. Results showed change in temperature (28.6-326^{0}C). pH (7.2-10.3) and TOC (24.2-41.3mg/g). Highest values were obtained nearest the waste pit while control soil had the least values. Total P, total N and C/N ratio were also lowest nearest the pit but cyanogenic potential was highest nearest pit. The soil heavy and trace metals (Hg. Cr, Pb andCd) were not affected except Ca which was highest near the pit and decreased away from it. Total heterotrophic bacteria had a count range of $3.7x10^4$-$6.6x10^6$ cfu/g. lipolytic bacteria, had $0.9x10^1$-$24x10^1$ cfu/g while nitrifying bacteria had 0.4-2.9x3.7x103 cfu/g. Phosphate-solubilizing bacteria had a range of $2.2x10^2$-$2.4x10^3$ cfu/g. In all cases, highest values were obtained 100m from the waste pit. followed by the control while the least was in the waste pit edge. Dehydrogenase activities followed the pattern of THB-highest in 100m, control, 10m. 5m and pit edge was the least. Urease had the opposite trend with highest nearest the pit (mg g-^{1}24^{-1}) and least in control (2.2). Acid phosphatase had decreasing activities towards the pit while alkaline phosphates had increasing activities in the same direction. This trend was pH dependent. Results indicated adverse effects of the CME on soil parameters and calls for regulations on the disposal of CME to avoid environmental degradation.

Keywords: Cassava, effluent, Bacteria, Enzyme, Soil , activities
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INTRODUCTION

Cassava (*Manihot esculenta* Crantz, synonymous with *Manihot utilissima* Rhol) belongs to the family Euphorbiaceae. It is mainly a food crop whose tubers are harvested between 7-13 months based on the cultivars planted (Cook, 1985; Taye, 1994). The tubers are quite rich in carbohydrates (85-90%) with very small amount of protein (1.3%) in addition to cyanogenic gloucoside (Linamarin and Lotaustiallin) (Nwabueze and Odunsi, 2007, Oyewole and Afolami, 2001). This high carbohydrate content makes cassava a major food item especially for the low income earners in most tropical countries especially Africa and Asia (Desse and Taye, 2001. Aderiye and Laleye, 2003).

The edible tubers are processed into various forms which include chips, pellets, cakes and flour. The flour could be fried to produce garri or steeped in water to ferment to produce foofoo when cooked (Oyewole and Odunfa 1992). The production and consequent consumption of cassava have increased extensively in recent times. This increased utilization of processed cassava products has equally increased the environmental pollution associated with the disposal of the effluents. The highly offensive odour emanating from the fermenting effluent calls for regulation in the discharge of the waste generated (Akani, *et al*., 2006,Adewoye. *et al*. 2005).

In most areas, cassava mills are mainly on small scale basis, owned and managed by individuals who have no basic knowledge of environmental protection. Though on small scale basis, there are many of them, which when put together, create enormous impact on the environment. This work therefore on the environment. This work therefore was aimed at assessing the impact of cassava mill effluents on soil physicochemical and biological indices of soil quality. This study will ascertain the health status of the cassava mill effluent impacted soil for human uses.

Materials and methods

The study area was Akaeze, a rural agrarian community of Ebonyi State, Nigeria. The community lies between latitude $5^0 53^1$N and longitude $7^0 37^1$. It is a tropical climate area with typical Guinea savanna vegetation characterized by very tall grasses and scattered trees. The people are mainly farmers with rice, cassava and yam being the main crops grown.

Sample collection

Soil samples were collected using Shiprek soil augar disinfected with cotton wool soaked in 70% ethanol at 0-15 cm depth. Four sampling areas of the mill were chosen. The areas sampled were the pit edge, 5m, 10m and 100m away while the sample from 25om away served as the control. At each sampling distance, three samples were collected and pooled to give a composite sample for that particular area. Sterile universal bottles were used to collect the soil samples for microbiological and enzymatic studies. Clear amber coloured glass bottles, rinsed thrice in distilled water solution, were used for the soil samples for physicochemical properties analysis. The biological indices (enzymes and bacterial diversity) were analyzed within 2-4 hours of collection while the physicochemical parameters were determined within 12-24 hours of collection.

Physicochemical properties

The soil temperature was determined *in-situ* using mercury in bulb thermometer inserted into the soil and allowed to stay for 5-10 minutes before reading was taken. The pH was determined using Jenway multipurpose tester (HANNA 1910 Modei) The organic matter content was estimated using the loss of ignition method (UNEP, 2004).

The heavy metals whose concentrations were analyzed include Pb, Cr, Cu and Cd while the trace elements were Mg, Ca, K and Na. The concentrations of these metals were determined using the AAS method as described in APHA (1998) methods.

