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Assessment of Microbial Dynamics in Soil under Peroxide-Aeration Treated Cassava Effluent: Impacts on Soil Health and Potential Plant Productivity

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ABSTRACT

This study assessed the impact of cassava processing effluent (CPE) treated via peroxide aeration on microbial soil dynamics, and its potential impact on plant productivity. Soil microbial counts, and soil respiration were examined under raw wastewater and peroxide-aeration combination treated effluent dilutions (0, 25, 50, 75, and 100%). The initial soil bacterial load in the soil of 3.20 x 10^8 CFU/g soil was increased to 8.90 x 108 CFU/g soil in raw wastewater, while dilutions using treated effluent exhibited modest increases from 6.55 to 6.69 x 10^8 CFU/g soil. The same pattern was observed in fungal populations, which increased from 3.00 to 64.00 x 10^8 CFU/g soil under raw wastewater and 18.6 to 19.00 x 10^8 CFU/g soil under dilution treatments. Soil respiration increased from 10.35 to 13.40 mg CO_2-kg^{-1} day^{-1} under the 50% dilution, indicating enhanced microbial activity. These results showed that reuse of cassava effluent can enhance soil microbial activities central to nutrient cycling and soil fertility. However, the highest microbial population and respiration under raw wastewater point toward the possibility of hazards such as microbial imbalance and toxic compounds like cyanide build-up. The moderate dilution levels, especially 50%, provided a compromise between stimulation of microbial activity and minimization of negative effects, which is aligned with sustainable agricultural management practice. It is concluded that careful dilution management of peroxide-aerated cassava effluent is key to maximizing soil health benefits while minimizing risks.

Keywords: Cassava processing, microbial activity, Peroxide-Aeration, pollution, wastewater

1. INTRODUCTION

The practice of using agro-industrial effluents to supplement agricultural irrigation water is being increasingly recognized as a means of nutrient recycling, water saving and waste disposal (Vivaldi et al., 2022; Muscarella et al., 2024). Cassava (Manihot esculenta Crantz) production causes the production of a significant amount of the effluents containing organic matter, cyanogenic compounds and minerals that may result, if untreated, in environmental pollution (Olaniyan et al., 2025; Kolawole, 2014). Nevertheless, when well-managed, cassava processing effluent (CPE) can serve as soil amendment to enrich it with nutrients and favor its biological functioning, (Onwuka et al., 2025).

Microbial communities in the soil are highly relevant for preservation of soil health and play a significant role in the decomposition of organic matter, nutrient cycling, and growths promotion of plants (Marzouk et al., 2025). Applied treated effluent may modify microbial communities and activities, and in some cases may enhance beneficial while reducing pathogenic microorganisms (Xiao et al., 2024). However, the availability of nutrients and the risk of microbial toxicity from residual compounds in treated CPE have been weakly documented, especially in tropical agro-ecosystems.

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Most previous studies have concentrated predominantly on the physico-chemical impacts of effluent reuse to soil (Abegunrin et al., 2016) but less has been made to study the microbial dynamics in relation to plant productivity response. The importance of the role of treated CPE in the structure and function of soil microbes needs to be determined for sustainable land utilization and food production.

This research aimed to evaluate the effects of treated cassava effluent reuse on soil microbial dynamics, soil health indicators, and plant productivity parameters under controlled conditions.

2. MATERIALS AND METHODS

2.1 Wastewater Treatment Procedure

Cassava processing effluent (CPE) used was collected from a cassava processing factory near the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria in air-tight opaque containers and kept in ice bags. The pH of the wastewater was subsequently adjusted between 10.8 and 11 by adding 0.5 M of NaOH to the effluent. Cyanide oxidation was induced using the addition of hydrogen peroxide (50% concentration) at a dosage of 0.5 g H2O2/g. A retention time of 3 hours was then allowed to enable oxidation and cyanide decontamination and then aeration for 2 hours. The wastewater was then transferred into the aeration chamber where the water was agitated for an hour using the paddle system. Oxidized effluent in the aeration chamber was replaced at a constant rate of 0.25 l/min to avoid overloading the system as recommended by (Omotosho et al., 2018). After aeration, wastewater was fed into a sand filter at a rate of 0.6 L/min. The effluent obtained was then later analyzed for physical, chemical and biological parameters using standard methods

