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Evaluation of Microbial Loads and Physico-Chemicals of Cassava Mill Effluent Simulated Soil

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BEA and AYI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DOE and AAB managed the analyses of the study. Author BEA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Evaluation of microbial loads and physico-chemicals of cassava mill effluent simulated soil was carried out using standard microbiological and biochemical techniques. This was to determine the effect of cassava mill effluent (CME) on rhizosphere microbial loads, physicochemical properties, nitrogenous salt and heavy metals. The results showed that CME effect on the physicochemical determinants (pH, Ca, Mg, K) and heavy metal determinant (Fe, Zn, Co, Ni, Pb and Mn) was concentration dependents. The nitrogenous salts (NO_3, NH_4^+ and NO_2) levels progressively increased with no significant differences ($p>0.05$ ANOVA). The microbial isolates were: *Saccharomyces* sp, *Mucorindicus, Fusarium* sp and *Gliocladium* sp for the fungal group. The bacterial group were *Chromobacterium* sp, *Corynebacterium* sp, *Bacillus* sp, *Acinetobacter* sp and *Escherichia coli* while the nitrogen-fixing bacterial group were *Azotobacter* sp*., Azospirillum* sp*., Frankia* sp*., Bradyrhizobium* sp*., Hebaspirillum* sp., Cyanobacteria (or blue green algae), *Anabaena*

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sp, *Nostoc* sp., *Clostridium* sp. and *Rhizobium* sp. There was no significant differences ($p>0.05$) in the rhizosphere microbial load across the concentration gradient at the CME-simulated plot phyto-remediated by *Centrosema pubesscens and Calopogonium mucunoides.* Agricultural wastes such as cassava mill effluent should be properly treated before discharging to the environment in other to prevent the loss of nitrogen-fixing bacteria and total heterotrophic bacterial genera that could be of immense importance to man.

Keywords: Simulated-soil; nitrogen-fixing bacteria; heavy metal; nitrogenous salts and cassava mill effluent (CME).

1. INTRODUCTION

With the fast industrialization going on in Nigeria, environmental pollution by effluents has increased greatly and the incessant discharge of effluents from industries such as agriculture, chemical, textile and petroleum industries etc. pollutes water bodies and soil environment [1-4]. According to Okunade and Adekalu, [4] the properties and quantities of the discharged liquid wastes varies from industry to industry depending on the water need of the industry, average daily production and product. They further said that it is hazardous to the soil or aquatic environment, if billions of gallons of waste water produced on daily basis from cassava processing mills in Nigeria are not treated before being released to the environment [1,3,4].

Soil is a large and diverse environment, in both time and space and microbial activities are concentrated at localized spots around and on organic residues. Thus, the introduction of a mixed bag of compounds can bring about a great alteration in the structure and function of the microbial community [5]. Soil is an important ecological system of terrestrial ecosystem. There is a direct effect of effluents on microbial community, organic matter and minerals of soil [6]. Soil is a specie-rich environment that contains all the major groups of tiny creatures. The soil microbiota are very helpful in the breaking down and in the manufacturing of organic compounds. They are equally involved in the weathering of primary minerals and in the cycling of plant nutrients [7]. Soil organisms are made up of the micro flora (bacteria and fungi) and the soil fauna (protozoa and invertebrate groups such as nematodes, mites and earthworms) [7].

Processing of cassava tubers generates both liquid and solid residues that are harmful to the environment. There are two vital biological wastes generated from the processing of cassava, they are the liquid squeezed out of the fermented parenchyma mash and the cassava peels. Cassava mill effluents are liquid wastes from the cassava mill that are usually discharged on land or water in an unplanned manner. The cassava peels obtained from its processing are normally discarded as wastes and allowed to rot in the open field with a small portion used as animal feed, thus resulting in health and environmental hazards [8,3].

Cassava mill effluent has been found to increase the number of organisms in the soil ecosystem which may be associated with increase in the soil pH, organic carbon and total nitrogen [8]. Studies from the works of Akpoveta et al. (2010) and Okunade and Adekalu, [4] showed that the heavy metals useful for growth and survival of organisms are only required in low concentrations. The high concentration of heavy metals in the soil and aquatic environment is as a result of cassava processing activities that has led to bioaccumulation of metals in flora and fauna. Heavy metals from the effluent are not biodegradable so they accumulate in primary organs in the body and over time begin to fester, leading to various symptoms of diseases as reported by Siyanbola et al. (2011) and Okunade and Adekalu [4].

Several other researches had been carried out by Chaturvedi et al. [9], Sponza, [10], Adewoye et al. [1], Okunade and Adekalu, [4] and Agbo et al. [11] on the adverse effects of effluents in water and on soil environment. Raw or partially treated waste water can be harmful to both aquatic and terrestrial life by greatly affecting on the natural ecosystem and also long term health effects [4]. Bio-remediation is therefore the ideal technology for removing pollutants such as cassava effluent from the soil environment by the action of microbes.

2. MATERIALS AND METHODS

2.1 Study Area

This research work was carried out at the green house, research laboratory of Microbiology Department and postgraduate laboratory of Plant and Ecological Studies Department, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria. The cassava mill effluent (CME) samples were collected at Iwuru Obio Ntan community in Biase Local Government Area of Cross River State, Nigeria with longitude 05°25'15.310"N and latitude 008°10'48.594"E. Iwuru Obio Ntan community main relief feature is lowland type of landscape grouped under the coastal lowland of Southern Nigeria within the tropical rainforest region. The soil type is made up of precisely the red and brown soil with abundant free iron oxide. The topography and location factors and prevalence of the tropical rainy climate that is warm, humid and moist for most parts of the year, encourages their major occupation which is farming and marketing of their farm produce. The study site was similar to that described by Osakwe [12].

