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(RESEARCH ARTICLE)

Assessment of the effects of cassava mill effluent on the soil and its microbiota in Biase local government area of Cross river state, Nigeria

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Abstract

The effects of cassava mill effluent (CME) on the soil and soil micro-biota was investigated. Soil samples were collected from sites polluted with CME and also from an adjacent site not polluted with CME. Standard microbiological methods were used in the isolation, antibiotic susceptibility and enumerating the population of the microorganisms while the physico-chemical parameters of the soil were determined using standard chemical processes. The bacteria isolates were *Bacillus sp., Pseudomonas sp., Enterobacter sp., Escherichia coli, Corynebacterium sp.* and *Proteus sp. a*nd the fungal isolates were *Aspergillus sp., Candida sp., Mucor sp., Penicillium sp., Saccharomyces sp.* and *Rhizopus sp*. The mean aerobic bacterial count for the soil samples ranged from $7.1\pm0.11x10^5$ cfu/g for the control soil to $8.0\pm0.19x10^5$ cfu/g for the CME impacted soil. The mean heterotrophic fungal counts varied from $4.7\pm0.41x10^5$ cfu/g for CME impacted soil to $4.2\pm0.57x10^5$ cfu/g for the control soil. Susceptibility profile showed that *Bacillus sp.* from the CME impacted site had reduced susceptibility to levoflacin, amoxil, chloramphenicol, and Ciprofloxacin. *Pseudomonas sp.* from the same site had reduced susceptibility to streptomycin, ampicilin, tarivid, peflacin, augmentin and ciprofloxacin. *Escherichia coli* had reduced susceptibility to streptomycin, ampicilin, ciprorex and nalidixic acid. The heavy metals Cu, Zn, Ni, Co, and Fe content of the CME impacted soil increased greatly. Excessive application of CME had negative effects on the soil, hence the high concentration of heavy metals in CME dumpsites and high microbial presence. The study also showed that CME can induce the resistance potential of some soil microbes to antibiotics and seriously affect the physicochemical parameters of soil.

Keywords: Cassava mill effluent (CME); Microbiota; Antibiotic susceptibility; Physico-chemical analysis; Soil microbes

1. Introduction

Cassava effluent is a source of pollution, if discharged into the environment before proper treatment [1]. Pollution of our environment occurs when waste water discharged from cassava processing are allowed to spread slowly into the soil or flow into streams or when cassava roots are fermented in surface water like ponds and streams, upstream of drinking water sources [2, 3, 4, 5] reported that suspended solid particles are important pollutants as pathogens are carried on the surface and body of the particles.

Effluent is normally discharged beyond the "factory" wall to roadside ditches or fields and allowed to flow freely, or sometimes settles in shallow depressions, in places where traditional processing is practiced. Eventually this effluent will seep into the soil or flow into streams [6]. Starch processors in Colombia, usually return the waste water directly to streams and other surface water sources [1]. Oboh [2] and Kolawole [3] discussed broadly on the effluents obtained from the processing of cassava into various end products. Ehiagbonare *et al.,* [7] and Kolawole [3] examined the fate of

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cassava effluent on the environment and realized that the effluent had negative effects on water, soil, air, domestic animals and plants.

In spite of the negative effects of cassava effluent on our environment as reported by researchers, the treatment and disposal of cassava waste water from industrial sources still continues; because cassava waste water disposal is done improperly and allowed to build up over time. A lot of the cassava effluent arising from processing ends up with domestic waste, while others seep into the soil. A fair volume of the cassava effluent is carried in solution, others have become so finely divided that they exist in a colloidal state, while others go into suspension [3, 8]. Water pollution causes nearly 14,000 deaths per day, basically due to contamination of drinking water sources [3, 4, 8]. The discharge of waste products and contaminants into surface runoff get into rivers through drainage systems, leaching into liquid spills, groundwater and wastewater discharges and littering. Soil contamination in our case occurs when drains from cassava processing factory are released by spill or underground leakage. This cassava effluent has a negative effect on our environment [3].

Effluent wastes contain varying concentration of heavy metals either as simple metals or complexes [6, 9]. Oti [10] and Agwa *et al.,* [6] in their separate studies, reported that cyanide forms complexes with zinc to and hydrogen to form an acidic complex called hydrogen cyanide acid. Cassava must be nicely and well prepared before consumption, as wrong preparation of cassava can leave enough residual cyanide to cause serious cyanide intoxication, goitres, and even cause ataxia or partial paralysis [11].

