Onuoha *et al.*, (2023)
Biological and Environmental Sciences Journal for the Tropics 20(3) December, 2023
ISSN 0794 – 9057; eISSN 2645 - 3142
BEST JOURNAL 20(3): 69 - 79
Date received: 11/08/2023
Date accepted: 19/11/2023
https://dx.doi.org/10.4314/bestj.v20i3.7

Impact of Cassava mill Effluent on the Microbial Population and Distribution in Polluted Soils in Abakaliki, Ebonyi State, Nigeria

Nweke, R.N[1], Onuoha, S .C[*1], and Orji, J.O[2]
[1]Department of Biotechnology, Faculty of Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.
[2]Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
*Correspondence: sconuoha@yahoo.com; Tel: +2348032385682

ABSTRACT
Cassava mill effluent generated from cassava processing when discharged to the soil, alters the nature of soil properties and also become a major cause of environmental degradation. This research characterized bacteria and fungi distributed in cassava effluent polluted soils in Abakaliki, Ebonyi State, Nigeria. Soil samples were collected over a period of three months using sterile soil auger into sterile sample zip bag for analysis. The bacterial count from the different sites varied significantly ($p<0.01$). The total colony count of bacteria from the effluent obtained from rice mill cassava processing sites ranged from $4.0\pm0.2x10^6$ CFU/g to $8.6\pm0.8x10^6$ CFU/g. During the month of August, the bacterial loads also differed very significantly ($p<0.01$) ranging from $3.3\pm0.3x10^6$ CFU/g to $6.5\pm0.6x10^6$ CFU/g across the locations. Similarly, the fungal count in varied significantly ($p<0.01$) ranging from $1.7\pm0.1x10^6$ CFU/g to $3.4\pm0.3x10^6$ CFU/g across the locations in the month of June. The bacteria species isolated from the polluted soil samples include *E. coli*, *Bacillus* spp. *Staphylococcus* spp., *Klebsiella pneumoniae*, *Shigella* spp., *Pseudomonas aeruginosa* and *Proteus* spp. While the fungal species identified include *Articulospora inflata, Trichoderma spp*., *Aspergillus* spp. and *Penicillium* spp. These organisms were also present in the unpolluted soil samples except for *Articulospora inflata* which was solely isolated from polluted soil sample in some of the sampled sites. This study has shown that bacterial population was higher in cassava mill polluted soil compared to the unpolluted sites. Whereas, the fungal population indicated only a mild increase in the polluted compared to the unpolluted sites.

Keywords*:* Bacteria; Cassava effluents; Fungi; Polluted soil

INTRODUCTION
Cassava (*Manihot esculenta*) belongs to the Euphorbiaceae family and it is a major staple food in Africa, especially Nigeria (Afuye *et al.*, 2015). Nigeria is the largest producer of cassava while the greatest exporter of this crop is Thailand. Cassava can be processed into diverse traditional delicacies including garri, fufu, lafun and flour among others, some of which are fermented products (Oti, 2002). Among all the products processed from cassava, garri is the most common in Nigeria. Garri production is done in varying scales: small, medium and large (Uzoije *et al.*, 2011). Cassava processing into garri involves several unit operations vis-vis peeling, washing, grating, pressing and fermenting (dewatering), sieving, roasting and drying (Okafor and Uzuegbu, 2008).
However, cassava processing generates a large volume of effluent (wastewater) which is a source of land pollution that creates environmental nuisance if discharged into the environment (Eze, 2015).

Clusters and seasonal processing activities of cassava tend to generate more effluent than can be utilized or converted into other finished products, hence poses a big challenge to managed. Environment pollution occurs when wastewater from cassava production are discharged on land or waterways indiscriminately and this in turn, affects the biota especially in Southern part of the country where most of the mills are located (Olorunfemi *et al*., 2008).

In addition, pollution of the environment occurs when waste water discharged from cassava processing sites is allowed to percolate 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. 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 (Etta *et al.,* 2019). Eventually this effluent may be soaked into the soil or flow into streams. A lot of the cassava effluent arise from processing end up with domestic waste, while others seep into the soil. A fair volume of the cassava effluent is carried in solution, some have become so finely divided that they exist in a colloidal state, while others go into suspension (Agedah, 2015).

