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Microbiological and Physicochemical Assessment of Cassava Mill Effluent (CME) Polluted Soil in Umuagwo Ohaji-Egbema, Imo State, Nigeria

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Abstract

The microbiological and physicochemical quality of cassava mill effluent (CME) polluted soil was studied. A total of six soil samples (four CME polluted and two unpolluted) were collected, processed, and analyzed for total heterotrophic bacterial count (THBC), total fungal counts (TFC), total *Nitrosomonas* count (TNsC), total *Nitrobacter* counts (TNbC), total phosphate solubilizing bacterial count (TPSBC), and total lipolytic bacterial count (TLBC). Physicochemical analyses were also carried out to determine the soil's pH, total organic carbon, moisture content, nitrate, phosphate, and cyanide concentrations. The THBC had a range of $1.3x10^6$±2.36 cfu/g – $8.6x10^6$±2.12 cfu/g, TFC had a range of $1.87x10^3$±1.23 cfu/g – $4.0x10^3$±1.62 cfu/g, TNsC had a range of $8.2x10^4$±1.22 cfu/g - $3.2x10^6$ ±3.86 cfu/g, TNbC had a range of $1.95x10^4$ ±1.28 cfu/g – $1.0x10^6$ ±1.25 cfu/g, TPSBC had a range of $2.3x10^5$ ±1.16 cfu/g – $4.0x10^5$±0.8 cfu/g, while TLBC had a range of $0.85x10^3$±1.43 cfu/g – $2.2x10^4$±1.34 cfu/g. The CME-polluted soil recorded a higher microbial population compared to the unpolluted soil samples. The physicochemical analysis revealed a more acidic pH of 6.28±0.01 – 6.57±0.06 for the polluted soil compared to the unpolluted, which had a slightly alkaline pH. This study has, however, revealed that the microbial and physicochemical quality of soils can be affected by CME pollution.

Keywords: Cassava, Effluent, Mill, Microbiological, Pollution, Soil

Introduction

Cassava (*Manihot esculenta*) is a perennial crop of the Neotropical origin with its dominance in the tropics (Oboh, 2007) [24]. The only part of this crop consumed is the root, tuber, and sometimes the leaves. Cassava also served as a staple food for most Africans and is sometimes described as the bread of the tropics due to its wide usage (Adams *et al*., 2009) [1]. Cassava root is long with a firm, homogenous whitish flesh and brown on the outside. The flesh can be chalk-white or yellowish, while the peels covering the flesh are brown or earthy. Cassava roots are rich in starch and contain significant calcium.

Cassava effluent is a pale yellow or milky-colored turbid liquid with an earthy odor. It contains large floating or suspended solids and very small solids in colloidal suspension (Oboh, 2007) [24]. Cassava effluent may contain oil and grease from the lubricated parts of the grinding machine. Cassava processing is generally considered to contribute greatly to environmental pollution and aesthetic deterioration. Its fermentation and processing give rise to other products like garri, fufu, tapioca, flasks, and so on. Cassava effluent contains carbohydrates, largely known to encourage bacterial growth due to its fermentation potential. Cassava contains carbohydrates (about 65-70%), protein (about 1-3%), and calcium (about 0.3-1%), while fiber, other minerals, fat and cyanogenic glycoside make up the remaining (Nwabueze and Odunsi, 2007) [21]. Its high carbohydrate content makes it a major food base, especially for the low-income earners in tropical African countries like Nigeria (Desse and Taye, 2006) [10]. Cassava is particularly a family food crop in the tropics and has been regarded as a poverty-alleviating crop through its food, nutrition, and income provision. The elimination of cyanide from wet cassava mash is essential in the production of food-grade cassava products like starch, garri, flour, and fufu.