The frequent release of cassava effluent has caused the adverse effect of cassava waste to the environment and bio-diversities [12]. For instance the garri processing wastes discharged into the environment causes foul smell and produces unattractive sights [13]. Like every other waste, cassava mill effluent upsets the marine equilibrium [14], showing a reduction in dissolved oxygen, depression in pH values, elevation of BOD and nitrate values in the tropical streams of Southern Nigeria [6]. In the same vein the pH value of the cassava mill effluent polluted stream has been observed to be as low as 2.6 and is also known to spoil stream beds by the settled suspended solids of the effluent, thereby making marine lives such as fish breeding pretty difficult to survive [15, 16].

The present study was carried out to determine the physico-chemical properties and heavy metal content of the cassava mill effluent, to compare soil physico-chemical properties and heavy metal contents of cassava mill effluent impacted soil to that of un-impacted soil, to determine the effect of CME on the bacterial and fungal population and their diversity in the impacted soil when compared with the control or un-impacted soil and to determine the effect of CME on the antibiotic susceptibility profile of the microbes found at the impacted sites and then compared with those found at the un-impacted sites.

2. Material and methods

2.1. Sample collection

This study was carried out at Iwuru Obio Ntan community, Biase Local Government Area of Cross River State, with longitude 05^{o} 25' 15 310" N and latitude 008^{o} 10'48 594" E. The main relief feature in Iwuru Obio Ntan is lowland type of landscape classified under the coastal lowland of Southern Nigeria inside the tropical rainforest region [17]. The soil type there is made up of precisely the red and brown soil with abundant free iron oxide. The physical, relief features and location factors and prevalence of the tropical rainy climate that is warm, humid and moist in most part of the year, encourages their major occupation which is farming [17]. Control soil samples, free of cassava effluent, were collected between June, 2015 and January, 2016 in sterile and sealed bags with labels from control sites (fertile farmland) 50 metres away from the processing plant. Soil samples were also collected from soil impacted by cassava effluent at the processing plant, along the run off site of the hydraulic press in sterile and sealed bags with labels. Cassava mill effluent sample from already pressed cassava, at the hydraulic press were also collected in a sealed rubber container that was labelled and taken to the laboratory [18].

2.2. Microbial analysis

2.2.1. Serial dilution

The various soil samples were removed from the aluminum foil and sterile polythene bag and one (1.0) grams of each soil samples was weighed out and homogenized into 9.0 ml of sterile distilled deionized water and then mixed thoroughly, then 10-fold serial dilution was carried out by transferring 1ml of the aliquot into 9ml of sterile distilled

water contained in the test tube (diluents). 1 ml of the dilution that was less turbid was plated in petri-dishes (in duplicate) using pour plating technique [19].

2.2.2. Enumeration and identification

Identification and enumeration was done following the methods described by Mboto *et al.,* [20]. After incubation, the colonies were observed and then counted and recorded, then identification of pure culture from each sample was done by studying colony characteristics, microscopy and biochemical characteristics. Identification was accomplished by using binocular microscope of view.

2.3. Isolation and purification of the isolates

The bacterial and fungal colonies which developed were isolated and purified by repeated sub-culturing into nutrient agar plates and saboraud dextrose agar respectively, which were incubated at room temperature for 24 hours (for NA) and 72 hours for (SDA), from these pure cultures, stock cultures were prepared in agar slants in stock bottles (using nutrient agar for bacterial isolates and SDA for fungal isolates) for subsequent identification and characterization.

2.4. Characterization and identification of isolates

The bacterial species isolated were identified and also characterized after investigating their Gram reaction, their physiological characteristics and cell micro-morphology. Morphological characterization was based on the colony morphology which includes, colour elevation, consistency and moisture contents (wet or dry colony) [18, 21].

2.5. Biochemical test

The identification of the bacterial isolates were further enhanced by biochemical characterization of the isolates. The biochemical tests carried out were: Gram staining, motility test, oxidase test, catalase test, citrate test, indole test, sugar fermentation test, methyl red test, Voges Proskaueur test and urease test.