2.2 Soil Treatment

Samples of soil (sandy loam soil) were collected from the top 12 cm layer (as suggested by Moore and Lawrence, 2013) from a research field at the IAR&T, Ibadan. The soil samples were passed through a 5 mm sieve (to remove plant residues and gravels) then placed in sacks and transferred to the to the screen house where sub-samples were obtained for laboratory analysis to determine of its physico-chemical characteristics. The soil was then put into the buckets before being compacted to a bulk density of 1.39 g/cm^3 which was the value obtained during the initial soil analysis, this was to simulate the bulk density of the soil at source.

2.3 Microbial Analysis Experimental Setup and Soil Treatment

The soil microbial analysis experiment was set up in a randomized block design (RBD) in 6 factors or treatments (0, 25, 50, 75 and 100% CPE concentrations) and raw CPE in a screen house as shown in Figure 1. The experiment was set up in four replicates giving a total of 24 experimental units. The control was taken as groundwater obtained from a borehole located around I.A.R.&T, Ibadan. The treatments were applied to each of the 10 kg potted soil samples at the rate of 0.9 L per day for a period of 90 days.

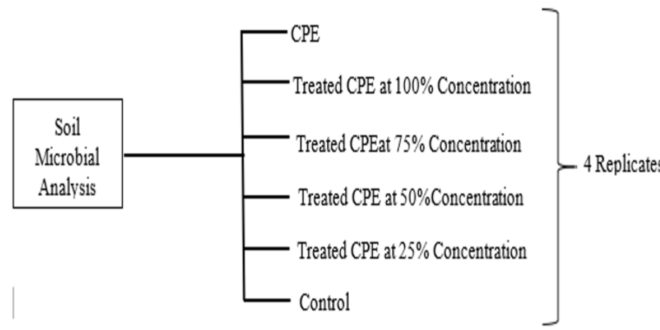

Figure 1: Schematic for soil microbial analysis experimental setup

2.4 Soil Respiration Measurement

A quantitative measure of microbial respiration rate in the soil i.e. Soil respiration, was measured using the alkali absorption technique by following standard protocols (Bauer 1991; Omokaro et al., 2024). Soil samples were collected at the start and completion of the treatment (12 weeks) from all treatment pots receiving effluents.

Soil sampling was done using soil corers (5 cm diameter × 10 cm depth). The collected soil cores were directly placed in airtight incubation jars with 20 mL of 0.5 M NaOH in a small vial suspended over the soil surface to trap CO_2 during incubation. Jars were incubated at 25 ± 2°C for 24 hours. After incubation, the remaining NaOH was titrated with 0.5 M HCl after adding 1 mL of $BaCl_2$ solution for precipitation of carbonate, as described by Zibilske (1994). Respiration in soil was determined as milligrams of CO_2-C per kilogram of dry soil per day using equation 1.

$$Soil\ respiration\ (mg\ CO_2 - C\ kg^{-1} day^{-1}) = \frac{(V_b - V_8) \times N\ \times 22 \times 1000}{Volume\ plated\ (mL)} \quad (1)$$

where;
V_b =volume of HCl used for blank (mL)
V_8 = volume of HCl used for sample (mL)
N = Dry weight of soil sample (kg)
T = Incubation time (days)

Water content was maintained at approximately 60% water-holding capacity throughout incubation to keep microbial activity from moisture limitation (Ulrik et al., 2000). Measurement was taken in triplicate per treatment for statistical accuracy and average determined.

2.5 Fungal Count Measurement

Fungal population in soil samples was enumerated using pour plate method on Potato Dextrose Agar (PDA), in line with Baek and Kenerley (1998) and Bhowmik (2018) with slight modifications.

