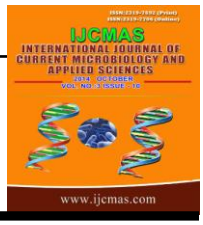
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 284-289**
http://www.ijcmas.com



Original Research Article

Effects of Cassava mill effluent on some groups of soil bacteria and soil enzymes

I.J.Ibe[1], J.N.Ogbulie[2], D.C.Odum[1], C.Onyirioha[1], P.Peter-Ogu[1] and R.N.Okechi[3*]

[1]Department of Microbiology, Federal Polytechnic Nekede, Owerri
[2]Department of Microbiology, Federal University of Technology, Owerri
[3]Department of Biotechnology, Federal University of Technology, Owerri
**Corresponding author*

Keywords

Cassava mill effluent, nitrifying bacteria, urease, impacted soil.

A B S T R A C T

The effects of cassava mill effluent on soil bacteria and enzymes were investigated. Soil samples were collected from sites polluted with cassava mill effluent (CME) and also from an adjacent site not polluted with CME during the dry and rainy seasons. Standard microbiological methods were used in enumerating the population of the microorganisms while the activity of enzymes was evaluated using biochemical processes. The bacterial groups studied include phosphate-solubilizing bacteria, nitrifying bacteria, cellulolytic bacteria, lipolytic bacteria and total heterotrophic bacteria. Nitrifying and lipolytic bacteria were most adversely affected in both the dry and rainy seasons. The activity of the enzymes decreased in the impacted soil as compared to the control, except that of urease which increased from 2.70±0.15 mg g^{-1} 24^{-1} to 5.80±0.19 mg g^{-1} 24^{-1} and from 2.80±0.05 mg g^{-1} $2h^{-1}$ to 5.20±0.21 mg g^{-1} $2h^{-1}$ in the dry and rainy seasons respectively. Excessive application of Cassava mill effluent had negative effects on soil

Introduction

Kolwan *et al*., (2006) defined soil as the top layer of the earth's lithosphere, formed from withered rock that has been transformed by living organisms. Soil formation is the result of the combined action of weathering and colonization of geological material by microbes (Willey *et al*., 2008). Soil has many layers, with the topsoil being the most productive. The biological components of the topsoil consist mainly of soil organisms, especially microorganisms which are key players in the cycling of nitrogen, sulphur and phosphorus, and the decomposition of organic residues. These affect nutrient and carbon cycling on global scale (Bunning and Jimenez, 2003). The topsoil receives the greatest impact from pollutants.

Present day industrial activities release substantial amounts of pollutants into the

natural environment. Such pollutants include crude oil and its refined products, palm oil mill effluent, cassava mill effluent, wastewater from agro-allied industries, refineries, human and animal wastes, laundering, car washing, wood waste and mining effluents, (Walsh *et al*., 2002, Wade *et al*., 2002, Ojumu, 2004, Arimoro and Osakwe 2006). Soil pollution causes imbalance in soil flora and fauna, which leads to reduced soil fertility. This is mostly because microorganisms which are involved in all nutrient cycling are destroyed. Cassava composes approximately 57% of tropical root and tuber production (Nwoko *et al*., 2009). Nigeria is the world's largest producer of cassava, *Manihot esculenta* (Crantz) (FAO, 2004). In Nigeria, cassava can be converted to diverse traditional delicacies which include; garri, fufu, lafun flour etc some of which are fermented products (Oti, 2002). Among all the products processed from cassava, garri is the most common in Nigeria. Garri production is done in varying scales; in a small, medium and large scale. Most garri processing plants in Nigeria produce between 7-10 million tonnes of garri annually (FAO, 2004). Much waste from cassava mills are generated which are usually discharged on land or into water bodies indiscriminately and this in turn affects the biota (Olorunfemi *et al.,* 2008). Cassava (*Manihot esculenta* Crantz) processing into garri involves several unit operations, including peeling, washing, grating, pressing, etc. Traditional garri production is associated with the discharge of large amounts of water, hydrocyanic acid, organic matter in the form of peels, and sieves from the pulp as waste products. Continuous discharge of these wastes has accentuated the adverse effect of cassava waste to the environment and biodiversities (Goodley, 2004). When these waste products are improperly disposed they generate offensive odours and unsightly scenarios (FAO, 2004; Okafor, 2008). The major component of the effluent from garri processing industries is cyanide and in most cases, the effluent is channeled into pits where it continues to accumulate and sink gradually into the surrounding soils thereby posing a serious health and environmental hazard (Okafor, 2008).