2.6. Antibiotics susceptibility testing

The susceptibility of the bacterial isolates to various conventional antibiotics was determined using Muller Hinton agar while employing the disk diffusion method. The antibiotics used include; Amoxil (AMX), Chloramphemcol (CHL), Ciprofloxacin (CPX), Erythromycin (ERY), Gentamycin (GEN), Norfloxacin (NFX), Rifampicin (RFP), Stremtomycin (STR), Ampliclox (APX) and levofloxacin (LEV). The commercial antibiotics disks were introduced on an agar plates previously seeded with 18hours broth culture of test organism. The plates were incubated at room temperature for 24 hours. The inhibition zones were measured in millimeters [22].

2.7. Physical analysis of soil and effluent samples

2.7.1. Soil digestion technique

After oven drying, the sample was bended into a fine powder from which 0.50 g of the powdered tissue was weighed into a conical flask and placed in a fume cupboard. The fume cupboard was used due to the emission of toxic fumes from the digestion process. Aqua region (1:4 r:r Nitric: perchloric) i.e. 30 ml Nitric to 10 ml perchloric. The mixture was placed in a hot air oven at 100 °C until digestion is completed. The mixture was then made up to 100 ml by adding 60 ml of demonized water. Heavy metals were analyzed using UV-visible spectrophotometer (model: HACH DR 5000) [23, 24].

2.7.2. Temperature

This was determined with the aid of a thermometer, the thermometer was inserted into the effluent and soil solution sample in a beaker and the reading was taken [19, 25].

2.7.3. pH

The pH was determined with the aid of a pH meter (model HACH SENSION +). The pH meter probe was inserted into the effluent and soil solution sample in a beaker, the read key was pressed and the pH reading was noted [19, 25].

2.7.4. Conductivity

Conductivity meter (Model: Orion 3 Star) was used for this. The conductivity meter probe was rinsed with distilled water and inserted into the sample in a beaker, the conductivity reading was displayed [19, 25].

2.7.5. BOD

BOD meter (Model: HACH HQ40d) was used to determine this parameter. The meter probe was rinsed with distilled water and inserted into the sample. BOD reading was displayed on activation of the read key [19, 25].

2.8. Chemical analysis of soil and effluent sample

2.8.1. Total iron

The desired test was selected, then the multi-cell adapter with the I-inch square cell holder was inserted, there after clean square sample cell was filled with 10ml of the sample. Contents of one ferrover iron reagent powder pillow was added to the sample cell and swirled well to mix. The formation of orange colouration indicated the presence of iron [23, 24, 26].

2.8.2. Manganese

The desired test was selected, then multi-cell adapter with I-inch square cell holder was inserted, there after square sample cell was filled with 10ml of the sample. Contents of one buffer powder pillow, citrate type of manganese stopper was added. Then contents of one sodium periodate powder pillow was added to the sample cell stopper and inverted to mix. Formation of violet colour indicated the presence of manganese [23, 24, 26].

2.8.3. Copper

A square sample cell was filled with 10ml of the digested effluent impacted soil sample. The control sample was inserted into the cell holder with the fill line facing the user. Zero was pressed on the time with the display showing 0.00mg/lcu. Within 30 minutes after the timer expired, prepared samples were inserted into the cell holder with the fill line facing the user. Then results were taken in mg/l Cu [23, 24, 26].

2.8.4. Nitrate

The test was selected, then the multi cell adapter with I-inch square cell holder was inserted facing the user. The square sample cell was filled with 10ml of the sample. Contents of one nitra Ver 5 nitrate reagent powder pillow was added. Then OK was pressed on the time. A one minute reaction period began, then the cell was shake vigorously until the time expired. The TIMER>OK was pressed again. Then a five-minute reaction period began. An amber colour developed showing the presence of nitrate [23, 24].

2.8.5. Nitrite

The test was selected, then the multi cell adapter with I-inch square holder was inserted. The square sample cell was filled with 10ml of the sample. Contents of Nitri Ver 3 Nitrite reagent powder pillow was added and swirled to mix. A pink colour developed showing the presence of nitrite [23, 24].

2.8.6. Phosphorus

A square sample cell was filled with 10 ml of the sample. The blank was inserted into the cell holder. The ZERO was pressed on the button, with the display showing 0.00mg/l $P0_4^3$. The prepared sample was wiped and inserted into the cell holder with the fill line facing the user. Then results were taken in mg/l PO_4^{3-} [19, 23, 24].

