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EFFECTOFCASSAVAMILLEFFLUENTONMICROBIALPOPULATION AND COMPOSITION IN A SOIL PLANTED TO MAIZE (*Zea mays* L.)

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ABSTRACT

Experiment was carried out at Ambrose Alli University Teaching and Research Farm, Ekpoma, Edo State, to determine the effect of cassava mill effluent (CASME) on microbial population and composition in a soil planted to maize (*Zea mays*) in the screen-house and field. In the screen-house, cassava mill effluent (CASME) had five levels (0, 20, 40, 80 and 120 ml/kg soil) that were fitted into Completely Randomized Design and replicated thrice. For the field experiment, CASME also had five levels (0, 2, 025; 4,050; 8,100 and 12,150 L/ha) fitted into Randomized Complete Block Design and replicated three times. In both experiments, maize variety Swan-1 was used as test crop. The number and types of bacteria were determined in composite soil samples before the CASME was applied, eight (8) weeks after application in the screen-house and in the field and at the end of the experiment (15 weeks after application in the field). Results revealed that the pre-treated soil had less populations of bacteria ($31x10^3$ cfu/g) and fungi ($21x10^3$ cfu/g) when compared to 16×10^4 cfu/ml and $4x10^4$ cfu/ml of CASME. At eight weeks in the screen-house, the population of bacteria and fungi increased with increased level of application. The species of bacteria and fungi that predominated were *Bacillus subtilis* and *Aspergillus niger*, respectively. In the field at eight (8) weeks and fifteen (15) weeks after application, results showed that the total viable counts for bacteria and fungi in soils increased with increased level of application of cassava mill effluent. *Bacillus subtilis* and *Penicillium notatum* were the dominant species of bacteria and fungi, respectively.

INTRODUCTION

Improved soil fertility through the application of fertilizer is an essential factor that enables the world to feed the billons of people that are added to its population (Brady and Weil, 2002). Soil fertility is usually maintained by the addition of organic and inorganic fertilizer.

Man's industrial and economical activities usually produce wastes which eventually get disposed on land. Nigeria, which is the world largest producer of cassava, (*Manihot esculenta* Crantz) (FAO, 2004), has consistently generated so much wastewater from cassava mills which are normally discharged on land or water indiscriminately and this in turn affects the biota (Ogboghodo *et al.,* 2001). Various studies by soil microbiologists on hydrocarbons degradation in soil revealed the presence of microflora in the soil that are capable of degrading a wide variety of hydrocarbons. However, heavy metal load in the soil reduces the functioning of soil biota resulting in reduced microbial activity (Kandiler, 1996).

Ogboghodo *et al.* (2006) reported that bacteria and fungi populations increased with time as rates of cassava mill effluent increased while some bacteria present at the beginning of the experiment and up to the sixth week after pollution with the effluent became extinct at the end of the experiment. They also observed that the relative abundance of different microbial pollutions of soil before pollution was in the following order: *Klebsiella* > *Escherichia* > *Bacillus* >*Pseudomonas*. That of fungi was *Mucor* >*Penicillium*>*Aspergillus*. Ogboghodo *et al.* (2001) stated that although *Mucor* species wereprominent fungi species, in both non-effluent and cassava mill effluent affected soils, it occurred more frequently in the effluent treated soils. Other fungal isolates found in the soils included: *Pennicillim* spp, *Aspergillus* spp and *Rhizopus* spp. Only few workers have isolated microorganisms from soils affected with cassava mill effluents. Therefore, the objective of this study was to assess the number and types of bacteria and fungi in the effluent affected soils cultivated with maize both in the screen-house and in the field.

MATERIALS AND METHODS

The screen-house experiment was carried out before that of the field. Both experiments were carried out in the Teaching and Research Farm of Ambrose Alli University, Ekpoma, Edo State, Nigeria. Ekpoma is a town situated at about 85km north of Benin City and south of Auchi in Edo state. It is located between latitude 6^0 41 ^{1}N - 6^0 49 ^{1}N and longitude 6^0 00 ^{1}E – 6^0 14 ^{1}E.