This work was aimed at determining the effect of cassava mill effluent on some groups of soil bacteria and soil enzymes.

Materials and Methods

Soil Sample Collection

Soil samples were collected using disinfected trowel from 0-15 cm depth, in soils polluted with cassava mill effluent, CME, as well as from soils not polluted with the agricultural wastes (which served as control). A set of soil samples was collected during the dry season (December – March) and another set of soil samples was collected during the rainy season (April – October). The soil samples were collected from Mmahu in Ohaji/Egbema LGA noted for garri processing in Imo State. The co-ordinates of Ohaji/Egbema Local Government Area are longitude $4^{o}49'$ N and latitude $6^{o}34'$ E.

Preparation of Soil Samples for Analysis

Bulked or composite soil samples from impacted and control sites were air-dried, ground, sieved (2mm) and stored at room temperature (28 ± 2^{O}C) for 24h.

Microbiological Analysis

The populations of different groups of bacteria were estimated using different microbiological methods.

Estimation of Total Heterotrophic Bacteria (THB)

Population of heterotrophic bacteria in the impacted and control soil samples were estimated as described by Okpokwasili and Okorie (1988).

Estimation of Nitrifying Bacteria (NB).

An aliquot (0.1ml) of 10^{-3} dilution was inoculated on mineral salt agar medium solidified with 1% Difco Noble Agar, using spread plate technique. Bacterial growth was obtained after 48-72h incubation at room temperature (28±2^{o}C) (Nwaugo *et al*., 2004).

Estimation of Lipolytic Bacteria (LB)

Aliquots of diluted soil samples were plated on tributyrin agar for 24h, and the formation of halo zone around the colonies on tributyrin agar was considered as positive result for the test (Mohan *et al*., 2008).

Enumeration of Phosphate Solubilizing Bacteria (PSB)

About 0.1ml of 10^{-3} dilution of soil sample was inoculated on NBRI-BPB medium solidified with 1% Difco Noble Agar using spread plate method, and incubated at 30^{o}C for 3 days. Production of yellow halos around the colonies was taken as positive result (US Patent, 2003).

Enumeration of Cellulolytic Bacteria (CEB)

The organisms were enumerated by plating serially diluted soil samples on cellulose agar which contained carboxy methyl cellulose (CMC) (Hatami *et al*., 2008).

Enzyme assay

Acid and Alkaline Phosphatases

The colorimetric estimation of the p-nitrophenol released by phosphatase activity when soil is incubated with buffered (pH 6.5 for acid phosphatase activity and pH 11 for alkaline phosphatase activity) sodium p-nitrophenyl phosphate solution and toluene, as described by Alef and Nannipieri (1995) was employed.

Lipase Assay

The method described by Onilude *et al*., (2010) was employed in this assay.

Urease Assay

The method used was based on the determination of ammonia released after incubation of soil samples with urea solution for 2h at 37^{o}C (Alef and Nannipieri, 1995).

Cellulase Assay

The enzyme was assayed based on the determination of released reducing sugars after the incubation of soil samples with carboxy methyl cellulose salt solution (CMC) for 24h at 50^{o}C (Alef and Nannipieri, 1995).

Assay for Dehydrogenases

The assay method as described by Cassida *et al*., (1964) was used. The assay involved colorimetric estimation of 2, 3, 5-triphenyl formazan (TPF) produced by the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) by soil microorganisms.

Statistical Analysis

SPSS was used to carry out a paired sample T-test to analyze the data and make inferences.