2.8.7. Potassium

The test was selected and the multi cell adapter with I-inch square cell holder inserted. A graduated mixing cylinder was filled with 25ml of the sample. Contents of potassium 1 reagent pillow was added along with the contents of one potassium 2 reagent pillow stopper and inverted several times to mix. The blank was wiped and inserted into the cell holder with the fill line facing the user. Zero was pressed on the time with the display showing 0.0mg/lk II. Within seven minutes after expires, the prepared sample was wiped and inserted into the cell holder with the fill line facing the user. Then results were taken in mg/l K [23, 24].

2.8.8. Ammonia

The test was selected and the multi cell adapter with one inch square holder was inserted. The square sample cell was filled with 100 ml of the sample. Contents of one ammonia salicyclate powder pillow was added to each cell stopper and then shook to dissolve. Then the OK button was pressed. A 15 minute reaction period began. A green color developed showing the presence of ammonia-nitrogen. When the timer expired, the blank was inserted into the cell holder with

the fill line facing the user. Zero was pressed on the timer with the display showing 0.00 mg/l NH_3-NII. The sample was wiped and inserted into the cell holder and results were taken in mg/l NH_3 [23, 24].

2.8.9. Zinc

10 ml of the sample solution was poured into a square sample cell. With the use of a plastic dropper, 0.5 ml of cyclohexanone was added to the solution in the graduated cylinder. Then OK was pressed on the timer. A 30 second reaction period began, during the period the prepared sample in the cylinder was shaked vigorously. A colour change was observed, which depending on the zinc concentration could be reddish orange, brown or blue [23, 24, 26].

2.8.10. Cobalt

The test was selected, then a multi-cell adapter with I-inch square cell holder was inserted. The square sample cell was filled with 100 ml of the sample. The second square sample cell was also filled with 10 ml of the sample marked with deionized water. Then contents of one phthalate-phosphate reagent powder pillow were added to each cell. Using the plastic dropper, 0.5 ml of 0.3% PAN indicator solution was added to each cell-stopper; and inverted several times to mix. Then the OK button was pressed on the timer. A three minutes reaction period began. During the reaction period, the sample solution varied from green to dark red, depending on the chemical make-up of the sample. When the timer expired, the contents of one EDTA reagent powder pillow was added to each cylinder and shaked to dissolve. The blank was wiped and inserted into the cell holder. The zero button was pressed with the display showing 0.00 mg/l co 12. The prepared sample was wiped and inserted into the cell holder. Then finally, results were taken in mg/l Co [23, 24, 26].

2.8.11. Calcium

Spectrophotometry method was used, 0.1 ml of the sample was placed in a test tube using pipette and 0.5 ml of calcium reagent Ca^{-1} was added and mixed. 0.4 ml each of calcium reagent Ca^{-2} and Ca^{-3} were also added to the test tube and mixed. The sample was allowed to stand for 8mins to elicit full colour development and then filled into a reaction cell placed in the spectrophotometer where the calcium concentration was displayed [23, 24, 25].

2.8.12. Sodium

Spectrophotometry method was used, 0.5 ml of sodium reagent Na-1K was placed in a reaction cell and 0.5 ml of the water sample added to it and mixed. A reaction time of 1 minute was allowed before reaching the concentration of sodium from the spectrophotometer [23, 24, 25].

2.8.13. Magnesium

Spectrophotometry method was used, 1 ml of the sample was placed in a reaction cell and mixed 1 ml of Magnesium reagent (Mg-1K) added to it. This was allowed to stand for 3 minutes and there after 0.3 ml of Magnesium reagent (Mg-2K) added, mixed and placed in the spectrophotometer. Magnesium concentration was read at a wave length of 568 nm [23, 24, 25].

2.8.14. Nitrogen

Spectrophotometry method was used, 5 ml of the water sample was placed in test tube and 1 micro spoonful of nitrite reagent NO_2-AN was added and shaken to dissolve. A time of 10 minutes was allowed before reading out the nitrite concentration in the sample (Tiimub *et al.,* 2012). This reading gives nitrite value and to get the nitrogen value, the nitrite value is multiplied by a constant 0.3045. Therefore nitrogen is NO_2 x 0.3045 [19, 23, 24].

2.9. Statistical analysis

Data obtained from microbial plate counts to determine the total heterotrophic bacterial count (THBC) and total hetrotrophic fungal count (THFC), physico-chemical analysis of the POME, polluted soil and control soil measurement of zones of inhibition of antibiotics on isolates from polluted and unpolluted soil was expressed as mean ± SE of duplicates. The data collected were analyzed using SPSS version 21. Replicate readings obtained from physico-chemical analysis were subjected to one way ANOVA at 95% level of significance. Mean + or – SD with probability < 0.05 were considered significant.