2.5.1 Sample Preparation

All treatment pots were sampled at 0–15 cm depth using a sterile soil auger. The collected samples were put into sterile polyethylene bags and brought to the laboratory for instantaneous analysis.

2.5.2 Serial Dilution and Plating

Ten grams of each fresh soil sample was shaken in 90 mL sterile distilled water in a 250 mL conical flask for 30 minutes at 150 rpm to dislodge fungal spores and hyphal fragments. The dilutions were carried out to 10^{-4}.From every dilution, 1 mL aliquots were plated in triplicates on streptomycin containing 0.05 g/L sterile PDA to suppress bacterial growth. The plates were incubated at 28 ± 2°C for 2–5 days.

2.5.3 Counting and Identification

Characteristic fungal morphology colonies appearing after incubation were counted as colony-forming units (CFU) per gram dry soil. The fungal colonies were counted using colony counter, and results were expressed as shown in Equation 2.

Representative fungal colonies were sub-cultured for further morphological identification using lactophenol cotton blue staining under a light microscope, following protocols by Mahalingam et al. (2022).

$$Fungal\ count\ (CFU\ g^{-1}\ soil) = \frac{Number\ of\ colonies\ \times Dilution\ factor}{Volume\ plated\ (mL)} \quad (2)$$

2.5.4 Quality Control

All glassware and media were sterilized at 121°C for 15 minutes in accordance with recommendations by Shareef (2019). Control plates (media only) were incubated alongside sample plates to check for contamination.

2.6 Bacterial Count Measurement

Bacterial population in soil samples was determined using the serial dilution and pour plate method on Nutrient Agar (NA), following standard protocols as described by Nweke and Onuoha, (2024) and Odoacbuchi et al. (2020).

2.6.1 Sample Collection and Preparation

Soil samples (0–15 cm depth) were collected from each treatment plot using a sterile auger and stored in sterile polyethylene bags. In the laboratory, 10 g of each soil sample was weighed into 90 mL of sterile physiological saline (0.85% NaCl) in a 250 mL Erlenmeyer flask. In accordance with Wu et al., (2025) the mixture was shaken for 30 minutes at 150 rpm to dislodge soil bacteria.

2.6.2 Serial Dilution and Plating

Serial dilutions were prepared up to 10^{-6}. From appropriate dilutions, 1 mL aliquots were transferred into sterile Petri dishes in triplicate. Sterile, molten Nutrient Agar cooled to approximately 45°C was poured into each plate, and the contents were gently swirled to mix. Plates were incubated at 28 ± 2°C for 24–48 hours.

2.6.3 Counting and Expression of Results

Visible bacterial colonies were counted using a digital colony counter, and counts were expressed as colony-forming units (CFU) per gram of dry soil, using Equation 3.

$$Bacteria\ count\ (CFU\ g^{-1}\ soil) = \frac{Number\ of\ colonies \times Dilution\ factor}{Volume\ plated\ (mL)} \quad (3)$$

2.6.4 Quality Assurance

All media and glassware were sterilized at 121°C for 15 minutes. Control plates were included to monitor contamination. All counts were conducted in triplicate for statistical reliability. Soil moisture content was determined and adjusted to dry weight basis before calculation, in accordance with the method of Black (1965).

3. RESULTS AND DISCUSSION

3.1 Microbial Activities

Cassava processing effluent is known to exert strong influences on soil microbial communities due to its acidity, organic richness, suspended solids, and microbial load. The observed differences between raw and peroxide-aeration treated effluent in this study as shown in Table 1 have important implications for microbial dynamics in soil, soil health, and potential plant productivity. The raw effluent exhibited a low pH (4.5), which can adversely affect soil microbial diversity by suppressing beneficial bacteria and favoring acid-tolerant or pathogenic microorganisms. Research has shown that acidic conditions are known to inhibit enzymatic activities, reduce nutrient mineralization, and negatively impact soil fertility (Du et al., 2024). In contrast, the treated effluent had a near-neutral pH (7.1), which is more favorable for a broad range of soil microorganisms, including nitrifying bacteria, decomposers, and plant growth-promoting rhizobacteria. This pH adjustment suggests that peroxide-aeration treatment may enhance microbial balance and functionality when such effluent is applied to soil.