2.2 Simulation Experiments

A medium sized green house with length 287 inches, width 264 inches and height 75 inches was built by the researchers for the purpose of this simulation experiment. The soil was collected from a garden free of recorded cases of industrial or agricultural effluent contamination. The legumes *Centrosema pubescens* and *Calopogonium mucunoides* (Fig. 1 and Fig. 2) both from the family *Leguminosae* and sub-family *Faboideae* were used for this study. The plants were selected based on the fact that they grow as legumes and fix nitrogen to the soil thereby improving the nutrient content of the soil for good growth and development of other plants.

Fig. 1. *Centrosema pubescens* plant, notice the slender stem, flower, triple leaflets and long pods with seeds

Fig. 2. *Calopogonium mucunoides* plant, a hairy annual or short-lived perennial trailing legume

Fig. 3. Simulation experiment in-progress; Notice the legumes arranged in column with the varying concentration of effluent labeled on the bucket

Ten kilograms of soil sample was dispensed into seven 22 x 19cm porous-bottomed transparent plastic buckets for *Centrosema pubescens* and *Calopogonium mucunoides* respectively. The test plants were obtained from Longitude 05°25'11.279"N and Latitude 008°10'59.836"E, Ephraim Street, Iwuru Obio Ntan community in Biase Local Government Area of Cross River State, Nigeria. They were identified by taxonomists of the Department of Plant and Ecological Studies, University of Calabar, Calabar, Nigeria. Water required for moistening the test soil prior to pollution, control soil and to vary the concentration of the effluents was collected from the University of Calabar water treatment plant. This was used to adequately stimulate the soil in the transparent buckets on daily basis to maintain a permanently wet environment [13].

Prior to simulated pollution, the plants were allowed to adapt to the new environment by formation of new buds and leaves. The 10 kg soil containing the test plants in the porous-bottomed transparent buckets was simulated with cassava mill effluent collected from Iwuru Obio Ntan community in Biase Local Government Area of Cross River State, Nigeria (Fig. 3).

Twelve (12) buckets were simulated with varying concentrations of the cassava mill effluent namely: 10%, 20%, 30%, 40%, 50% and 100% vol/vol pollution for *Centrosema pubescens* and *Calopogonium mucunoides*. The other two (2) buckets without effluent treatments (0%) served as the controls for *Centrosema pubescens* and *Calopogonium mucunoides*. The plants were watered with the corresponding concentration of effluent and observed daily for 14 weeks and the effects of the effluents on the plants studied. At maturity, they were harvested and analyses were carried out to evaluate the rhizosphere organisms, rhizosphere soil physico-chemical properties, nitrogenous salt levels in the simulated soils and metal loads as described by AOAC [14]; Sofowora [15]; Egwaikhide and Gimba [16]; Mofunanya et al. [17]; Gafar et al. [18]; Chandrappa et al. [19], Mofunanya and Nta [20]; John et al. [13]; Agbo and Mboto [21] and Agbo et al. [11].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Microbiology of cassava mill effluent-simulated soil

3.1.1.1 Fungal isolates from cassava mill effluent-simulated soil

The fungal isolates from the cassava mill effluent simulated soil were virtually the same across the

concentration gradients of 0%, 10%, 20%, 30%, 40%, 50% and 100%, the only difference was that different fungal genera was found in plots where different leguminous plants were used for the simulation experiment. *C. mucunoides* at cassava mill effluent (CME) impacted plots (0%-100%) had *Saccharomyces* sp. and *Mucor indicus* as it predominant fungal isolate while the *C. pubescens* from the same site had *Fusarium* sp. and *Gliocladium* sp. as its predominant fungal isolates. The results for the cultural characterization and identification of fungal isolates of 0-100% concentration CME-simulated soil phyto-remediated with *Calopogonium mucunoides* and *Centrosema pubescens* are presented in Table 1.

3.1.1.2 Bacterial Isolates from cassava mill effluent-simulated soil

Cassava mill effluent (CME)-simulated soil (0%-100%) had *Chromobacterium* sp. and *Corynebacterium* sp. as it predominant bacterial isolates from the *Centrosema pubescens* plots. *Calopogonium mucunoides* plots had *Bacillus* sp*., Acinetobacter* sp. and *Escherichia coli* as it bacterial isolates. The results of the biochemical characteristics of bacterial isolates from CME-simulated soil are presented in Table 2.

3.1.1.3 Nitrogen-fixing bacterial isolates from cassava mill effluent-simulated soil

Same nitrogen-fixing bacteria were isolated from all the cassava mill effluent simulated plots (0%-100%) irrespective of the legume planted for the phytoremediation exercise. The nitrogen-fixers isolated were: *Azotobacter* sp*., Azospirillum* sp*., Frankia* sp*., Bradyrhizobium* sp*., Hebaspirillum* sp., Cyanobacteria (or blue green algae) and *Anabaena* sp. Other isolates were *Nostoc* sp*., Clostridium* sp. and *Rhizobium* sp. *Azotobacter* species was Gram positive, motile and possessed catalase and oxidase enzymes. It was able to produce acid and gas from mannitol, glucose and sucrose. *Clostridium* species was a spore former, Gram positive, liquefied gelatine, produced only acid from lactose but produced acid and gas from glucose. *Frankia* species was a spore former, Gram positive, possessed catalase enzyme and produced only acid from all the sugars tested.