Results and Discussion

Seasonal bacterial loads: During the dry season, THB had the highest value (6.7 x 10^6±0.13 cfu/g) in the control as well as in the impacted soil (3.7 x 10^4 ±0.26 cfu/g). NB had the least population (0.6 x 10^1 ±0.03cfu/g) in the polluted soil, followed by LP with 0.9 x 10^1 ±0.02 cfu/g. PSB decreased from 2.9 x 10^4±0.19 -2.4 x 10^2±0.19 cfu/g, and CEB from 2.3 x 10^4 ±0.05 to 2.4 x 10^3 ±0.19 cfu/g. During rainy season, THB decreased from 2.1 x 10^7±0.18 cfu/g in the control to 4.3 x 10^4±0.08 cfu/g in the impacted soil. THB had the highest population (2.1 x 10^7±0.18 cfu/g) in the control while LB had the lowest (2.4 x 10^4 ±0.19 cfu/g).

Enzymes activity: Apart from urease which increased from 2.70±0.15 mg g^{-1} 24^{-1} to 5.80±0.19 mg g^{-1} 24^{-1}, the other enzymes followed the same trend of decrease in activity. Dehydrogenase had the highest activity in the control (33.50±0.18 mg g^{-1} $6h^{-1}$) but decreased significantly to 16.40±0.19 mg g^{-1} $6h^{-1}$ in the polluted soil (p=0.05). Lipase showed the least enzymatic activity both in the control (2.40±0.19 g^{-1} 30 min) and in the impacted soil (1.10±0.01 g^{-1} 30 min). During the rainy season, the activities of the enzymes decreased in the impacted soil except urease whose activity increased from 2.80±0.05 mg g^{-1} $2h^{-1}$ (control) – 5.20±0.21 mg g^{-1} $2h^{-1}$ (impacted) The activity of dehydrogenase was highest in both the control and the impacted soil, though it decreased significantly from 34.32±0.13 mg g^{-1} $6h^{-1}$ in the control to 20.17±0.05 mg g^{-1} 6h-1 in the impacted soil. Lipase showed the least activity in the control (2.50±0.02 g^{-1} 30 min) and in the impacted soil (1.40±0.19g^{-1} 30 min).

The population of the different bacterial groups decreased in the impacted soil, as shown in Table 1. The high content of cynogenic glucoside, example, linamarin, in the CME may have adversely affected the microbial load. Nitrifying bacteria had the least count while THB had the highest in the impacted soil. Nitrifying bacteria were negatively affected by the alkaline environment. THB were generally higher in population than the other bacterial groups. Nwaugo *et al*., (2008), Pelczar *et al*., (2003), Karin (2006) and Onyeagba *et al*., (2002) reported similar results. This is because THB is a summation of all viable bacteria while the other ones are fractions of THB. On the other hand nitrifying bacteria are very sensitive to environmental stress and had very low population. This agrees with Nwaugo *et al*., (2009). But Ogboghodo *et al*., (2009) reported that CME increased the population of soil bacteria and fungi. This could be due to differences in concentration of the effluent. As shown in Table 2, CME decreased the enzymatic activities of the soil enzymes, except that of urease. Lipase showed the least activity and dehydrogenase showed the highest activity, generally in all the polluted soils. Nwaugo *et al*., (2009) obtained similar results. The decrease in dehyrdogenase activity in this work is contrary to the observation of Achuba and Pereiemo-Carke (2008), who reported an increase in the dehydrogenase activity in spent engine oil-polluted soil. A decrease in the population of THB in the impacted soil correlated with decrease in activity of dehydrogenase; in agreement with the findings of Lee *et al*., (2002), and Oliviera and Pampulha (2006). Increased urease activity observed in this work agrees with Nwaugo *et al*., (2008). The alkaline environment may have encouraged the activity of the enzyme (urease).