Soil enzymes activities

The enzymes whose activities were assessed in this study include dehydrogenase, urease, and the phosphatase (acid and alkaline). The soil was dried at room temperature for 24 hours and passed through 0.5mm sieve . The sieved soil was used for the analysis.

Dehydrogenenase activity was determined by the method involving the reduction of TTC (Triphenyl tetrazolum chloride) to triphenyl formazon (TPF) after incubation of the TTC amended soil at 30^0C for six hours as described by Alef (1995). The urease activity was determined calorimetrically according to the method of Kandeler and Gerber (1988). The soil was incubated for 24 hours at 37^0C with urea amendment and the result was expressed as NH_3-N dry soil. Activities of both acid and alkaline phosphatases were determined as described by Tabatabai (1997) which involved the use of P-nitrophenyl phosphate and read at 410nm. While acid phosphatase activity was determined at pH 6.8, that of alkaline phosphatase was determined at Ph 11.5.

Microbial diversity

The biological loads of various groups of bacterial species were determined using the culture techniques involving different selective culture media. Five different soil samples were analyzed for the bacteria diversity as described earlier in sample collection. Each sample was inoculated particular the media using the spread plate techniques as described by Chessbrough (2001) after ten-fold serial dilution. The bacterial groups were total heterotrophic bacteria (THB) whose presence was assessed with Tryptone Soy Agar, lipoytic bacteria with Tributyrin Agar and nitrifying bacteria (NB), assessed with modified mineral salt agar. The phosphate-solubilizing Agar was used to assess the phosphate solubilizing bacteria (PSB) as described in US patent (2003).

Cyanogenic potentials of the impacted water.

Cyanogenic poyentials of the impacted water was determined using modified pricate paper kits method as described by Bradburg *et al.*, (1999). 2mls of the water sample was put into 250ml conical flask containing 25ml distilled water. A strip of the test paper was soaked in the alkaline sodium picrate solution and fixed into the conical flask with the cork (stopper). The flask was allowed to stand at room temperature for 18-24 hours. The paper was removed and eluted in 60ml water. The absorbance of the water was read at 540nm using spectrophotometer (Unican Heλ105y England). The results were recorded as mg HCN/L.

Statistical analysis

The results obtained in this study were subjected to standard statistical analysis by the use of correlation analysis, standard deviation and ANOVA. This was to determine the significance of the results.

Results

The results of the physicochemical properties are shown in Table 1. There were two clear trends-either decreasing or increasing gradients with distance away from the waste pit. The pH with a range of 7.2-10.3 temperature (28.6-32.6^{0}C) and TOC (24.2-41.3 mg/g) were highest at the pit edge but lowest in the control soil sample. Total P (1.78-5.61mg/g) and total N (2.10-3.07 mg/g) had a similar trend. The 100m away and control soil samples values were not statistically different (P =0.05) where distance affected the other results significantly p=0.05). the C/N ratio decreased significantly (p=0.05) away from the edge of the waste pit (Table 1). The cyanogenic potential which was highest at the pit edge, decreased with distance away from the pit.

The results of the metal concentrations of the various heavy metals and trace metals are shown in Table 2.

There were no significant changes in the concentrations of the metals determined except in Ca (p=0.05). The highest calcium concentration was observed in the pit edge (2.1mg/g) followed by the 5m (1.97mg/g) while the least was at the control soil (250m away).

In analysis of bacterial diversity, results obtained indicated that THB had the highest counts in all the soil samples analyzed. This group (THB) like all other bacterial groups, increased from the pit edge soil ($3.7x10^4$ cfu/g lowest) to $7.4x10^6$ (highest) cfu/g in the 100m away soil. The 100m away soil value of THB was more than the control soil values. The LB ($0.9x10^1$-$2.4x10^3$ cfu/g) NB ($0.4x10^1$ - $2.9x10^3$ cfu/g) and PSB ($2.2x10^2$ - $2.4x10^4$cfu/g) (Table 3) had similar prevalence with distance away from the pit. Though most of the bacterial groups had higher prevalence in the 100m soil than control soil sample, only that of THB was statistically significant (p=0.05).