Environmental pollution occurs when waste water from cassava processing facilities is allowed to spread slowly into the soil or flow into streams or when cassava roots are fermented in surface water like ponds and streams, a common practice in some communities where potable water is in short supply (Oboh, 2007) [24]. This practice also harms human, animal, and plant life,

both aquatic and terrestrial. The effluent from this industry could be a real problem because of its high levels of cyanide and biological oxygen demand content. The frequent release of cassava effluent has caused the adverse effect of cassava waste on the environment and bio-diversities (Adewoye, 2005 [2]; Kolawole, 2014). Besides affecting bacterial diversity, it also causes environmental fouling or aesthetic deterioration of the impacted environment (FAO, 2004) [15].

The microbial quality of soil is greatly influenced by the quality of effluent that is often discharged onto the soil. Industrial effluent, with its attendant heavy metals, organic matter, and some foreign chemicals, can cause pollution in the soil (Igbinosa and Igiehon, 2015) [17]. In Nigeria, cassava mill effluent, often discharged onto the soil, is rich in organic matter. This practice is prevalent because the soil, which is depleted by leaching and constant cropping, produces poor yield or no yield at all. In most cases, the soil is left uncultivated for about four years to regain fertility. The increase in small, medium, or large-scale cassava industries or cottage-industry clusters exacerbates the problem (Osakwe, 2012) [26]. The impact of cassava mill effluent on soil quality is a cause for concern because the microbiological quality of soil is important to preserve soil productivity.

Ohaji Egbema, located in Imo State, Nigeria, is heavily impacted by cassava processing activities with several small to medium-scale cassava processing plants. The discharge of CME into the surrounding soil has raised concerns about the region's microbiological and overall soil quality. Understanding the extent of contamination and its potential impacts is crucial for developing appropriate management strategies and ensuring the sustainability of agricultural practices (Agwaranze *et al.*,2018) [4].

Several studies have explored the microbiological quality of CME-impacted soil in different regions. The researchers found that CME application significantly changed soil pH, organic matter content, and microbial composition. These alterations can have positive and negative consequences on soil fertility and overall ecosystem functioning. It is clear that the microbiological quality of CME-impacted soil in Ohaji-Egbema, Imo State, demands further investigation. This study aims to investigate the microbiological quality of CME-impacted soil in Umuagwo Ohaji-Egbema Imo State Nigeria and provide valuable insights for sustainable agricultural practices in the region. By understanding the microbial dynamics, it will be possible to evaluate the risks associated with CME discharge and develop appropriate management strategies to mitigate potential adverse effects.

Materials and Methods

Study Area

This study was conducted at the cassava mill plant in Umuagwo community Ohaji-Egbema Imo state. The global position system (GPS) was used to determine the coordinates of the sample points, 5˚18'N, and 6˚56'E, 5˚11'N and 6˚37E, 5˚35'N and 6˚37E, 5˚15'N and 6˚35'E. The area is a tropical rainforest region. The major occupation of people in this area is farming, and the basic product they cultivate is cassava, which has caused an increase in the number of cassava mill plants in that area.

Umuagwo is characterized by two distinct seasons: wet and dry. The relative humidity and temperature of the area usually range between 50-90% and 28±2°C, respectively, all year round. The generated cassava mill effluents are discharged into the farm and streams without treatment.

Sample Collection and Processing

Four sets of soil samples were collected from cassava effluent polluted sites and two sets of samples from an unpolluted site that served as control, all from the Umuagwo community in Ohaji Egbema. Topsoil was collected from different points at a 0-30 cm depth using a soil auger. Soil samples were also collected 1000 m away from the cassava mill effluent polluted site, which served as the control sample. All soil samples were put into a dark polyethylene bag and labeled samples A1, A2, A3, and A4 for the contaminated samples and C1 and C2 for the control samples. All samples were transported to the laboratory in an ice chest at a temperature of 4°C for analysis.

Enumeration of culturable microbial population

The total culturable heterotrophic bacterial counts (TCHBC) and total heterotrophic culturable fungal counts (TCHFC) were carried out using nutrient agar (Accumedia, Sweden) and Sabouraud dextrose agar (Accumedia, U.S.A a subsidiary of Neogen) medium respectively. The media was prepared following the manufacturer's instructions.