Experimental Methods: Core surface soil samples (0 - 15 cm) collected randomly from 18 points from Ambrose Alli UniversityTeaching and Research Farm were bulked together to form a composite sample. Cassava mill effluent was applied at treatment rates of 0, 20, 40, 80 and 120 ml/kg of soil and fitted into Completely Randomized Design and replicated thrice. The treated soils were left for 3 weeks to equilibrate before maize seeds were planted. A total of 15 plots cultivated with maize each measuring 2.25 m^2 were used for the field experiment. CASME was applied after land preparation at treatment rates of 0, 2,025; 4,050; 8,100 and 12,150 L/ha. Plots were also left for three weeks before planting.

Chemical Analyses of the Effluent

Cassava mill effluent was analyzed for its chemical properties using standard laboratory procedures (Rhoades, 1982; Anderson and Ingram, 1989). The parameters analyzed include pH, organic C, Total N and available P, exchangeable cations (Ca, K, Mg and Na), micro-nutrients (Mn, Fe, Cu) and heavy metals (Cr, Ni and Cd).

Microbiological Analyses of Effluent and Soil Samples

Microbiological analyses of cassava mill effluent was carried out. Soil samples were also analyzed for their microbiological properties before the application of cassava mill effluent, 8 weeks after application and at the end of the experiment (fifteen weeks after application). These were done according to the methods of Cowan and Steel (1993) and Robert *et al.* (2004). The bacterial isolates were identified using standard biochemical tests (Robert *et al*., 2004; Cheesebrough, 2006). The test employed include morphological characteristics, colonial, gram stain, catalase, urease, oxidase, motility and sugar fermentation (sucrose, fructose, manitol, lactose, glucose, galactose, and maltose). The fungal isolates were identified with the aid of macroscopic and microscopic examinations and confirmed using the Atlas of Mycology (Robert *et al.,* 2004) based on morphology, and substrate mycelium.

RESULTS AND DISCUSSION

The chemical properties of cassava mil effluent used are shown in Table 1. The results of the analyses of the fresh effluent and the soil before the effluent was applied to the soil planted to maize (Table 2) revealed that the soil had less populations of bacteria (31x10^3 cful/g) and fungi (21x10^3 cfu/g) when compared to 16 x10^4 cfu/ml and 4 x10^4 cfu/ml of cassava mill effluent.

Table 1: Chemical properties of the effluent used for experiment.

Parameters	Cassava Mill Effluent (CASME)
pH	4.30
Ava. P (mg/L)	21.00
Total N (mg/L)	0.15
Exchangeable cation (mg/L)	
Ca	334.00
Mg	115.00
K	89.00
Na	213.35
Available micro nutrient and heavy metals (mg/L)	
Mn	113.31
Fe	78.08
Zn	21.50
Cu	8.31
Pb	0.49
Ni	1.32
Cr	0.99
Cd	0.02

Ava. P: Available P.

Table 2: Bacteria and fungi populations/identification on effluents and soil before application.

Types of Sample	Bact popn (cfu/ml)	Bacteria Identification	Fungi popn (cfu/ml)	Fungi Identification
CASME	16x10^4	*Bacillus sphericus*	4x10^4	*Candida spp*
		Bacillus cereus		
		Pseudomonas spp		
Soil	31x10^3 (cfu/g)	*Baccillus* spp	21x10^3	*Candida* spp
		Clostridium spp		*Penicillium* spp
		Pseudomonas spp		
		Staphylococcus spp		
		Micrococcus spp		

Table 3: Effects of cassava mill effluent (CASME) on bacteria and fungi populations/ identification at eight (8) weeks after application in the screen-house

Trt CASME (ml/kg)	Bact Popn (cfu/g)	Bacteria Identification	Fungi popn (cfu/g)	Fungi Identification
0	$2x10^{8}$	*Bacillus subtilis*	$24x10^{7}$	*Trychophyton spp*
				Aspergillus niger
20	$68x10^{7}$	*Bacillus subtilis*	$42x10^{7}$	*Aspergillus niger*
		Enterobacter aerogenes		
		Proteus mirabilis		
40	$72x10^{7}$	*Bacillus subtilis*	$44x10^{7}$	*Aspergillus niger*
		Proteus mirabilis		
80	$88x10^{7}$	*Bacillus subtilis*	$46x10^{7}$	*Aspergillus niger*
		Proteus mirabilis		
120	$104x10^{9}$	*Bacillus subtilis*	$48x10^{7}$	*Aspergillus niger*
		Proteus mirabilis		

Table 4: Effects of cassava mill effluent (CASME) on bacteria and fungi populations/identification at eight (8) weeks after application in the field.