3.1.2 Microbial loads of cassava mill effluent-simulated soil

The microbial loads at the plots that *Centrosema pubesscens* was used to phyto-remediate CME-simulated soil was $9.70\pm0.25x10^5$ $cfug^{-1}$, $2.30\pm0.12x10^5$ $cfug^{-1}$ and $2.38\pm0.02x10^6$ $cfug^{-1}$ for bacteria, fungi and nitrogen fixing bacteria respectively at the control plot. At the 30% CME-simulated plot, the microbial loads was $5.80\pm0.20x10^5$ $cfug^{-1}$, $4.30\pm0.20x10^5$ $cfug^{-1}$ and $1.95\pm0.012x10^6$ $cfug^{-1}$ for bacteria, fungi and nitrogen-fixing bacteria respectively. There was no significant differences ($p>0.05$) in the rhizosphere microbial load at the 30% CME-simulated plot phyto-remediated by *Centrosema pubesscens.*

At the 50% CME simulated plot, the bacterial load was $7.70\pm0.26x10^5$ $cfug^{-1}$, the fungal load was $4.60\pm0.20x10^5$ $cfug^{-1}$ while the nitrogen fixing bacterial load was $1.06\pm0.02x10^6$ $cfug^{-1}$. Statistical analysis also showed that there was no significant differences ($p>0.05$) in the rhizosphere microbial load at the roots of *Centrosema pubesscens* used for the phyto-remediation of 50% CME-simulated plot. The microbial load at the 100% CME-simulated plots were $9.60\pm0.20x10^5$ $cfug^{-1}$, $5.30\pm0.20x10^5$ $cfug^{-1}$ and $9.67\pm0.12x10^5$ $cfug^{-1}$ for bacteria, fungi and nitrogen fixing bacteria respectively. Once again, there was no significant differences ($p>0.05$) in the rhizosphere microbial load at the 100% CME-simulated plot. The results of the microbial loads of CME-simulated plots phyto-remediated by *Centrosema pubescens* are presented in Fig. 4.

The CME simulated soil plots phyto-remediated by *Calopogonium mucunoides* had the microbial load of $9.23\pm0.25x10^5$ $cfug^{-1}$, $1.17\pm0.06x10^6$ $cfug^{-1}$ and $2.59\pm0.01x10^6$ $cfug^{-1}$ for bacteria, fungi and nitrogen-fixing bacteria respectively for the control (0%) plot. The 30% CME simulated soil plot had bacterial load of $6.57\pm0.20x10^5$ $cfug^{-1}$, fungal load of $3.40\pm0.20x10^5$ $cfug^{-1}$ and nitrogen-fixing bacteria load of $1.93\pm0.05x10^6$ $cfug^{-1}$. There was no significant differences ($p>0.05$) in the microbial load at the root of *Calopogonium mucunoides* used to phyto-remediate 30% CME-simulated plot.

At the 50% CME simulated plot phyto-remediated by *Calopogonium mucunoides*, the microbial load was $5.1\pm0.16x10^5$ $cfug^{-1}$, $3.97\pm0.16x10^5$ $cfug^{-1}$ and $1.78\pm0.02x10^6$ $cfug^{-1}$ for bacteria, fungi and nitrogen-fixing bacteria respectively. Statistical analysis also revealed that there was no significant differences ($p>0.05$) in the rhizosphere microbial load at the 50% CME-simulated plot phyto-remediated by *Calopogonium mucunoides.* The bacterial load at the 100% CME-simulated plot was $3.4\pm0.2x10^5$

$cfug^{-1}$, the fungal load was $5.3\pm0.06x10^5$ $cfug^{-1}$ while the nitrogen-fixing bacterial load was $1.39\pm0.02x10^6$ $cfug^{-1}$. The cassava mill effluent was highly acidic, as such it had negative effect on the nitrogen-fixing bacterial population. The nitrogen-fixing bacteria population progressively decreased from $2.59\pm0.01x10^6$ $cfug^{-1}$ at the control plot to $1.39\pm0.02x10^6$ $cfug^{-1}$ at the 100% plot. The fungal population increased progressively from the control plot to the 100% plot. Once again, statistical analysis showed that there was no significant differences ($p>0.05$) in the rhizosphere microbial load at the 100% CME-simulated plot phyto-remediated by *Calopogonium mucunoides.* The results of the rhizosphere microbial loads in CME simulated soil phyto-remediated by *Calopogonium mucunoides* are presented in Fig. 5. Analysis showed that there was non-significant ($p>0.05$) student-test mean comparisms of the different treatments (10, 20, 30, 40, 50 and 100%) against the control.

3.1.3 Physicochemical characteristics of CME-simulated soil

The physicochemical parameters of the CME simulated soil varied across the concentration gradients. The pH of the *C. pubescens* plots were 6.38 ± 0.04, 6.58 ± 0.04, 6.68 ± 0.04, 6.78 ± 0.04, 6.75 ± 0.07, 6.65 ± 0.07 and 7.05 ± 0.07 for the control, 10%, 20%, 30%, 40%, 50% and 100% CME simulated plots. The textural class of the soil was greatly affected too. For the *C. pubescens* plots, the control, 10%, 20% and 30% simulated plots had loamy soil texture, whereas 40%, 50% and 100% simulated plots had sandy soil texture. The effective cation exchangeable capacity for the *C. pubescens* plot increased progressively with increase in the concentration of CME to the soil. Elements like calcium (Ca), magnesium (Mg) and potassium (K) increased in concentration in the soil with increase in percentage of CME pollution. Same was applicable for the *C. mucunoides* plots. Table 3 presents the physicochemical properties of cassava mill effluent (CME) simulated soil. Statistical analysis revealed that physico-chemical properties of cassava mill effluent (CME) (10, 20, 30, 40, 50 and 100%) simulated soil phyto-remediated with *C. mucunoides* showed no significant difference ($p>0.05$) when compared with the control. The plots phyto-remediated by *C. pubescens* showed that the physico-chemical parameters analyzed at 10% and 20% CME-simulated soil showed no significant differences ($p>0.05$) when compared with the control soil. However, at 30, 40, 50 and 100% CME-simulation, the mean values for the physico-chemical parameters analyzed were significantly different ($p<0.05$) from the control.