A volume of 200 µL each of 10^{-3} – 10^{-6} dilutions of the individual samples was spread onto the corresponding medium. The inoculated plates were then incubated at 30 ^{0}C for 24 h for TCHB and 48 h for TCHF. After this, agar plates with discrete colonies ranging from 30 – 300 were selected (APHA, 2005) [8], and the total viable cells were estimated in Cfu/g.

Similarly, the total culturable *Nitrosomonas* count (TCNsC), the total culturable *Nitrobacter* count (TCNbC), the total culturable phosphate solubilizing bacterial count (TCPSBC), and total culturable lipolytic bacterial counts (TCLBC) were determined.

Nitrosomonas counts were carried out using the Winograsky media phase 1 (formulated using 2g$(NH_4)_2SO_4$, 1g K_2HPO_4,0.5g $MgSO_4.7H_20$, 2g NaCl, 0.4g $FeSO_4.7H_20$,0.01g $CaCO_3$ and 15g agar powder) dissolved in 1000 mL distilled water and autoclaved at 121˚C, 15psi pressure for 30 mins then dispensed into sterile Petri dishes to solidify. The compound $(NH_4)_2SO_4$ is used in this media as a differential compound to indicate the presence of *Nitrosomonas*, an Ammonia oxidizer.

Nitrobacter counts were carried out using the Winograsky media phase 11 (formulated using 0.1g KNO_2, 1g Na_2C0_3, 0.5g of NaCl, 0.4g $FeSO_4.7H_20$, 15g of agar powder) dissolved in 1000 mL distilled water and autoclaved at 121˚C, 15 psi pressure for 30 mins then dispensed into sterile Petri dish to solidify. KNO_2 was introduced as a differential compound for *Nitrobacter*, an $N0_2$ oxidizer.

Pikovaskay's medium (formulated with 5g $Mgcl.6H_20$, 0.25g $MgSO_4.7H_20$, 15g $(NH_4)_2SO_4$, 5g $Ca(PO_4)_2$, 15g agar powder) dissolved in 1000 mL distilled water and autoclaved at 121˚C,15psi pressure for 30 mins then dispensed into the sterile Petri dish and allowed to solidify was used in the isolation of phosphate solubilizing bacteria.

The tributyrin agar was used, as described by Ejimofor *et al.* (2023) [11], to estimate the total culturable lipolytic bacterial counts.

The inoculated plates were incubated at 30˚C for 24-48 hours for total culturable heterotrophic bacteria and total culturable lipolytic bacteria, 72 hours for fungal counts, and 7 days for nitrifying and phosphate solubilizing bacteria.

After this, the agar plate with discrete colonies ranging between 30 to 300 colonies was selected as described by (Agwaranze *et al.*, 2018) [4]. The total viable cells were estimated in CFU/gram.

Physicochemical Analysis

The individual samples were analyzed for pH, moisture content, total organic carbon, hydrogen cyanide concentration, nitrate, and phosphate concentrations. pH was determined using a pH meter (HANNA H18441) according to the method of Maduwuba (2022) [19]. Moisture content was determined using the method described by AOAC (2002) [7] and Brown (2011) [9] with slight modification. Nitrate and phosphate concentrations were determined using the method described by APHA (2005) [8]. Total organic carbon (TOC) was determined using the wet oxidation method, as described by Schumacher (2002) [27] and Maduwuba (2024) [20]. Hydrogen cyanide concentration was monitored by adopting the method according to Akwaranze *et al.* (2018) [4].

Result and Discussion

The total culturable heterotrophic bacterial count (TCHBC) revealed that sample A1 had a TCHBC of 8.6 $x10^6$ ±2.12 cfu/g, sample A2 had 2.4 $x10^6$ ±1.67 cfu/g, sample A3 had 3.0 $x10^6$ ±1.83 cfu/g, sample A4 had 4.3 $x10^6$ ±1.42 cfu/g while the control samples C1 and C2 had $1.3x10^6$ ±2.36 cfu/g and 1.5 $x10^6$ ±2.85 cfu/g respectively. Sample A1 revealed the highest TCHBC of $8.6x10^6$ ±2.12 cfu/g, while sample C1 recorded the lowest TCHBC of $1.3×10^6$ ±2.36 cfu/g, as represented in Fig 1.