3. Results

A total of three (4) samples were analyzed microbiologically and physico-chemically, out of which two (2) soil samples were from the cassava mill effluent dumpsites, and two (2) soil samples from fertile un-impacted farmland. After carrying out serial dilution and plating using pour plate method. Isolates showed growth on nutrient agar medium and SDA medium and thus were confirmed through several biochemical tests and through their cultural and microscopic appearances. The isolates were identified using keys provided in the Bergey's manual of determination bacteriology [27]. The isolates obtained were *Bacillus sp.* (28.6%), *Pseudomonas sp.* (28.6%), *Enterobacter sp.* (7.1%), *Corynebacterium sp.* (14.3%), *Proteus sp.* (7.1%) and *Escherichia coli* (14.2%). After purification and sub-culturing, the fungi isolates were further identified using colony morphology and microscopy. Eleven (11) species of fungi were isolated and they include; *Aspergillus sp.* (18.2%), *Penicillium sp.* (27.2%), *Mucor sp.* (9.1%), *Candida sp.* (18.2%), *Saccharomyces sp.* (18.2%) and *Rhizopus sp.* (9.1%).

3.1. Bacterial and fungi enumeration

The average microbial count of bacteria and fungi as presented in figure 1 below. The total mean heterotrophic bacterial count ranged from $7.0\pm0.11x10^5$ cfu/g, for the control soil while the effluent and polluted soil were $5.1\pm0.28x10^5$ cfu/g and $7.8\pm0.19x10^5$ cfu/g respectively.

The fungal mean ranged from $4.2\pm0.57x10^5$ cfu/g for the control soil while the effluent and polluted soil were $4.4\pm0.41x10^5$ cfu/g and $3.9\pm0.42x10^5$ cfu/g respectively. The impacted soil had the highest count of $7.8\pm0.19x10^5$ cfu/g for the microbial count. From the bar chart, it was observed that bacterial counts were higher than the fungal counts. In both cases, the bacteria and fungi counts at the impacted and un-impacted soils were higher than that at the cassava impacted soils.

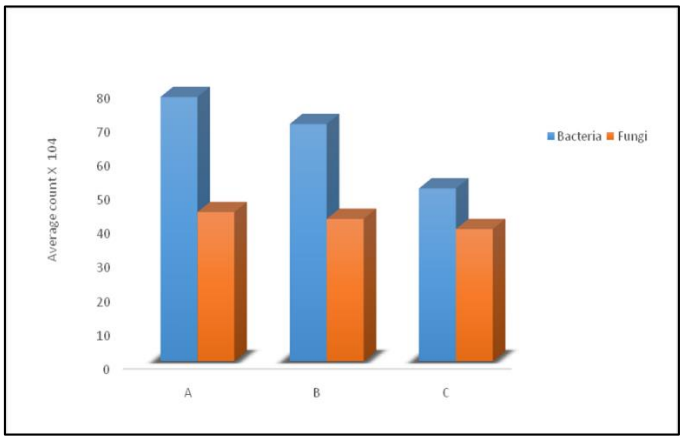

Figure 1 Bacterial and fungal counts at the impacted soil, un-impacted soil and cassava effluent (A Impacted soil, B - Un-impacted soil and C - Cassava effluent)

3.2. Physico-chemical analysis

The results for the physicochemical analysis for the cassava impacted soil, un-impacted soil and cassava effluent samples are presented in table 1. Duplicate data obtained for each of the parameters were analyzed using One-Way ANOVA at 0.05 (95%) level of significance. Mean ± Standard deviations across the rows with probability values less than 0.05 were considered significant. The cassava effluent sample was acidic with the pH value of 3.13 while the values for un-impacted and impacted soil samples were slightly above neutral with pH 7.87 and 7.21, respectively. Nitrogen concentration was highest in cassava effluent sample (797.00 (mg/l), with 95.15 (mg/l) and 7.51 (mg/l) for un-impacted and impacted soil, respectively. Potassium was atleast seven fold higher in cassava effluent than in impacted soil and un-impacted soils samples. Magnesium levels were almost similar in the cassava effluent (657.75 mg/l) and impacted soil (660.85mg/l) and lower for un-impacted soil 36.95 (mg/l).