Table 1: Comparison of Results from Treatment Methods with NESREA Effluent Discharge Standard to Water Bodies

Parameter	NESRA Standard	Raw Wastewater	Treated Effluent (Pilot Scale)
pH	7	4.5	7.1
Colour^{m}	7	3.5	2.6
Phenols	0.2	0.11	ND
Total Dissolved Solids	2,000	615	610.44
Total Suspended Solids	30	2,330	1,993.24
BOD	50	108	60
Total Coliform Countn (CFU/g of soil)	400	3.5 x 10^{4}	3.77 x 10^{2}
Organisms Identified	-	Aeromonas sp: Enterobacter sp	Enterobacter sp

*All units mg/l unless otherwise stated; ^{m}Lovibond Units; ^{n}Most Probable Number (MPN)/100 ml; **ND:** Not detected

The reduction in colour following treatment indicates decreased concentrations of complex organic and colloidal materials. In soil systems, excessive coloured organic compounds from untreated effluent can clog soil pores, reduce aeration, and limit microbial respiration (Bodor et al., 2024). The improved clarity of the treated effluent implies reduced physical interference with soil structure, thereby supporting aerobic microbial processes that are critical for nutrient cycling and organic matter decomposition.

Phenolic compounds, although present at low concentrations in the raw effluent, were completely removed after treatment. Phenols are known to exert antimicrobial effects, often inhibiting beneficial soil microorganisms even at trace levels (Dembińska et al., 2025). Their absence in the treated effluent suggests reduced toxicity to soil microbial communities, enabling more stable microbial populations and improved biological activity that support soil health and plant growth.

Total Dissolved Solids (TDS) levels remained low and relatively unchanged after treatment, indicating minimal risk of salinity stress to soil microorganisms and plant roots. This is advantageous, as research has shown that elevated TDS can disrupt microbial osmotic balance and impair nutrient uptake by plants (Weber-Scannell and Duffy, 2007; Ashie et al., 2024). However, Total Suspended Solids (TSS) remained high despite treatment. High TSS can influence soil microbial dynamics by increasing organic particle deposition, potentially stimulating heterotrophic microbial growth but also risking anaerobic microzones that may favor undesirable microbial pathways if not well managed.

The reduction in Biochemical Oxygen Demand (BOD) from 108 to 60 mg/L reflects partial stabilization of organic matter. Studies have shown that in soil, lower BOD inputs reduce excessive microbial oxygen demand, preventing localized oxygen depletion that could suppress aerobic microbial populations essential for nutrient mineralization (Seo et al.,2007; Merchant et al., 2025). Furthermore, the dramatic reduction in total coliform counts following treatment indicates improved hygienic quality of the effluent, reducing the likelihood of introducing pathogenic microorganisms into soil ecosystems.

3.2 Soil Bacterial Count

The results of soil bacteria count as shown in Table 2 demonstrate clear shifts in soil microbial dynamics following the application of cassava processing effluent (CPE) and its treated derivatives.

Table 2: Bacterial Count of the soil at the start and finish of the treatment (10^8 CFU/g soil)

Parameter	R W	Control	25%	50%	75%	100%
Initial	3.20	3.20	3.20	3.20	3.20	3.20
Harvest	9.81	6.55	6.56	6.65	6.67	6.69

Baseline soil bacterial count was relatively low (3.20 x 10^8 CFU/g soil), reflecting typical microbial loads prior to external inputs. Following treatment, the application of raw CPE resulted in the highest bacterial population (9.81 x 108CFU/g soil), a significant increase compared with the control (6.55 x 10^8 CFU/g soil). This pronounced rise is consistent with earlier findings that cassava effluents, being rich in soluble carbohydrates and organic matter, stimulate rapid microbial proliferation (Odoacbuchi et al., 2020; Nweke and Onuoha, 2024). However, such excessive microbial activity may also signal ecological imbalance, as uncontrolled bacterial dominance has been associated with soil acidification, oxygen depletion, and the suppression of beneficial microbial groups (Atasoy et al., 2024).