Trt CASME(L/ha)	Bact Popn (cfu/g)	Bacteria Identification	Fungi Popn (cfu/g)	Fungi Identification
0	$28x10^{4}$	*Bacillus subtilis*	$18x10^{7}$	*Aspergillus niger*
				Trychophyton spp
2,025	$44x10^{4}$	*Bacillus subtilis*	$28x10^{7}$	*Candida albicans*
4,050	$56x10^{4}$	*Bacillus subtilis*	$36x10^{7}$	*Rhizopus oryzae*
				Penicillium notatum
8,100	$96x10^{4}$	*Bacillus sustilis*	$45x10^{7}$	*Penicillium notatum*
12,150	$126x10^{4}$	*Bacillus subtilis*	$51x10^{7}$	*Penicillium notatum*
				Aspergillus niger.

At eight weeks in the screen-house, cassava mill effluent increased the population of bacteria and fungi in soils planted to maize as the level of application increased when compared to the control (Table 3). This could be regarded to as destabilization of the soil ecological balance as a result of effluent application. This was in agreement with what was earlier reported by Adesomoye *et al.* (2006). It was also observed that there were various types of bacteria species with the highest at 20 ml/kg effluent rate. For bacteria, *Bacillus subtilis* predominated while for fungi, *Aspergillus niger* was the most frequent and the order of abundance include *Bacillus subtilis*> *Proteus mirabilis* > *Enterobacter* aerogenes. Environmental stress brought about by the application of cassava mill effluent could be responsible for the reduction in types of fungi species observed in the effluent affected soils. This was in line with what was reported by other workers (Adesemoye *et al.,* 2006; Ogbohodo *et al.,* 2006).

In the field, the results of microbiological analyses of the various soil samples planted to maize at 8weeks after application of cassava mill effluent revealed that total bacteria and fungi populations obtained from the effluent affected soils were more than that in soil without the effluent (Table 4). The total viable bacteria and fungi counts in the effluent affected soils increased with increased level of effluent application with the highest counts of 126 x 10^3 L/ha when compared to the control of 31 x 10^3 and $21x10^7$ cfu/g respectively. It could be that the organisms present in the cassava mill effluent and organisms autochthonous to the soil engaged in competition after application of the effluent. Guided by the law of survival of the fittest (Madigan *et al.*, 2003), those that were not able to survive the new condition (pH 4.30) were probably eliminated. The relative abundance of fungi in soil were in the following order: *Penicillium* > *Aspergillus*> *Rhizopus* > *Candida*. This findings was in conformity with what Ogbohodo *et al.* (2001) earlier reported with cassava mill effluent.The presence and abundance of *Bacillus subtilis* observed in the effluent affected soil may be borne out of the fact that this organisms were indigenous to the soil. This was in agreement with what was reported by Atlas and Bartha, (2007).

At 15 weeks after application of cassava mill effluent, the total bacteria and fungi counts were high in effluent affected soils when compared to what were recorded in non-effluent affected soil (Table 5). However, the population of these

Table 5: Effects of cassava mill effluent (CASME) on bacteria and fungi populations/identification at fifteen (15) weeks after application in the field.

Trt CASME (L/ha)	Bacteria Popn (cfu/g)	Bacteria Identification	Fungi Popn (cfu/g)	Fungi Identification
0	$3x10^7$	*Bacillus subtilis*	$2x10^7$	*Penicillium notatum*
		Escherichia coli		
2,025	$42x10^6$	*Bacillus subtilis*	$26x10^6$	*Penicillium notatum*
		Klebsiella aerogenes		
4,050	$54x10^6$	*Bacillus subtilis*	$32x10^6$	*Aspergillus terrus.*
8,100	$8x10^6$	*Bacillus subtilis*	$4x10^7$	*Penicillium notatum*
		Klebsiella aerogenes		
12,150	$115x10^6$	*Bacillus subtilis*	$41x10^6$	*Microsporum ferrugineum*

organisms declined when compared to what was obtained in 8 weeks after application. The highest bacteria and fungal counts of 115 x 10^6 and 41 x 10^6 cfu/g were obtained at 12,150 L/ha CASME.