Table 4 shows the soil enzymes activities of the five soil samples analyzed. Though all the enzymes showed some form of gradient in their activities, highest values were obtained at the 100m away soil samples. Urease (5.1 mg g^{-1} 24^{-1}) and alkaline phosphatase (6.7 umol-p-nitrophenol) had their highest activities in the waste pit edge which gradually decreased till the lowest in the control soil with 2.2 mg g-1 $24h^{-1}$ and 2.7 umol-p-nitrophenol respectively. Dehydrogenase and acid phosphatase activities were highest in the 100m soil samples which gradually decreased with distance to the waste pit. The lowest values of dehydrogenase was 16.10 while the highest was 39.40. Lowest acid phosphatase value was 0.41 umol-nitrop[henol. While dehydrogenase and acid phosphatase activities correlated positively with THB urease correlated negatively with THB but followed the trend of pH changes and total organic carbon

TABLE 1: Physicochemical Parameters of Soil

Parameter	Edge	5m	10m	100m	Control
pH	10.3	10.1	8.4	7.6	7.2
Temp. ^0C	32.6	30.1	29.2	28.7	28.6
TOC	41.3	35.0	30.8	24.4	24.2
P	1.78	2.12	2.73	5.19	5.61
N	2.10	2.16	2.28	3.91	3.07
C/N Ratio	19.48	15.30	13.12	8.01	7.87
Cyanogenic	5.21	4.66	3.22	0.92	0.62
NH_3-N	5.2	4.3	2.7	0.79	0.72

Values are means of three times sampling

TABLE 2: Metal Concentration found in the Soil

Parameter	Edge	5m	10m	100m	Control
Pb mg/g	0.108	0.102	0.100	0.097	0.097
Cr mg/g	0.03	0.02	0.02	0.01	0.01
Cdmg/g	0.012	0.012	0.012	0.012	0.012
Cu mg/g	0.112	0.112	0.113	0.112	0.12
K mg/g	0.23	0.21	.27	0.39	0.42
Na mg/g	0.11	0.10	0.09	0.09	0.09
Ca mg/g	2.1	1.97	1.61	1.42	1.47
Zn mg/g	1.41	1.61	1.51	1.35	1.37

Values are means of three times sampling results

TABLE 3: Bacteria diversity (cfu/g) of the various soil Samples.

	Locations				
Group	Edge	5m	10m	100m	Control
THB	$3.7x10^4$	$3.9x10^4$	$3.9x10^5$	$3.4x10^6$	$6.7x10^6$
LB	$0.9x10^1$	$0.9x10^1$	$1.7x10^2$	$2.4x10^3$	$2.2x10^3$
NB	$0.4x10^1$	$0.4x10^1$	$0.4x10^2$	$2.7x10^3$	$2.9x10^3$
PSB	$2.2x10^2$	$2.4x10^2$	$2.1x10^3$	$2.x10^4$	$2.3x10^4$

Values are means of three times sampling results

THB = Total Heterotrophic Bacteria
LB = Lipotytic Bacteria
NB = Nitrifying Bacteria
PSB = Phosphate Solubilizing Bacteria

TABLE 4: Enzymatic activities evaluated in the soil samples

Enzyme	Edge	5m	10m	100m	Control
Dehydrogenase mg $g^{-1}6h^{-1}$	16.10	20.30	28.75	39.40	37.50
Urease mg $g^{-1}24^1$	5.1	4.8	3.6	2.4	2.2
Acid phosphatase umol-p-nitrophenol	0.41	1.2	2.7	3.3	3.6
Alkaline phosphatase umol-p-nitrophenol	6.7	6.4	5.1	3.1	3.4

Discussion and conclusion

Results obtained in this study suggest adverse effects of the impact of cassava mill effluent on soil physicochemical and biological parameters. Analysis of these results showed that pH and temperature were hgighest very close to the waste pit. The same pit edge soil had highest organic carbon and cyanogenic potential but was lowest in P and Nwaugo *et al*, (2008a) and Akani *et al*, (2007) had reported similar situations in their studies. The TOC and low C/N ratio observed with increasing effects of the cassava mill effluent (CME) could be attributed to the contents of the effluents. CME is known to be high in organic carbon, which could then reduce the C/N ratio of impacted soil but increased the TOC. Shanhinroknsar *et al*, (2008) and Nattipong and Alissara (2006) agreed that soil impaction with organic matter results in decreased C/N ratio especially if the impacting material has low N content. This agrees well with this study, CME has very low protein but high carbohydrate contents hence the observed low C/N ratio.

The high cyanogenic potentials observed near the waste pit agreed with studies of Aderiye *et al*, (2005) and Nwabueze and Odunsi (2007). These researchers had reported values of 2.91-4.11 and 2.17-5.0 mg HCN kg from cassava peels and tuber pastes respectively. The high cynogenic potential had been attributed to the high cynogenic glucosides (Linamarin and lotaustain) contained in cassava. The values obtained by Aderiye *et al*, (2005) and Nwabueze and Odunsi (2007) do not differ much from the results obtained in this study, especially as it was cassava effluents that was studied too. The high change in pH and temperature very close to the waste pit could be attributed to the high oxidative and reductive biochemical transformations taking place there. The break down of organic matter in the effluent was exothermic, which caused the increase in temperature while the metabolism of the little protein content released ammonia (NH_3. The ammonia dissolved in the available moisture to cause the reported increase in pH value. A similar observation had been reported by Nwaugo *et al*, (2008a) and Chinyere (2001) in similar studies on cassava effluent.