Table.1 Effect of Cassava Mill Effluent on Microbial Load of Soil (cfu/g) in Different Seasons

Parameters	Dry Season Control	Dry Season Impacted Soil	Rainy Season Control	Rainy Season Impacted Soil
Nitrifying Bacteria	$2.4x10^4$±0.02	$0.6x10^1$±0.03	$3.2x10^4$±0.25	$1.1x10^1$±0.02
Phosphate Solubilizing Bacteria	$2.9x10^4$±0.19	$2.4x10^2$±0.19	$3.3x10^4$±0.06	$2.5x10^2$±0.17
Total Heterotrophic Bacteria	$6.7x10^6$±0.13	$3.7x10^4$±0.26	$2.1x10^7$±0.19	$4.3x10^4$±0.08
Cellulolytic Bacteria	$2.3x10^4$±0.05	$2.4x10^3$±0.19	$2.9x10^4$±0.19	$2.8x10^3$±0.08
Lipolytic Bacteria	$2.2x10^4$±0.02	$0.9x10^1$±0.02	$2.4x10^4$±0.19	$1.1x10^1$±0.01

Table.2 Effect of Cassava Mill Effluent on Soil Enzymes Relative to the Control

Enzymes	Dry Season Control	Dry Season Impacted Soil	Rainy Season Control	Rainy Season Impacted Soil
Dehydrogenase (mg g^{-1} $6h^{-1}$)	33.50±0.18	16.40±0.19	34.32±0.13	20.17±0.05
Acid Phosphatase (µmol-p-nitrophenol)	3.40±0.05	2.40±0.19	3.60±0.13	2.60±0.01
Alkaline Phosphatase (µmol-p-nitrophenol)	3.10±0.19	2.80±0.12	3.70±0.05	2.90±0.02
Urease (mg g^{-1} $2h^{-1}$)	2.70±0.15	5.80±0.19	2.80±0.05	5.20±0.21
Cellulase (mg g^{-1} $6h^{-1}$)	3.20±0.03	2.10±0.01	3.20±0.19	2.90±0.31
Lipase (g^{-1} 30 min)	2.40±0.19	1.10±0.01	2.50±0.02	1.40±0.19

References

Achuba, F. I and Peretiemo - Clarke, B. O. 2008. Effects of Spent Engine Oil on Soil Catalase and Dehydrogenase activities. *International Agrophysics* 22,1-4.

Arimoro, F. O and Osakwe, E. I. 2006. The influence of sawmill wood wastes on the distribution and population of microinvertebrates at Benin River, Niger Delta Area, Nigeria. *Chemistry and Biodiversity Journal 3:* 578-592.

Bunning, S and Jimenez, J. J. 2003. Indicators and Assessment of Soil Biodiversity Soil ecosystem functioning for farmers and Govt. FAO, Land and Water Development Division. Rome, Italy. Pg 1 – 21.

Cassida, L. E., J. D. Klein and Santoro, D. 1964. Dehydrogenase activity. *Soil Science* 98: 371-374.

FAO 2004. The global cassava development strategy. Published by Food and Agricultural Organization.

Hatami,S., H. A. Alikhami,, H. Besharati,, N. Salehrastin,, M. Afrousheh, and Yazdani, J. .Z.. 2008. Investigation of Aerobic Cellulolytic Bacteria in some of North Forest and Farming Soils. *American-Eurasian Journal of Agriculture and Environmental Sciences*, 3(5): 713- 716.

Karin, N. 2002. Impact of organic waste on structure and function of soil bacterial communities. PhD Thesis. Swiss University of Agricultural Sciences. Uppsala, Sweden.

Kolwzan, B., W. Adamiak, K. Grabas and Pawleczyk, A. 2006. Introduction to Environmental Microbiology. Oficyyna Wydawnicza Politechniki Wroclawskiej, Wroclaw.

Lee, I. K., Y. Chang, Y. Bac, B. Kin and

Baek, H. 2002. Heavy metals concentrations and enzyme activities in soil from a contaminated Korean shooting range. *Journal of Biosensors and Biomineralization*. 94: 406-4-11.