3.1.4 Nitrogenous salts levels in Cassava Mill Effluent (CME)-simulated soil

The nitrogenous salts analyzed at the CME simulated soil are nitrate [NO_3], ammonium salt [NH_4^+], and nitrite [NO_2]. The nitrate levels in the CME simulated soil increased progressively from 88.53 ± 0.02 $mg100g^{-1}$ to 173.81 ± 0.03 $mg100g^{-1}$ for the *C. pubescens* plot and 104.47 ± 0.02 $mg100g^{-1}$ to 147.60 ± 0.05 $mg100g^{-1}$ for the *C. mucunoides* plot. The ammonium salt content also increased progressively from 166.62 ± 0.03 $mg100g^{-1}$ to 249.96 ± 0.08 $mg100g^{-1}$ for *C. pubescens* plot and 187.42 ± 0.01 $mg100g^{-1}$ to 229.10 ± 0.02 $mg100g^{-1}$ for the *C. mucunoides* plot. The same was applicable for the nitrite level in the soil. Data analysis revealed that there was no significant differences ($p>0.05$) at both the *C. mucunoides* and *C. pubescens* plots nitrogenous salts levels in CME-simulated soil when compared with the control soil. Table 4 presents the nitrogenous salts levels in cassava mill effluent (CME) simulated soil. Different superscripts represent non-significant ($p>0.05$) student-test mean comparisons between nitrogenous salts levels in cassava mill effluent (CME) simulated soil against the control soil.

3.1.5 Metal loads of Cassava Mill Effluent (CME)-simulated soil

The metals considered in the analysis were iron, zinc, cobalt, nickel, lead and manganese. The iron (Fe) loads in CME simulated soil decreased with increase in the percentage of CME pollution at the *C. pubescens* plots while that at the *C. mucunoides* increased with increase in the concentration of CME pollution. Zinc (Zn) levels in the CME simulated soil decreased with increase in the percentage of CME pollution at the two leguminous plant plots.

There was a steady rise in the concentration of lead (Pb) at the *C. pubescens* plot from the control to 50% CME-polluted soil plots but dropped at the 100% polluted soil. At the *C. mucunoides* plot, lead (Pb) levels decreased with increase in the percentage of CME pollution. Data analysis revealed that

there was no significant differences ($p>0.05$) in the metal loads of cassava mill effluent (CME)-simulated soil across all the concentration gradients and in both *C. pubescens* and *C. mucunoides* plots. Table 5 presents metal loads of cassava mill effluent (CME)-simulated soil.

3.2 Discussions

The results of the physicochemical parameters monitored during the simulation study period revealed that CME-simulated soils showed a continuous increase in soil pH when compared to values from their respective control soil. There was a continuous increase in the soil electrical conductivity in CME-simulated soils when compared to values obtained from their respective control soil (0%). The result equally showed a continuous decrease in the available phosphorus in CME-simulated soil when compared to values obtained from their respective control soil (0%). The results obtained from this present studies are in variant with that of John et al. [13].

The total nitrogen in CME-simulated soil fluctuated across the concentration gradients. Similar physico-chemical result trend was observed by John et al. [13] during their study on the "fate of nitrogen-fixing bacteria in crude oil contaminated wetland Ultisol". There were significant changes in physico-chemical properties of the effluent simulated soils, this changes agreed with that of the study reported by Brooks et al. [22]; Iyakndue et al. [23] and Agbo et al. [11].

The concentration of lead at *Centrosema pubescens* plot of CME-simulated soils increased with corresponding increase in pollution levels. The concentration of nickel at *Calopogonium mucunoides* plot of the CME-simulated soils increased with corresponding increase in pollution levels. The results showed that the concentration of zinc in CME-simulated soils decreased with increase in pollution levels. The concentration of iron in the *Calopogonium mucunoides* plot of CME-simulated soil increased with corresponding increase in pollution levels. The results also showed that the concentration of manganese at *Calopogonium mucunoides* plots of CME-simulated soils increased with corresponding increase in pollution levels. The increase in the concentration of heavy metals at the simulated plots agrees with the report of Qishlaqi and Moore [24]. They suggested that the increase in heavy metal could be as a result of the use of wastewater for irrigation and probably due to extensive use of organic fertilizers and/or solid manure.

Brooks et al. [22] reported a decrease in the concentration of manganese with increase in the concentration of pollution levels that differed from that of this present study. Iyakndue et al. [23] on the other hand reported an increase in the concentration of manganese with corresponding increase in pollution levels, their results agreed with that of the present study. John et al. [13] reported that the lowest concentrations of metals were detected in unpolluted soils (control) which did not agree with the results in the present study. This was due to the fact that they studied the effect of hydrocarbon on soil microbiota while this present study looked at the effect of cassava mill effluent on the soil microbiota. Akpan et al. [25] reported that the pH of soils around the point of effluent discharge indicated a general high tendency for high availability of metals. They further reported that this could increase the risk of heavy metals uptake by crops around the effluent dumpsites.