The relative abundance of bacteria in the effluent affected soil was in the following order: *Bacillus* > *Klebsiella* > *Escherichia coli* while that of fungi was *Penicillium*> *Aspergillus* > *Microsporium* and most fungi isolates were soil – inhabiting microorganisms (Atlas and Bartha, 2007). Some bacteria and fungi species which were present at the beginning of the experiment up to 8weeks after application of the effluent became extinct at the end of the experiment while new species of microorganisms were isolated from the effluent affected soil. This further confirmed the findings of Ogboghodo *et al.* (2006) when they used cassava mill effluent.

CONCLUSION

Microbiological analyses of cassava mill effluent and soil sample before the application of the effluent showed that the effluent contained higher number of bacteria species (16 x10^4 cfu/ml) and fungi (4 x10^4 cfu/ml) when compared to that obtained from the soil (31x10^3 and 21x10^3 cfu/g). It was also observed that the soil had more diversity of bacteria and fungi species than that of the effluent.

In the screen-house and in the field, the soil planted to maize at 8 weeks after the application of cassava mill effluent showed increased population of microorganisms with increased level of effluent application.

At 8 weeks, after the application of effluent, the soil planted to maize revealed reduction in bacterial species diversity while at 15 weeks, after the effluent application there was increase in the diversity of bacterial species. In the field experiment, *Bacillus subtilis* and *Penicillium notatum* predominated. In the screen-house, *Bacillus subtilis* and *Aspergillus niger* were more frequent in soils planted to maize.

REFERENCES

Adesemoye, A.O., Opere, B.O. and Makinde, S.C.O. (2006). Microbial content of abattoir waste water and its contaminated soil in Lagos, Nigeria. *African Journal of Biotechnology* 5 (20): 1963-1968.

Anderson, J.M. and Ingram, J.S.I. (1989). *TSBF: A handbook of Methods of Analysis.* CAB International. Walling – Ford, U.K. pp 38-39.

Atlas, R.M. and Bartha, R. (2007). *Microbial Ecology: Fundamentals and Applications.* 4th edition Benjamin/Cummings Publishing Company, Inc India pp 58-66.

Brady, N.C and Weil, R.R. (2002). T*he Nature and Properties of Soil.* 13th Edition. Dorling Kindresly Publishers (India) Pvt, Ltd. Pp 608-609

Cheesebrough, M. (2006). *District Laboratory Practice in Tropical Countries.* Part 2. Low Price Edition. Cambridge University Press, London pp 79-201.

Cowan and Steel (1993). *Manual for the identification of medical bacteria.* Third edition. Cambridge University press pp 50-76.

FAO, (2004). Cassava Industrial Revolution in Nigeria. *Codex Alimentarius Commission* XII, supplementary 4. Rome.

Kandiler, F. (1996). Influence of heavy metals on the functional diversity of soil microbial communities. *Biology and fertility in Soils* 23 (3): 299-306.

Madigan, M.T.; Martinko, J.M. and Parker, J. (2003). *Brock biology of microorganism. International* edn. Prentice Hall Intern. Ltd, London. pp 1020-1040.

Nwoko, C.O. (2010). Evaluation of palm oil mill effluent to maize (*Zea mays* L.), crop yields, tissue nutrient content and residual soil chemical properties. *Australian Journal of Crop Science* 5: 28-32.

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

Ogboghodo, I.A., Oluwafemi, A.P. and Ekeh, S.M. (2006). Effects of polluting soil with cassava mill effluent on the bacteria and fungi populations of a soil cultivated with maize. *Environmental Monitoring and Assessment* 116: 419-425.

Rhoades, J.D. (1982). In: *Methods of Soil Analysis*, part 2. Second Edition A.L. Page, R.H. Miller and D.R. Keeney (eds). American Society of Agronomy, Madison U.S.A pp54-60.

Robert, A.S., Ellen, S.H. and Jens, C.F. (2004). *Introduction to Food and Airborne Fungi.* Seventh Edition, *Centraal bureau Voor schimmkultures*. *The Netherlands* pp 23-306.