The discharge of waste products and contaminants into surface runoff get into the rivers through drainage systems, leaching into liquid spills as well as groundwater and wastewater discharges. The effluent include the milky colloid pressed out of the fresh tuber paste, the latex, the wash water, etc. Reports have shown that cassava effluent contains harmful cyanides, copper, mercury and nickel which have the capacity to affect native microbiota (Aiyegoro *et al.,* 2007). The risk to human lives, microbes and aquatic organisms constituted by industrial and gaseous effluents cannot be over-stressed (Okafor, 2011).

The effluent from cassava greatly affected the activities of the microorganisms in the polluted soil and the soil became more acidic in nature (Akpan *et al.,* 2011). Food and Agriculture Organization FAO (2008) found that the total bacteria (*Lactobacillus plantarium*, *Pseudomonas aeruginosa*, *Bacillus spp.* and *Viltro* spp.) obtained from the contaminated soil with cassava wastewater was more than that in the soil without contaminant. According to Nwankwo *et al.* (2005), organisms isolated during the fermentation of cassava tubers, as practiced for "fufu" production included *Bacillus subtilus*, *Pseudomonas alkaligenes*, *Lactobacillus plantarium*, *Leuconostic mensenteriodes* and *Pseudomonas aeruginosa*. Hence, pollution from such effluent could result to a serious imbalance in the living and non-living entities of the ecosystem. This could also lead to a reduction in the soil fertility. This study is aimed to isolate and characterize bacteria and fungi present in soil from selected cassava effluent polluted soil in Abakaliki, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out in ten (10) selected cassava mill plants including Abofia, Agbaja, Echara Unuphu, Igwe-Okpo, Ndiebor, Mgbabor, Obegu, Onuebonyi, Sharon, and Rice mill all in Abakaliki, Ebonyi State. Ebonyi State is located approximately within latitude 6^{o} 20'N and longitude 8^{o} 06'E in the derived savannah of South-Eastern part of Nigeria at an elevation of 117 m. The rainfall pattern is bimodal (April-July and September - November) with a short spell in August referred to as August break and annual rainfall of about 1,800 - 2,000 mm. The average temperature is between 25 ^{o}C in January, 34 ^{o}C in June and 30 ^{o}C in November and the relative humidity is between 60 - 80 % (Oboh, 2006; Ude, 2011).

Abakaliki has a population of about one hundred and thirteen thousand, one hundred and thirty (113,130) people (Uhegbu *et al*., 2012). The major occupations of the people in this are farming and trading; there are also civil servants and students.

Culture media

The culture media used for this study include Nutrient agar, Mannitol salt agar, *Salmonella-shigella* agar, Mac Conkey agar, and potato dextrose agar. All the media were manufactured by (Titan Biotech, India) and prepared according to the manufacturer's instructions.

Samples collection

A total of 60 soil samples were aseptically collected using a well sterilized soil auger into a sterile zip bags from a depth of 0-20 cm (Akpan *et al.,* 2011) from soil polluted with cassava effluent in selected communities in Ebonyi State. The samples collected were carefully labeled and conveyed to the Applied Microbiology Laboratory, Ebonyi State University, Abakaliki for further processing and microbiological analysis using standard procedures. Additionally, soil samples free of cassava effluents were collected from control sites outside the processing plant. All samples were collected between the months of June and August, 2022.

Sample preparation

One gram (1 g) each of the soil sample was added into a sterile test tube and 9 mL of distilled water was added to make a stock solution. Dilutions were prepared from the stock solution to 10^{-10}. Diluted samples were used for microbial analysis according to the method of Akpan *et al.* (2011).

Determination of microbial Load and identification of species of Microorganisms in the soil samples

Each of the soil samples was allowed to air-dry at room temperature in the laboratory. Then 1 g of the sample was suspended into 9 mL of distilled water and 1 mL from the 1^{st} to the 5^{th} dilution was plated onto nutrient agar and potato dextrose agar respectively by pour plate techniques. The plates were incubated at 37 ^{o}C for 24 h for bacterial culture and 72 h for fungal culture. The colony count was performed with conventional plate count method using colony counter (Cheesbrough, 2006). Based on the colonial morphology, suspected colonies of *E. coli*, *Proteus* and *Klebseilla* spp were subcultured on MacConkey and Eosin methylene blue (EMB) agar for further identification and differentiation (Onuoha *et al,* 2016). Similarly, colonies suspected to be *Staphylococcus aureus* were sub-cultured on mannitol salt agar while *Salmonella-Shigella* agar was used for the isolation of *Shigella* spp. Meanwhile, potato dextrose agar was used for the identification of fungi**.** The suspected colonies of bacteria species (*E. coli, Klebsiella, Shigella, Proteus, Pseudomonas, Staphylococcus, Bacillus* spp.) and fungi spp. were sub-cultured and stored on nutrient agar and potato dextrose agar slants respectively from which Gram staining and biochemical tests were carried out for further identification.