The results of the nitrogenous salt levels in the effluents simulated soils revealed that the concentration of nitrate-nitrogen, ammonium-nitrogen and nitrite-nitrogen increased with corresponding increase in pollution levels during the simulation study period. John et al. [13] reported a decrease in the concentration of nitrate-nitrogen in their simulated soils; this may be as a result of the different pollutant used in their own study as against that used in this present study. John et al. [13] also reported that their control soil sample contained a very high amount of the ammonium-nitrogen and nitrate-nitrogen while nitrite-nitrogen was not detected in their simulated and control soil. This was not the case in this present study, because the concentrations of all the nitrogenous salts studied increased with corresponding increase in pollution levels from the 0%-100% concentration. Akpan et al. [25] reported that exposing nitrogen-fixing bacteria to effluents could affect nitrogen-fixing capabilities within the roots of legumes. This may result in reduced plant vigor and productivity, as well as significant crop loss.

Table 1. Cultural characteristics and identification of fungal isolates from CME-Simulated Soil

Sample code		Cultural characteristics of isolate	Microscopic features of isolates	Possible Organism
CM(CME) 0-100%	1	White to creamy coloured smooth soft and had bacteria-like appearance	Oval and elongated cell with buds attached or protruding from the mother cell and in chains	*Saccharomyces* sp
	2	Fast growing colony, fluffy white in appearance	Aseptate broad hyphae, large spherical head produced by the conidiosphore	*Mucor indicus*
CP(CME)	1	Rapid growing colony, woolly to cotton-like in appearance	Conidiosphore occurred in sickle cell shape and are multicelled	*Fusarium* sp
0-100%	2	Rapid growing colony that was wolly and became empacted with greem patches	Vertically arranged conidiosphore, flask-shaped phialidesand clustering conidia	*Gliocladium* sp

Key: CM – Calopogonium mucunoides, CP – Centrosemapubescens, CME – Cassava mill effluent, sp –Species

Table 2. Biochemical characteristics of bacterial Isolates from CME-simulated soil

Sample code	Isolate No	Cultural characteristics of bacterial isolates	Cell morphology	Gram Rxn	Motility	Indole	Ornithine	Methyl Red	Vogues Proskauer	Citrate	Catalase	Oxidase	Coagulase	Urease	Lactose	Sucrose	Glucose	Gas Production	H_2S	Probable organism
CP(CME) 0-100%	1	Circular, convex smooth and purple colony	Rod	-	+	-	-	+	-	+	+	+	-	+	+	+	+	-	-	*Chromobacterium* sp
	2	Raised, translucent grey Colony	Short straight rod	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	*Corynebacterium* sp
CM(CME)	1	Large, irregular, flat, dry Colony	Bacilli rod	+	+	-	+	-	+	+	+	+	-	-	-	-	+	-	+	*Bacillus* sp
0-100%	2	Red colony, mucoid and spreading	Rod in pairs	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	-	*Acinetobacter* sp
	3	Convex, circular, mucoid smooth colony	Short rod	-	+	+	+	+	-	-	+	-	-	-	+	+	+	+	-	*Escherichia coli*

Key: CP – Calopogonium mucunoides, CM – Centrosemapubescens, CME – Cassava mill effluenty, sp – Specie, + - Positive, - - Negative

Table 3. Physicochemical properties of Cassava Mill Effluent (CME)-simulated soil

	Control		10%		20%		30%		40%		50%		100%	
Parameters (mg/kg)	**CP**	**CM**	**CP**	**CM**	**CP**	**CM**	**CP**	**CM**	**CP**	**CM**	**CP**	**CM**	**CP**	**CM**
Sand (%)	88.00±0.25	87.11±0.60	88.29±0.03^{ab}	87.35±0.08^{ab}	88.51±0.02^{ab}	87.50±0.04^{ab}	89.33±0.03^{aa}	89.43±0.03^{ab}	90.21±0.15^{aa}	89.54±0.13^{ab}	90.21±0.04^{aa}	90.08±0.11^{ab}	90.15±0.04^{aa}	88.90±0.31^{ab}
Clay (%)	7.92±0.11	9.23±0.13	8.03±0.10	9.28±0.09	8.11±0.15	9.47±0.06	8.00±0.10	8.63±0.16	7.28±0.10	8.05±0.15	6.98±0.04	7.08±0.11	7.16±0.23	6.97±0.05
Silt (%)	4.08±0.37	3.67±0.47	3.68±0.13	3.38±0.18	3.39±0.17	3.03±0.10	2.67±0.17	1.95±0.13	2.52±0.25	2.42±0.28	2.82±0.00	2.85±0.21	2.69±0.18	4.14±0.26
TC	LS	LS	LS	LS	LS	LS	LS	LS	S	S	S	S	S	S
pH	6.38±0.04	7.05±0.07	6.58±0.04	7.18±0.04	6.68±0.04	7.25±0.07	6.78±0.04	7.05±0.07	6.75±0.07	6.88±0.04	6.65±0.07	6.38±0.04	7.05±0.07	6.73±0.04
EC (ds/m)	0.18±0.01	0.49±0.01	0.18±0.01	0.45±0.01	0.17±0.01	0.44±0.01	0.17±0.01	0.36±0.01	0.27±0.01	0.27±0.01	0.32±0.01	0.15±0.01	0.67±0.01	0.44±0.01
OM (%)	0.18±0.01	0.49±0.01	0.18±0.01	0.45±0.01	0.17±0.01	0.44±0.01	0.17±0.01	0.36±0.01	0.27±0.01	0.27±0.01	0.32±0.01	0.15±0.01	0.67±0.01	0.44±0.01
TN (%)	0.09±0.01	0.12±0.01	0.15±0.01	0.11±0.01	0.28±0.01	0.12±0.01	0.58±0.01	0.10±0.01	0.84±0.01	0.10±0.01	1.13±0.01	0.12±0.01	0.07±0.01	0.13±0.01
AP (mg/kg)	22.05±0.07	78.71±0.06	65.61±0.08	23.18±0.04	53.56±0.06	24.05±0.10	24.98±0.04	41.19±0.04	23.89±0.02	31.34±0.54	24.08±0.11	25.98±0.04	19.39±0.08	22.72±0.05
Ca(Cmol/kg)	3.23±0.04	4.43±0.04	4.81±0.08	3.71±0.01	6.50±0.02	5.53±0.04	8.13±0.11	7.03±0.04	11.43±0.01	10.92±0.07	13.55±0.04	14.38±0.04	13.98±0.04	13.18±0.04
Mg(Cmol/kg)	1.07±0.01	1.52±0.02	1.14±0.02	1.81±0.01	1.74±0.01	1.94±0.02	2.34±0.01	2.44±0.02	3.44±0.02	3.73±0.04	4.41±0.01	4.33±0.04	4.10±0.03	4.23±0.04
Na(Cmol/kg)	0.03±0.00	0.04±0.00	0.08±0.01	0.09±0.01	0.10±0.01	0.08±0.01	0.10±0.01	0.09±0.01	0.10±0.01	0.08±0.01	0.09±0.01	0.07±0.01	0.10±0.01	0.08±0.01
K (Cmol/kg)	0.09±0.01	0.07±0.01	0.11±0.01	0.16±0.01	0.14±0.01	0.19±0.01	0.18±0.01	0.25±0.01	0.24±0.02	0.35±0.01	0.27±0.01	0.41±0.02	0.66±0.02	0.47±0.02
EA	0.97±0.01	0.83±0.04	1.03±0.01	0.91±0.01	1.07±0.01	0.97±0.01	1.11±0.01	1.03±0.01	1.22±0.02	1.16±0.02	1.27±0.01	1.29±0.01	1.12±0.01	2.55±0.01
ECEC	5.33±0.01	6.80±0.02	8.89±0.03	10.12±0.15	10.13±0.04	12.31±0.02	12.47±0.02	13.61±0.03	15.33±0.04	18.34±0.01	19.61±0.03	20.43±0.03	19.94±0.03	20.49±0.03
BS (%)	82.01±0.01	88.23±0.04	84.90±0.01	89.63±0.02	86.31±0.01	90.32±0.02	87.74±0.02	91.01±0.01	90.61±0.02	92.35±0.04	93.46±0.03	93.73±0.04	94.37±0.04	87.49±0.03
THC (mg/kg)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Different superscripts represent non-significant ($p > 0.05$) student-test mean comparisms while similar superscripts represent significant ($p < 0.05$) student-test mean between physico-chemical properties of cassava mill effluent (CME) simulated soil against control soil.