Calcium, sodium and electrical conductivity were higher for the effluent and un-impacted soil than that of the impacted soil. However, the electrical conductivity and calcium were highest for the effluent while sodium was highest in the un-impacted soil sample. Biological oxygen demand was at 5 days determined at 28^{o}C and were 4.54, 5.73 and 7.18, respectively for un-impacted, cassava effluent and impacted soils in ascending order. Dissolved solids were highest in effluent (478.75 mg/l) and least in the impacted soil. The concentration of copper was two and three fold higher in

effluent sample than in un-impacted and impacted soil samples, correspondingly. The concentration of zinc was fairly constant across all three samples examined.

However, iron, cobalt and ammonia concentrations were highest in cassava effluent. In the un-impacted soil sample, cobalt and nitrate was below detection levels (BDL) as well as in the un-impacted soil samples. Cassava effluent had the highest amount of nitrate of 12.01 mg/l while un-impacted soil had the highest concentration of nitrite (88.05 mg/l).

Table 1 Physicochemical analysis of cassava effluent, impacted and un-impacted soils samples

Parameters	Un-impacted soil	Impacted soil	Cassava effluent
pH	7.87±0.01[a]	7.21±0.01[b]	3.13±0.01[c]
Nitrogen (mg/l)	95.15±0.10	7.51±0.01	797.00±1.41
Phosphorus (mg/l)	288.85±0.10	79.51±0.01	432.00±1.41
Potassium (mg/l)	66.65±0.10	56.25±0.10	459.00±1.41
Magnesium (mg/l)	36.95±0.10	660.85±0.10	657.75±0.21
Calcium (mg/l)	34.15±0.10	6.71±0.01	64.30±1.41
Sodium (mg/l)	53.75±0.10	4.51±0.01	30.90±0.14
Conductivity(µs/cm)	26.35±0.10	8.99±0.01	46.20±0.01
BOD (28 ^{o}C)	4.54±0.01	7.18±0.01	5.73±0.01
TDS (mg/l)	57.12±0.01	4.51±0.01	478.75±0.01
Copper (mg/l)	8.40±0.01	14.73±0.01	29.34±0.01
Zinc (mg/l)	9.76±0.01	11.13±0.01	10.17±0.00
Nickel (mg/l)	0.66±0.00	0.68±0.00	1.75±0.00
Cobalt (mg/l)	BDL[d]	0.78±0.00	8.80±0.01
Iron (mg/l)	13.95±0.01	18.09±0.01	31.95±0.01
Ammonia (mg/l)	3.76±0.01	3.50±0.00	10.50±0.01
Nitrate (mg/l)	BDL[d]	1.10±0.01	12.01±0.01
Nitrite (mg/l)	88.05±0.01	0.61±0.01	6.10±0.01

[a,b,c] Represent Mean±SD readings that are significant ($p < 0.05$) across the rows for each of the parameters and samples while [d] equals below detection level, respectively.

3.3. Antibiotic susceptibility test

The results of the sensitivity testing on gram negative and gram positive organisms isolated from different soil samples are presented in figures 2 and 3. The antibiotics discs used for this study were Streptomycin, Ampicillin, Ciprorex, Ampicillin, Tarivid, Nalidixic Acid, Perflacine, Gentamicin, Augmentin, Ciprofloxacin, Septrin, Levoflacin, Ampiclox, Rifampin, Amoxil, Norfloxacin and Erythromycin. *Pseudomonas sp., Proteus sp.* and *Escherichia coli* isolated from all three sites are tested against the antibiotics. Ciprofloxacin showed the most consistent activity against the test isolates with zones of inhibitions of 25 mm and 24 mm against *Pseudomonas* and *Escherichia coli*, respectively. The least zone of inhibition (6 mm) against *Pseudomonas, E. coli*, and *Proteus* species were shown by Tarivid and Septrin, Nalidic acid and Perflacine, and Nalidic acid, respectively. *Pseudomonas* species isolated from impacted and effluent soil samples were most sensitive to the test antibiotics.

The sensitivity of the gram positive organisms is presented in and figure 3. The highest zone of inhibition of 24 mm was observed for *Bacillus sp.* isolated from cassava effluent impacted soil with Ciprofloxacin while the least zone of inhibition observed was *Enterobacter sp* isolated from same site. The most isolated species was *Bacillus sp.* from all three location followed by *Corynebacterium* and *Enterobacter* in terms of frequency of occurrence. Levoflacin, Streptomycin and Gentamicin all showed zones of inhibitions that were ≥10 mm to ≤ 20 mm on all test isolates. However, the remaining antibiotics had zones of inhibitions that were ≥ 6 mm to ≤ 24 mm.