The total culturable fungal counts (TCFC) revealed that sample A1 recorded a TCFC of $2.8x10^3$±2.19 cfu/g, sample A2 recorded a TCFC of $2.2x10^3$±1.46 cfu/g, sample A3 recorded $4.0x10^3$±1.62 cfu/g, sample A4, $3.5x10^3$±2.00 cfu/g while sample C1 and C2 recorded a TCFC of $2.1x10^3$±2.53 cfu/g and $1.87x10^3$±1.23 cfu/g respectively. The highest TCFC was observed in sample A3, while sample C2 recorded the least TCFC, as represented in Fig 2.

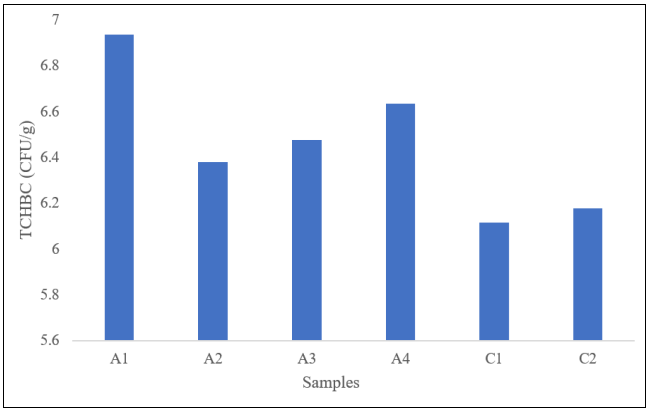

Fig 1: Total culturable heterotrophic bacterial counts in all the samples

The total culturable phosphate solubilizing bacterial counts (TCPSBC) revealed the presence of phosphate solubilizing bacterial population as 2.3 $x10^5$ ±1.16 cfu/g for A1, $3.4x10^5$±1.35 cfu/g for A2, $2.8x10^5$ ±1.68 cfu/g for A3, $4.0x10^5$±0.8 cfu/g for A4 and no-growth for the control samples C1 and C2. The highest TCPSBC of $4.0x10^5$±0.8 cfu/g was observed in sample A4, as shown in Fig 3.

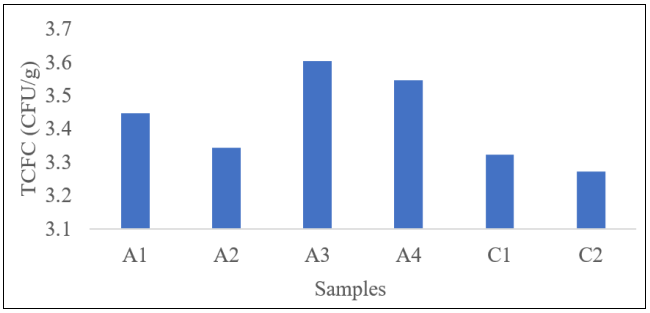

Fig 2: Total culturable fungal counts of all the samples

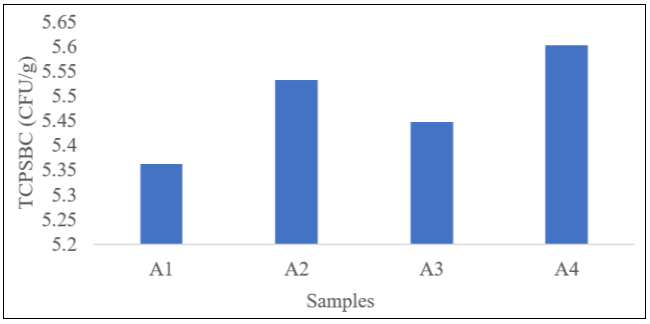

Fig 3: Total culturable phosphate solubilizing bacterial counts in all the samples