Key: CP – Centrosema pubescence, EC - Electrical conductivity, Ca – Calcium, EA – Exchangeable acidity, ND – Not detected, CM – Calopogonium mucunoides, OM – Organic matter, Mg – Magnesium, ECEC – Effective Cation Exchangeable Capacity, LS – Loamy soil, mg/kg – Milligram per kilogram, TN – Total nitrogen, Na – Sodium, BS – Base saturation, TC - Textural class, AP – Available phosphorus, K – Potassium, THC – Total hydrocarbon

Table 4. Nitrogenous salt levels in Cassava Mill Effluent (CME)-simulated soil

Level of pollution (%)	Legume	Nitrate [NO_3] mg/100g	Ammonium [NH_4^+] mg/100g	Nitrite [NO_2] mg/100g
0	*Centrosema pubescens*	88.53±0.02	166.62±0.03	2.90±0.04
	Calopogonium mucunoides	104.47±0.02	187.42±0.01	3.51±0.03
10	*Centrosema pubescens*	98.40±0.03^{ab}	171.80±0.01^{ab}	3.15±0.01^{ab}
	Calopogonium mucunoides	112.41±0.03^{ab}	192.63±0.02^{ab}	3.73±0.02^{ab}
20	*Centrosema pubescens*	103.29±0.02^{ab}	174.40±0.03^{ab}	3.30±0.02^{ab}
	Calopogonium mucunoides	116.39±0.04^{ab}	192.66±0.02^{ab}	3.83±0.01^{ab}
30	*Centrosema pubescens*	108.24±0.02^{ab}	177.04±0.03^{ab}	3.43±0.03^{ab}
	Calopogonium mucunoides	120.32±0.01^{ab}	197.86±0.03^{ab}	3.98±0.08^{ab}
40	*Centrosema pubescens*	118.08±0.02^{ab}	182.25±0.03^{ab}	3.70±0.03^{ab}
	Calopogonium mucunoides	128.30±0.04^{ab}	203.07±0.02^{ab}	4.16±0.03^{ab}
50	*Centrosema pubescens*	127.91±0.03^{ab}	187.45±0.03^{ab}	3.99±0.06^{ab}
	Calopogonium mucunoides	136.23±0.02^{ab}	208.27±0.03^{ab}	4.37±0.02^{ab}
100	*Centrosema pubescens*	173.81±0.03^{ab}	249.96±0.08^{ab}	5.45±0.06^{ab}
	Calopogonium mucunoides	147.60±0.05^{ab}	229.10±0.02^{ab}	4.70±0.05^{ab}

Different superscripts represent non-significant (p>0.05) student-test mean comparisms between nitrogenous salts levels in cassava mill effluent (CME) simulated soil against the control soil