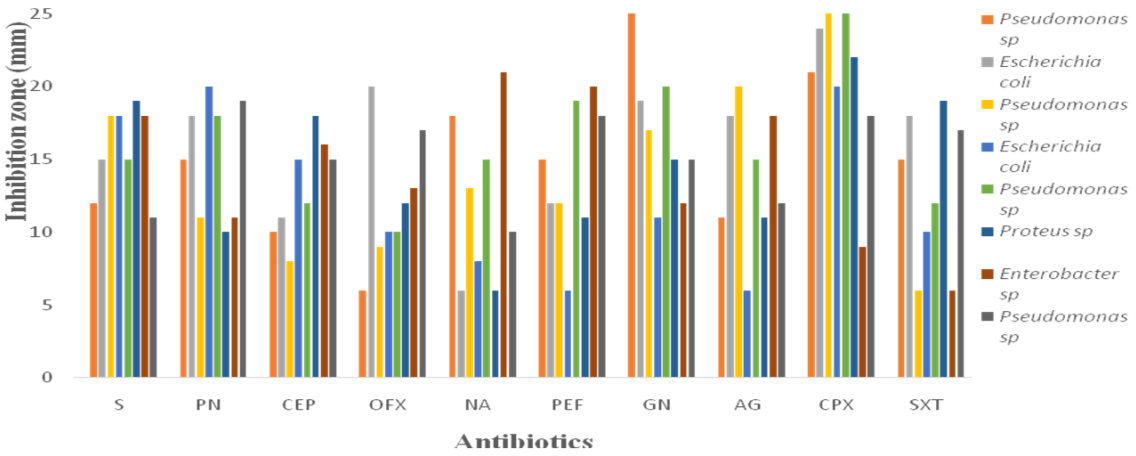

Figure 2 The sensitivity of different gram negative organisms and antibiotics

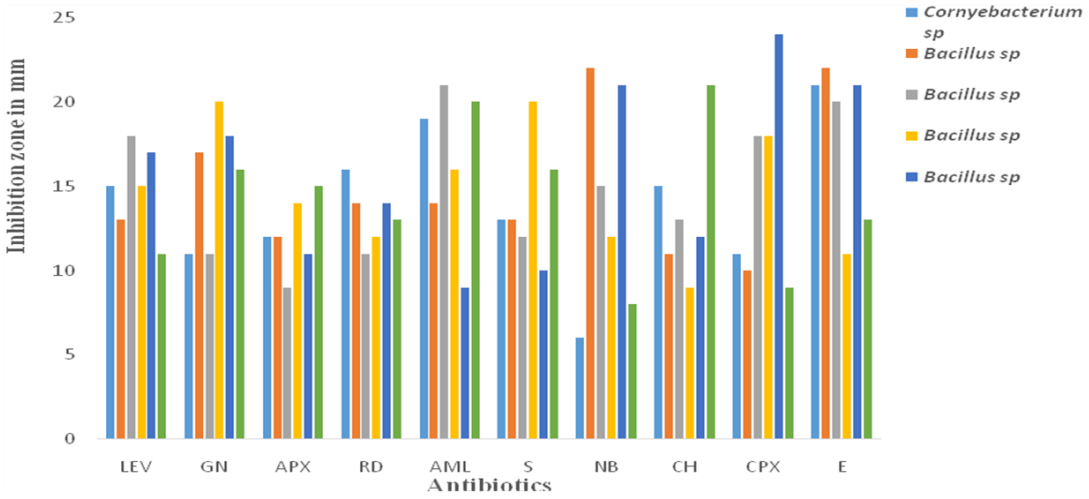

Figure 3 The sensitivity of different gram positive organisms and antibiotics

4. Discussion

The following bacterial isolates were found in the course of the research; *Bacillus sp.* (28.6%), *Pseudomonas sp.* (28.6%), *Corynebacterium sp., Escherichia coli, Proteus sp.,* and *Enterobacter sp*. Eleven (11) species of fungi were equally isolated which are; *Penicillium sp., Mucor sp., Candida sp., Saccharomyces sp., Aspergillus sp.,* and *Rhizopus sp.* These isolated bacterial and fungal species were equally isolated by other authors [23, 24, 28]. These microbial isolates were more in number in the cassava effluent impacted soil sites than in the un-impacted soil and in the cassava effluent. Cassava effluent impacted soil had a significantly increased bacterial count of $7.8\pm0.19\times10^5$ cfu/g while the fungal counts for the impacted and un-impacted were $4.4\pm0.41\times10^5$ and $4.2\pm0.57\times10^5$ cfu/g respectively. This goes to indicate that the cassava effluent has impacts on the fungal diversity of the effluent impacted soil. Fungal counts were less than the bacterial counts ($P < 0.05$); and this is in agreement with the report of [28, 29]. The increase in the fungal growth in the impacted soil experiments may also be due to the acidic pH of the soil which is 7.21±0.01.

These bacteria species may have picked up the genetic traits from their environment that helped them to survive in such an acidic environment [28, 30]. The low pH of the soil could explain the presence of cyanogenic glycosides in the cassava effluent impacted soil. Factors like low clay content, low pH and high negative soil charges were reported as soil conditions that increase cyanide mobility [28, 29]. In addition, the high organic carbon contents of the cassava effluent may have contributed to the proliferation of these aerobic microorganisms as reported by [28, 29, 30]. Top soil was indicated to harbor the richest microbial diversity because it contains a higher amount of organic matter and oxygen which decreases with depth [28, 31].

The total nitrogen recorded in this present study was possibly due to nitrogen mineralization as a result of the breakdown or degradation of organic matter. Electrical conductivity was used as a means of assessing soil salinity. The values obtained for the electrical conductivity in the soil samples may be as a result of the increase in the concentration of soluble salts [28]. The heavy metal content for all the cassava effluent impacted soils were significantly higher than the levels observed in the control soils ($P > 0.05$). This means that the soils receiving the cassava effluent have some levels of heavy metal enrichments. The high concentration of heavy metals like Nickel, Iron, Copper, Zinc and Magnesium in the cassava effluent impacted soils could also be attributed to the wearing off or abrasion of the cassava milling machine parts and emission of the metals through the exhaust of the machine [28, 32]. In low pH soils, the solubility of Magnesium decreases and it becomes less available.