By contrast, soils amended with treated CPE at varying concentrations (25–100%) recorded bacterial counts ranging from 6.56 to 6.69 x 10^8 CFU/g soil, values comparable to the control (6.55 x 10^8 CFU/g soil). This outcome indicates that effluent treatment effectively moderated microbial stimulation, preventing the excessive proliferation observed with raw CPE. Similar trends were reported by Ajao et al. (2025), who observed that effluent bioremediation or dilution significantly reduced microbial distortions while maintaining soil fertility potential. The narrow range across concentrations further suggests that treatment not only detoxifies effluent but also stabilizes nutrient release, ensuring that bacterial activity remains within ecologically sustainable thresholds.

These findings have important implications for soil health and crop productivity. While raw CPE provides abundant nutrients that can enhance short-term microbial growth, its unregulated application may compromise soil microbial balance and long-term fertility (Etinosa and Ozede 2015). Conversely, treated CPE supports microbial populations at levels comparable to unamended control soils, a condition that favours soil nutrient cycling without inducing adverse ecological effects. This aligns with studies indicating that moderate microbial abundance, rather than uncontrolled proliferation, optimally supports soil enzymatic activity and plant nutrient uptake (Cheng et al., 2024).

3.3 Soil Fungal Count

The fungal population dynamics observed in this study provide important insights into the ecological effects of cassava processing effluent (CPE) reuse on soil quality. At baseline, soil fungal count was low (3.00 x 10^8 CFU/g soil), reflecting the typical microbial equilibrium of untreated soil.

Following effluent applications, soils amended with raw CPE exhibited a sharp increase in fungal abundance (64.00 x 10^8 CFU/g soil), significantly higher than both the control (20.00 x 10^8 CFU/g soil) and treated effluent

Table 3: Fungal Count of the soil at the start and finish of the treatment (10^8 CFU/g soil)

Parameter	R W	0%	25%	50%	75%	100%
Initial	3	3	3	3	3	3
Harvest	64	20	19	19	18.74	18.6

soils (18.60–19.00 x 10^8 CFU/g soil). This surge may be attributed to the high organic load of untreated cassava wastewater, which supplies fermentable substrates that favour rapid fungal proliferation (Nweke and Onuoha 2024). However, such elevated fungal counts are not necessarily beneficial; excessive fungal colonization has been associated with reduced microbial diversity and may encourage the growth of opportunistic or phytopathogenic strains (Mahalingam et al. 2022).

In contrast, treated CPE soils maintained fungal populations (18.60–19.00 x 10^8 CFU/g soil) close to the control (20.00 x 10^8 CFU/g soil) with the concentrations at 25 and 50% being closest (19.00 x 10^8 CFU/g soil). This indicates that effluent treatment not only reduced toxic cyanogenic compounds, which otherwise inhibit certain microbial groups (Berkinbayeva et al., 2025), but also stabilized fungal abundance at levels compatible with balanced soil ecology. Similar results were reported by Velmurugan and Pandian (2024), who found that effluent remediation through biological or physicochemical processes normalized fungal and bacterial populations, preserving ecological functions such as decomposition and nutrient cycling.

The reduction in fungal proliferation under treated CPE compared to raw effluent suggests a restorative effect on soil health. While fungi are key decomposers that drive organic matter breakdown, extremely high populations, such as those observed under raw CPE application, may disrupt plant–soil–microbe interactions and compromise crop productivity (Nweke and Onuoha 2024). By contrast, the near-control levels in treated CPE soils suggest that fungal roles in nutrient cycling can be maintained without risking soil pathogenicity. Importantly, maintaining fungal populations within moderate ranges supports optimal soil enzymatic activities and enhances nutrient availability for crops, thereby improving long-term plant productivity (Frac et al., 2018).