Table 5. Metal loads of Cassava Mill Effluent (CME)-simulated soil

Parameters	Control		10%		20%		30%		40%		50%		100%	
(mg/kg)	CP	CM	CP	CM	CP	CM	CP	CM	CP	CM	CP	CM	CP	CM
Iron (Fe)	421.43±0.04	455.13±0.02	377.18±0.02^{ab}	505.85±0.06^{ab}	355.06±0.03^{ab}	531.17±0.04^{ab}	332.95±0.05^{ab}	556.52±0.06^{ab}	278.71±0.06^{ab}	607.18±0.02^{ab}	244.44±0.04^{ab}	657.875±0.05^{ab}	118.06±0.10^{ab}	665.87±0.08^{ab}
Zinc (Zn)	30.68±0.11	30.80±0.06	29.52±0.03	30.24±0.04	28.97±0.02	29.96±0.03	28.43±0.04	29.71±0.02	27.33±0.03	29.13±0.03	26.24±0.02	28.59±0.02	23.12±0.03	5.79±0.02
Cobalt (Co)	4.22±0.03	6.62±0.03	4.39±0.02	6.12±0.03	4.44±0.01	5.82±0.03	4.52±0.02	5.52±0.02	4.69±0.05	5.04±0.02	4.83±0.04	4.42±0.02	6.42±0.02	6.82±0.02
Nickel (Ni)	4.94±0.02	2.71±0.04	4.57±0.03	4.26±0.04	4.38±0.01	5.05±0.03	4.20±0.03	5.84±0.01	4.94±0.01	7.42±0.04	5.71±0.04	8.96±0.02	8.57±0.01	5.66±0.02
Lead (Pb)	10.14±0.02	9.19±0.04	10.56±0.02	8.90±0.04	10.77±0.02	8.78±0.04	11.00±0.05	8.60±0.03	11.40±0.02	8.31±0.02	11.82±0.03	8.07±0.03	4.34±0.01	5.39±0.04
Manganese (Mn)	25.22±0.02	21.42±0.02	24.15±0.02	22.52±0.02	23.61±0.03	23.05±0.01	23.06±0.01	23.62±0.03	22.01±0.04	24.72±0.02	20.92±0.03	25.82±0.02	20.72±0.03	21.94±0.06

Different superscripts represent non-significant (p>0.05) student-test mean comparisms between metal loads of cassava mill effluent (CME) simulated soil against the control soil.
Key: mg/kg – Milligram per kilogram, CP – Centrosema pubescens, CM- Caloponium mucunoides

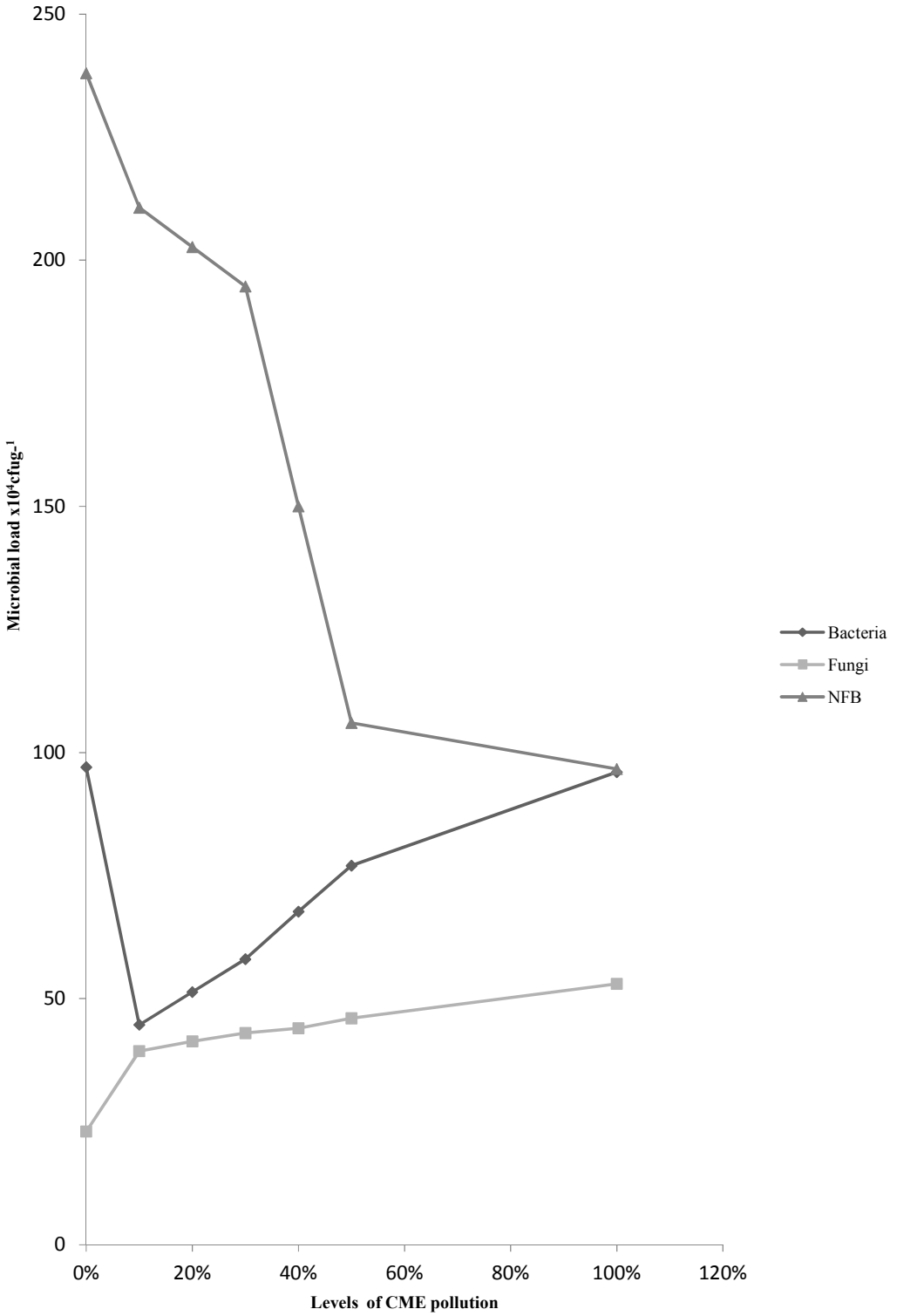

Fig. 4. Effect of graded concentrations of CME on soil microbiota using *C. pubesens* as phytoremediation agent