Nwaugo, V. O., G.C. Chinyere and Inyang, C. U. 2008. Effects of palm oil mill effluent (POME) on Soil Bacterial Flora and enzyme activities in Egbema. *Plant Products Research Journal*, 12:10-13.

Nwaugo, V. O., C.A. Etok, G. N.Chima and Ogbonna, C. E. 2009. Environmental impact of cassava mill effluent on physicochemical and microbial community structure and functions. *World Journal on Environmental Research*, 8(3):19-19.

Nwoko, C., S. Ogunyemi, E. E.Nwokocha and Nnorom, I. C. 2009. Evaluation of Phytotoxicity effect of POME and CME on tomato (*Lycopersium esculentum*) after pre-treatment options. *International Journal of Environmental Science and Development*, (1).

Ogboghodo, I. A., I. O. Osemewota, S.O. Eke and Iribhogbe, A. E. 2001. Effects of Cassava (*Manihot esculenta* Crantz) grating mill effluent on textural, chemical and biological properties of surrounding soil. *World Journal of Biotechnology*. 2: 292-301.

Okafor, J. O. 2008. Impact of effluents from garri processing industries on the environment in Bida, Niger state of Nigeria. *Journal of Engineering and Applied Sciences* 3 (6): 487-490.

Ojomu, T. V., A. A. Bello, J.A. Sonibara and Solomon, B. A. 2004. Evaluation of microbial systems for bioremediation of petroleum refinery effluents In Nigeria. *African Journal* of *Biotechnology* 4(1): 31-35.

Olorunfemi, D. I., E. O. Emoefe and Okieimen, F. E. 2008. Effect of cassava processing effluent on seedling height, biomass and chlorophyll content of some cereals. *Research Journal* of *Environmental Sciences*, 2 (3): 221-227.

Okpokwasili, G. C and Okorie, B. B. 1988. Biodegradation potentials of microorganisms isolated from car lubricating oil. *Tribiology International*, 21: 215-222.

Oliviera, A and Pampulha, M. E. 2006. Effects of long-term heavy metal contamination on soil microflora characteristics. *Journal of Biosensors Bioengineering* 102(6): 157-601

Onilude, A.. A., R. O. Igbinabolor and Wakil, S, N. 2010. Effect of varying relative humidity on the rancidity of cashew kernel oil by lipolytic organisms. *African Journal of Biotechnology*, 9(31): 4809-4896.

Onyeagba, R. A., V. O. Nwaugo, S. O. Obiekezie, C. E. Ogbonna and Verla, A. W. 2008. Phytoremediation of Pb and Zn polluted soil with *Chromolana odoranta* and *Talinum triangulare* in Ishiagu mining area, Ebonyi State, Nigreia. 32nd Annual Conference of Nig. Soc. For Microbiol. Abia State Univ., Uturu, 10th- 13th October 2008.

Oti, N. N. 2002. Discriminant functions for classifying erosion degraded lands at Otamiri, Southeastern Nigerian. *Journal of Tropical Agriculture and Food Environment* 3(1): 34-40.

US Patent. PatentStorm 6638730. Composition for qualitative screening ofphosphate solubilizing microorganisms and a qualitative method for screening microorganisms. Issued October 28, 2003.

Wade. J. W., E. Omoregie and Ezenwata, I. 2002. Toxicity of Cassava (*Manihotesculenta* Crantz) effluent on the Nile Tilapia, *Oreochromis niloticus (L)* under laboratory condition. *Journal of Aquatic Sciences* 17(2): 89-94.

Walsh, C., J. P. R. Gooderham, M. R. Grace, S. Sdraulig, M. I. Rosyidi and Lelono, A. 2002. The relative influence of diffuse and point-source disturbances on a small upland stream in East Java Indonesia: a preliminary investigation. *Hydrobologia Journal* 487: 183-192.

Willey, J. M., I. M. Sherwood and Woolverton, C. J. 2008. Prescott, Harley, and Klein's Microbiology 7th Edn. McGraw -Hill, New York. 2008. 1-1088.