3.4 *Soil Respiration*

Results of readings for soil respiration collected at the start and end of the treatment as shown in Table 4 revealed that the highest respiration rate was observed in soils amended with 50% treated CPE (13.40 CO_2-C kg^{-1} day^{-1}), suggesting that partial effluent application optimally balanced nutrient availability with microbial metabolism. This is consistent with previous findings that moderate organic waste amendments enhance soil microbial respiration by supplying readily degradable carbon substrates while avoiding the inhibitory effects of toxic compounds (Stefanovska et al., 2025; Omokaro et al., 2024).

Table 4: Soil respiration of the soil at the start and finish of the treatment (10^8 CO_2-C kg^{-1} day^{-1})

Parameter	R W	0%	25%	50%	75%	100%
Initial	10.35	10.35	10.35	10.35	10.35	10.35
Harvest	12.79	12.75	12.98	13.4	12.2	12.75

The highest respiration rate was observed in soils amended with 50% treated CPE (13.40 CO_2-C kg^{-1} day^{-1}), suggesting that partial effluent application optimally balanced nutrient availability with microbial metabolism. This is consistent with previous findings that moderate organic waste amendments enhance soil microbial respiration by supplying readily degradable carbon substrates while avoiding the inhibitory effects of toxic compounds (Stefanovska et al., 2025; Omokaro et al., 2024). By contrast, the slightly lower respiration rates observed under 75% treated CPE (12.20 CO_2-C kg^{-1} day^{-1}) may reflect microbial stress at higher effluent loads, possibly due to residual cyanogenic glycosides or osmotic imbalances, which have been reported in cassava effluent–impacted soils (Ndubbuisi and Chidiebere 2018; Okoye et al., 2023).

Interestingly, raw CPE (12.79 CO_2-C kg^{-1} day^{-1}) and the control (12.75 CO_2-C kg^{-1} day^{-1}) showed nearly identical respiration values. This may indicate that while raw effluent provides abundant organic substrates to fuel respiration, the presence of inhibitory compounds such as hydrogen cyanide offsets this potential, resulting in microbial activity similar to untreated soil. This observation aligns with earlier studies demonstrating that raw cassava effluent can simultaneously stimulate microbial growth while suppressing specific enzymatic activities, depending on the balance between nutrient enrichment and toxicity (Igbinosa, 2015).

From an ecological perspective, the elevated soil respiration in treated effluent-amended soils indicates enhanced microbial-driven carbon cycling, which is beneficial for nutrient mineralization and plant productivity (Curiel-Yuste, 2007). However, excessively high or suppressed respiration may signal microbial imbalance, with potential negative consequences for soil fertility. The near-control respiration values observed in treated CPE soils, particularly at 25–100% concentrations, suggest that effluent treatment effectively stabilized microbial activity, allowing soils to sustain functional respiration comparable to healthy controls.

In all, these results demonstrate that while raw CPE introduces ecological risks due to the interplay of nutrient overload and toxicity, treated cassava effluent supports balanced microbial respiration that can maintain soil health and potentially enhance crop productivity when applied at moderate concentrations.

4. CONCLUSION

The reuse of treated cassava processing effluent significantly influenced soil microbial dynamics, as indicated by increases in bacterial and fungal counts, as well as enhanced soil respiration rates. Moderate effluent dilutions, particularly at 50%, provided optimal conditions that stimulated microbial activity while minimizing potential negative effects associated with raw or undiluted effluent, such as toxic compound accumulation. These findings highlight the potential of using appropriately diluted cassava effluent as a soil amendment to support soil health and plant productivity. However, long-term application requires careful management to prevent adverse environmental impacts. Further research is recommended to explore crop yield responses, microbial community structure, and soil biochemical properties under sustained cassava effluent reuse in agricultural systems.

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