Observations in this study concerning metals suggest that cassava effluent did not cause much variation on heavy metals contents of the soil. Most of these metals (heavy and trace) had no change or very slight negligible change in concentrations. However, only the calcium content of the soil was significantly affected. This calcium change in soil impacted with cassava waste had been reported by Aderiye and Laleye (2003) and Adeyemo (2005). The observed increase could have resulted from the impact of the cassava mill effluent (CME)

. In biological indices analyzed, results showed that the bioloads of all the bacteria groups increased with distance away from the waste pit suggesting adverse growth conditions towards the pit. However, values obtained at 100m away were not statistically higher than those from control. This observation suggests that the high content of cassava mill effluent suppressed bacterial growth but at very low concentrations could be support bacterial growth. The high content of cyanogenic glycosides (linamarin and lotaustiallin) in the CME was not metabolized by many microorganisms but when in low quantity, could be easily metabolized by the few organisms capable of doing so, while other bacterial species depend on the non-toxic intermediates produced,. Nwaugo *et al* (2008a) and Karin (2006) reported that organic matter when added to the soil in small concentrations, encouraged bacterial growth. This observation agreed well with this study. Conditions and nutrients at 100m sampling point could have been optimum for bacteria growth, hence the results obtained.

Several authors (Nwaugo *et al*, 2007, Pelczar *et al*, 2003, Karin 2006, Onyeagba *et al*, 2008) have stated that total heterotrophic bacterial counts are in all cases higher than the other specific bacterial groups in the soil. Similarly, Nwaugo *et al*. (2008a) and Prescott et al, (2001) reported that very slight change in environmental factors affect nitrifying bacteria adversely. These observations are evident in this study. While THB were the most prevalent in all the soil samples analyzed, NB were trhe least. NB were the most adversely affected bacterial group in the study. Some bacterial species in the specialized group (LB, NB and PSB) may equally be found in the THB, making the THB more abundant than any other group.

Results from the enzyme activities analyzed showed some of the biogeochemical transformations taking place in the soil more vividly. The results correlated well with the findings in the bioloads of various bacteria groups. Dehydrogenase ,which is found in intact bacterial cell, had its highest activities in the 100m soil sample and lowest in the most impacted soil (pit edge). This was exactly the case of the THB bioload with 3.7×10^4 at the pit edge but 7.4×10^6 at the 100m sample spot. Olivera and Pampulha (2006), Lee *et al*, (2002) and Nwaugo *et al* (2008b) had reported a similar observation with dehydrogenase. Since dehydrogenase is found in living bacterial cells, it therefore follows that the more the bacterial cells, the higher the production and consequent activities of the enzyme.

The activities of urease were also significant in this study. Urease has its highest activities in the most impacted soil (pit edge) which had the highest TOC content and low N content. Shahinrokhsar *et al*, (2008) stated that high TOC stimulated urease activities while Cookson and Lepiece (1996) reported increased urease activities in N-content soil. The increased metabolism of TOC resulted in the release of high amount of NH_3 which impacted the characteristic urine (NH_3) odour around cassava mills.

Activities of acid and alkaline phosphatase were in opposite direction. While alkaline phosphatase activities increased towards the pit edge that of acid phosphatase decreased in that direction but increased in the opposite. This observation agreed very well with the prevailing environmental conditions especially the pH. Soil pH towards the waste pit was alkaline. The break down of the little protein content in the effluent which resulted in the production of NH_3 and consequent increase in soil pH made conditions favourable for alkaline phosphatase activities but unfavourable for acid phosphatase. Alkaline phosphatase operated best at the pH of 11.5 while acid phosphatase acts better at pH 6.8. the optimum pH of activities of thes enzymes could have been the major cause in th observed variations in the rates of activitie especially as they had similar rates in th control-soil and 100m away soil.

In conclusion, this study reveale adverse environmental effects of cassava mi effluents on soil physicochemical and biologica parameters. Again, it also calls for seriou rehabilitation, if the soil will be used fo agricultural and other purposes as the factor important in soil health are negatively affecte In addition, this study revealed absence o proper regulation in the disposal of wastes an industrial effluents and so calls for introductio of such regulations to control the disposal o wastes generated from such industries. Th becomes more glaring as most of these cassav mills are sited near residential areas.

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