The total culturable Nitrosomonas counts (TCNsC) revealed a high population of *Nitrosomonas* sp. Sample A1 recorded a TCNsC of 2.0 $x10^6$ ±4.13 cfu/g, A2 2.8 $x10^6$ ±3.25 cfu/g, A3 $3.2x10^6$ ±3.86 cfu/g, A4 $2.3x10^6$ ±1.17 cfu/g while samples C1 and C2 recorded $3.4x10^6$ ±1.01 cfu/g and 8.2 $x10^4$ ±1.22 cfu/g respectively as represented in Fig 4. Sample A3 recorded the highest TCNsC, followed by sample A2, while sample C2 had the lowest TCNsC.

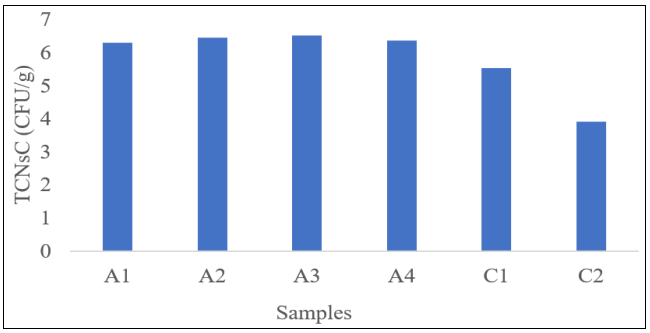

Fig 4: Total culturable Nitrosomonas counts in all the samples

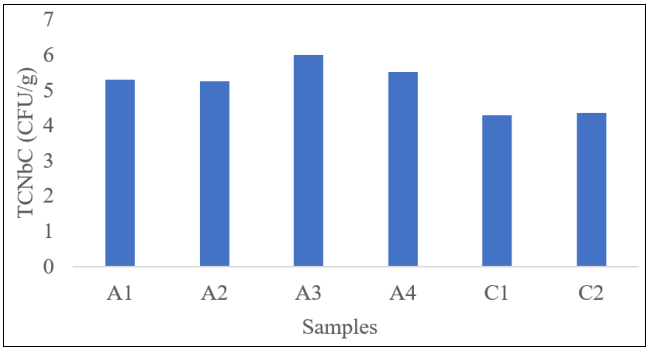

Fig 5: Total culturable Nitrobacter counts in all the samples

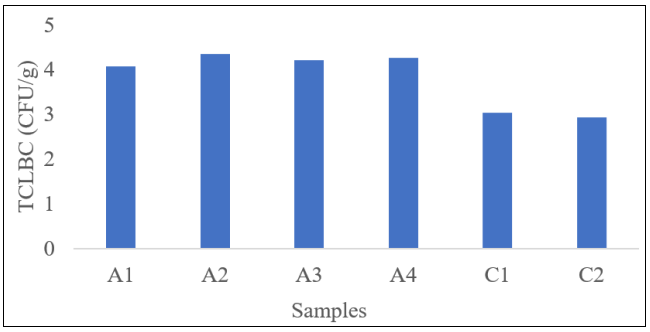

Fig 6: Total culturable lipolytic bacterial counts in all the samples

The total culturable Nitrobacter counts (TCNbC) revealed that the highest TCNbC of 1.0 $x10^6$ ±1.25 cfu/g was observed in sample A3 followed by samples A4 (3.2 $x10^5$ ±3.05 cfu/g), A1 (2.0 $x10^5$ ±1.91 cfu/g), A2 (1.8 $x10^5$ ±1.06 cfu/g), C1 (2.2 $x10^4$±2.81 cfu/g) and the lowest TCNbC was observed in sample C2 with 1.95$x10^4$ ±1.28 cfu/g as represented in Fig 5.