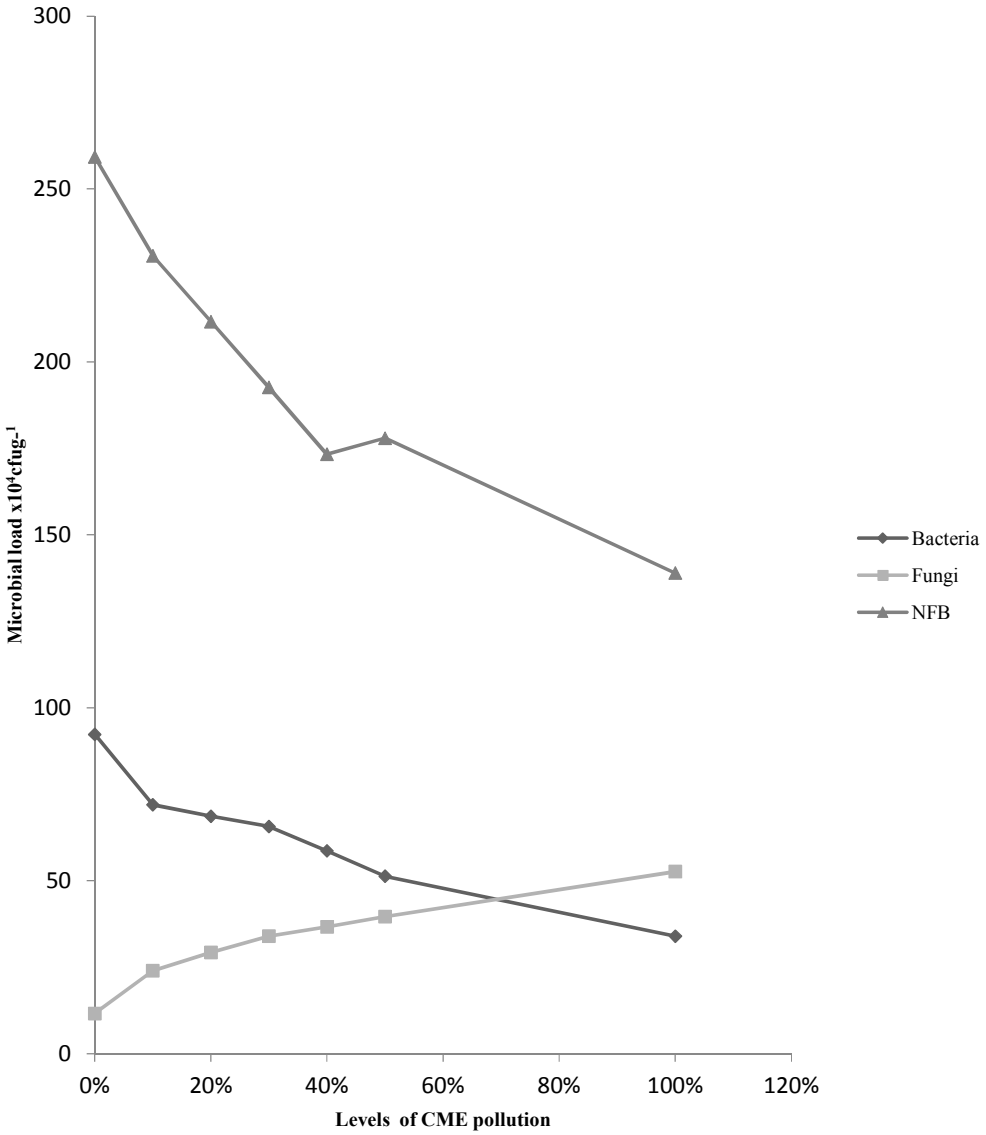

Fig. 5. Effect of graded concentrations of CME on soil microbiota using *C. mucunoides* as phytoremediation agent

The findings from this present study showed that the microbial (bacterial, fungal and nitrogen fixing bacterial) load from the CME-simulated soil increased with a corresponding increase in the concentration of pollutant, this agreed with the findings of Agbo et al. [11]. The increment in microbial population was probably as a result of increased nutrient and mineral content from the cassava mill effluent. This present findings agreed with that of Obire and Nwanbet [26], Eze and Okpokwasili [27] that reported gradual increase in microbial population in polluted soil. This increase in fungi load may be as a result of the acidic nature of the cassava mill effluent, some fungi do better in acidic environment than bacteria that can only survive in a neutral to alkaline pH environment.

The microorganisms identified from the CME-simulated soil sample were similar to those earlier reported by Agbo et al. [11]. *Corynecbacterium* sp. was the predominant organism identified from the CME-simulated soil sample. The effluent discharge distorted the microbial diversity of the ecosystem.

4. CONCLUSION

The results revealed that the physico-chemical parameters of the effluent simulated soil were altered. The findings from this study showed that the pH of soils around the point of effluent discharge indicated a general high tendency for high availability of metals. These metals could be toxic to the microorganisms present at that site of effluent discharge. The findings of this study also revealed that heavy metal contents in the effluent impacted soil increased greatly. The simulation experiment analysis also revealed that soil acidity influences many chemical and biological characteristics of soil including availability of nutrient and toxicity of metals which can also affect microbial community in many ways.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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