For the gram negative organisms, *Ciprofloxacin* showed the most consistent activity against the test isolates with zones of inhibitions of 25 mm and 24 mm against *Pseudomonas* and *Escherichia coli*, respectively. The least zone of inhibition (6 mm) against *Pseudomonas, E. coli*, *Enterobacter sp* and *Proteus* species were shown by Tarivid and Septrin, Nalidic Acid and Perflacine, and Erythromycin and Nalidic acid, respectively. *Pseudomonas* species isolated from impacted and effluent soil samples were most sensitive to the test antibiotics.

For the gram positive organisms, the highest zone of inhibition of 24 mm was observed for *Bacillus sp.* isolated from cassava effluent impacted soil with Ciprofloxacin. Levoflacin, Streptomycin and *Gentamicin* all showed zones of inhibitions that were ≥10 mm to ≤ 20 mm on all test isolates. However, the remaining antibiotics had zones of inhibitions that were ≥ 6 mm to ≤ 24 mm.

5. Conclusion and recommendations

The results of this present study shows that the cassava effluent has effects on the microbial diversity of the receiving soil. This is indicated by the significant increase in microbial population noticed in the microbial density of the effluent impacted soil and the simultaneous effect on the physical and chemical characteristics of the soil. The milling stage which is a large stage in processing cassava tuber to be ready for consumption releases the highest/ largest quantity of cassava mill effluent/ waste water. As a result of this, the use of cassava milling machine cannot be avoided. It is hereby recommended that the government of Cross River State and Nigeria at large should set aside, a large expanse of land away from residential areas where cassava processing milling machines should be located just as we have mechanic villages where mechanic workshops are located in some of our cities today. Those involved in the processing of cassava and the cassava farmers do not understand the implication of the dangers posed by cassava effluent because most of them are illiterates. Enlightenment campaigns by the government and agricultural extension workers, on detoxifying cassava effluent in accordance with standard and appropriate methods of disposal of both solids and cassava waste water are recommended for save and healthy environments. More research work should be done on the effects of cassava mill effluent on the surrounding water bodies and its effect on aquatic and human life.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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