The total culturable lipolytic bacterial counts (TCLBC), as presented in Fig 6 revealed that sample A1 recorded a TCLBC of 1.2 $x10^4$ ±1.52 cfu/g, sample A2 2.2 $x10^4$ ±1.34 cfu/g, sample A3 1.6 $x10^4$ ±1.69 cfu/g, sample A4 1.8 $x10^4$ ±1.23 cfu/g, sample C1 1.1 $x10^3$ ±1.37 cfu/g and sample C2 0.85 $x10^3$ ±1.43 cfu/g. The highest TCLBC was observed in sample A2, while sample C2 recorded the least TCLBC in all the samples.

The microbial populations of the CME-polluted soil samples were higher than those of the polluted samples (C1 and C2). This suggests that CME significantly impacts the microbial population and diversity of the receiving soil environment. This result agrees with the report of Agbo *et al*. (2019) [3], who also recorded a higher microbial population in CME-contaminated soils. The higher microbial population could also indicate that the CME-polluted soil samples contain more nutrients required to support robust microbial proliferation (Enerijiofi *et al*., 2017; Ezeigbo *et al*., 2014) [12, 13]. However, the result of the microbial population of this study is in contrast with the outcome of the study conducted by Nwakoby and Ejimofor (2003) [22], who reported a significant decrease in microbial population during the assessment of CME-impacted soil compared to the un-impacted soil.

The fungal counts were also lower than the bacterial counts, which may be a result of the soil pH. This also agrees with the report of Igbinosa and Igiehon (2015) [17] and Aiyegoro *et al*. (2007) [5], who reported a higher bacterial population in cassava mill effluent polluted soil than the fungal population.

Sample A3 recorded the highest total culturable heterotrophic bacterial counts, total *Nitrosomonas* counts, total *Nitrobacter* counts, and total fungal counts. This could be due to its low acidity, with a pH of 6.57±0.06 compared to other CME-polluted samples. Also, it recorded a high total organic carbon content, which played a role in the proliferation of microbial population. The presence of nitrifying bacteria and phosphate solubilizing bacteria could be an indication of active nutrient cycling such as nitrification, nitrogen fixation in the root noodles of plants present in the soil, and phosphate solubilization, which are pivotal for the survival of soil microbes and increased crop yield (Ezeogo *et al*., 2021) [14].

Table 1: Physicochemical Analysis of CME contaminated and uncontaminated soil samples

Parameters	A1	A2	A3	A4	C1	C2
pH	6.28±0.01	6.40±0.12	6.57±0.06	6.35±0.03	6.91±0.01	7.20±0.04
Moisture content (%)	25.81±1.45	32.25±2.14	30.45±1.88	29.6±1.73	17.57±0.92	16.98±1.23
TOC (%)	3.83±0.04	2.99±0.014	3.25±0.22	3.04±0.11	2.00±0.03	1.89±0.02
Nitrate (mg/kg)	2.68±0.01	2.54±0.13	3.20±0.04	2.87±0.15	1.44±0.01	1.39±0.012
Phosphate (mg/kg)	1.49±0.01	2.31±0.05	2.52±0.41	1.38±0.11	0.52±0.01	0.66±0.01
Cyanide Content (mg/kg)	110.7±5.23	102.3±4.04	128.62±4.00	117.21±7.21	1.25±0.02	0.99±0.01

Values represent the mean± standard deviation of duplicate sample analysis

The result of the physicochemical analysis is presented in Table 1. The pH analysis revealed an overall slightly acidic to alkaline pH in all the samples. Sample A1 recorded a pH of 6.28±0.01, sample A2 6.40±0.12, sample A3 6.57±0.06, sample A4 6.35±0.03, while samples C1 and C2 recorded a pH of 6.91±0.01 and 7.20±0.04 respectively. The highest pH was recorded in sample C2, while the lowest pH was recorded in sample A1. The pH of the soil played a vital role in the high concentration of cyanogenic glycosides in the soil. High cyanogenic glycosides can also cause low clay content, high negative soil charges, high electrical conductivity, low crop yield, and other conditions (Igbinosa and Igiehon, 2015; Fuller, 2004) [17, 16]. The ability of the microorganisms to strive at the pH range of 6.28±0.01 – 7.20±0.04, which is present in the soil samples, could have been made possible as a result of microbial adaptation mechanisms and the ability of the resident microbes to acquire genetic characteristics from the soil environment (Nwaugo *et al*., 2008) [23]. The pH of the CME-polluted soils was lower (slightly acidic) than that of the unpolluted soil. A similar pH was reported by Akin *et al*. (2006) [6] and Olusola (2009) [25] as a suitable pH for the growth of plants as nutrients are at optimum availability within the range of 6 and 7 in the soil ecosystem.

The moisture content revealed higher moisture content for the CME-contaminated soil samples than the control samples. Percentage moisture of 25.81±1.45 %, 32.25±2.14 %, 30.45±1.88 %, 29.6±1.73 % were recorded for samples A1, A2, A3, and A4 respectively while samples C1 and C2 recorded percentage moisture of 17.57±0.92 % and 16.98±1.23 % respectively. The highest percentage of moisture was recorded in sample A2.

The total organic carbon (TOC) revealed a TOC of 3.83±0.04 %, 2.99±0.014 %, 3.25±0.22 %, 3.04±0.11 %, 2.00±0.03 %, and 1.89±0.02 % for samples A1, A2, A3, A4, C1 and C2 respectively. The highest TOC was recorded in sample A1, while sample C2 recorded the lowest TOC. High total carbon content contributes to the growth and survival of high microbial life in the soil samples analyzed (Agbo *et al*., 2019) [3]. Studies have revealed that topsoil is the richest in microbial population and diversity due to its high total organic carbon and oxygen gradient. This decreases with

soil depth (Nwaugo *et al*., 2008) [23].

Nitrate concentration revealed that sample A1 had a nitrate concentration of 2.68±0.01 mg/kg, sample A2 2.54±0.13 mg/kg, sample A3 3.20±0.04 mg/kg, sample A4 2.87±0.15 mg/kg, sample C1 1.44±0.01 mg/kg and sample C2 1.39±0.012 mg/kg. The nitrate concentration recorded in this study could have resulted from the complete or partial mineralization process, which occurred during the degradation of the soil organic matter, mostly comprising of cassava mill effluent and other soil organic components.

Phosphate concentration revealed a higher phosphate concentration for the CME-contaminated soil samples than the control samples. Phosphate concentrations of 1.49±0.01 mg/kg, 2.31±0.05 mg/kg, 2.52±0.41 mg/kg, 1.38±0.11 mg/kg, 0.52±.0.01 mg/kg and 0.66±.01 mg/kg were recorded for samples A1, A2, A3, A4, C1 and C2 respectively.

The cyanide content revealed a high hydrogen cyanide concentration of 110.7±5.23 mg/kg, 102.3±4.04 mg/kg, 128.62±4.00 mg/kg, and 117.21±7.21 mg/kg for samples A1, A2, A3, and A4. The control samples C1 and C2 recorded very low cyanide concentrations of 1.25±0.02 mg/kg and 0.99±0.01 mg/kg, respectively. The high cyanogenic concentration in the polluted soil compared to the unpolluted soil could be attributed to high concentrations of cyanogenic glycosides from the processed cassava tubers, which entered the soil through leaching. The cyanogenic content of the CME-polluted soil is within the range of values obtained by Nwabueze and Odusi (2007) [21] and Agwaranze *et al*. (2018) [4] during their study.

Conclusion

This study has revealed that cassava mill effluent (CME) pollution can affect the microbiological and physicochemical quality of the soil. The microbiological analysis has shown an increased microbial population due to CME pollution. It was also discovered that the CME-polluted soils revealed a more acidic pH than the unpolluted soils. CME pollution can also affect the soil's total organic carbon, nitrate, and phosphate concentrations. This further shows that CME pollution can cause an ecological shift in the microbial community diversity of the soil environment. Therefore, there is a need for further studies on the dominant microbial community diversity present in CME-polluted soils.

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