RESULTS

Identification of the isolated microorganisms

The isolates were pre-identified based on their appearance on culture media. *Escherichia coli* was identified on EMB with greenish metallic sheen feature, *Klebsiella* spp. showed pink mucoid colonies on MacConkey agar, *Staphylococcus aureus* indicated golden yellow colonies on mannitol salt agar. Other isolates that were identified includes *Pseudomonas* spp. based on greenish colour, *Proteus* spp. based on swarming growth on plates, *Bacillus* spp. (milky colonies) and *Shigella* spp. which was smooth and pink colonies on *Salmonella-Shigella* agar (Table 1).

The isolates were further identified based on their biochemical characteristics. The *S. aureus* and *Bacillus* spp. were identified as Gram positive bacteria; they were methyl red, oxidase and indole negative; but catalase and citrate positive. On the other hand, *Pseudomonas*, *Klebsiella*, *Shigella* and *Proteus* spp. were found to be Gram negative bacteria; they are citrate, indole, catalase positive, except *Pseudomonas* spp. (indole negative) and all the Gram negative isolates identified in this study were methyl red negative as shown in Table 1.

Table 1. Morphological and biochemical identification of bacterial isolates

Morphology	Gram's staining	Methyl Red	Citrate	Catalase	Indole	Oxidase	Suspected organism
Pink, smooth	-	-	+	+	+	-	*E. coli*
Greenish	-	-	+	+	-	+	*Pseudomonas* spp.
Pink, mucoid, rough	-	-	-	+	+	-	*Klebsiella* spp.
Pink & Smooth	-	-	+	+	+	-	*Shigella* spp.
Swarming	-	-	+	+	+	-	*Proteus*
Milky	+	-	+	+	-	-	*Bacillus*
Golden yellow	+	-	+	+	-	-	*Staphylococcus aureus*

Key: + = Positive, - = Negative

Bacterial population across the locations

The total bacterial colony count of the cassava effluent polluted area showed that rice mill cassava processing site had the highest bacteria load of $8.6\pm0.8x10^6$ CFU/g followed by Abofia and Sharon ($5.8\pm1.3x10^6$ CFU/g and $5.8\pm1.2x10^6$ CFU/g) respectively while Igwe-Okpo cassava processing sites recorded the lowest bacterial population ($4.0\pm0.2x10^6$ CFU/g) as shown in Figure 1. A decline in bacteria load was recorded at rice mill cassava processing site in July, with the count ranging from $8.5\pm0.7x10^6$ CFU/g to $3.3\pm0.3x10^6$ CFU/g. Abofia recorded significantly higher bacterial count ($8.5\pm0.7x10^6$ CFU/g) followed by Mgbabor ($6.9\pm3.0x10^6$ CFU/g), Obegu (6.9 ± 0.7 $x10^6$ CFU/g) and Onuebonyi ($6.8\pm0.3x10^6$ CFU/g) but Echara Unuphu recorded significantly lowest bacterial load ($3.3\pm0.3x10^6$ CFU/g) (Figure 1). Meanwhile, in August, the bacterial loads across the location were significantly higher in Obegu cassava effluent pollution site ($6.5\pm0.6x10^6$ CFU/g) compared to other locations. This was followed by Abofia ($5.4\pm0.2x10^6$ CFU/g), Echara Unuphu ($5.1\pm1.5x10^6$ CFU/g) and Onuebonyi ($5.0\pm0.7x10^6$ CFU/g) while Mgbabor recorded the lowest colony forming units ($3.3\pm0.3x10^6$ CFU/g) as shown in Figure 1. There was a significant difference ($p<0.05$) in the bacterial colony count across the locations within the three months.

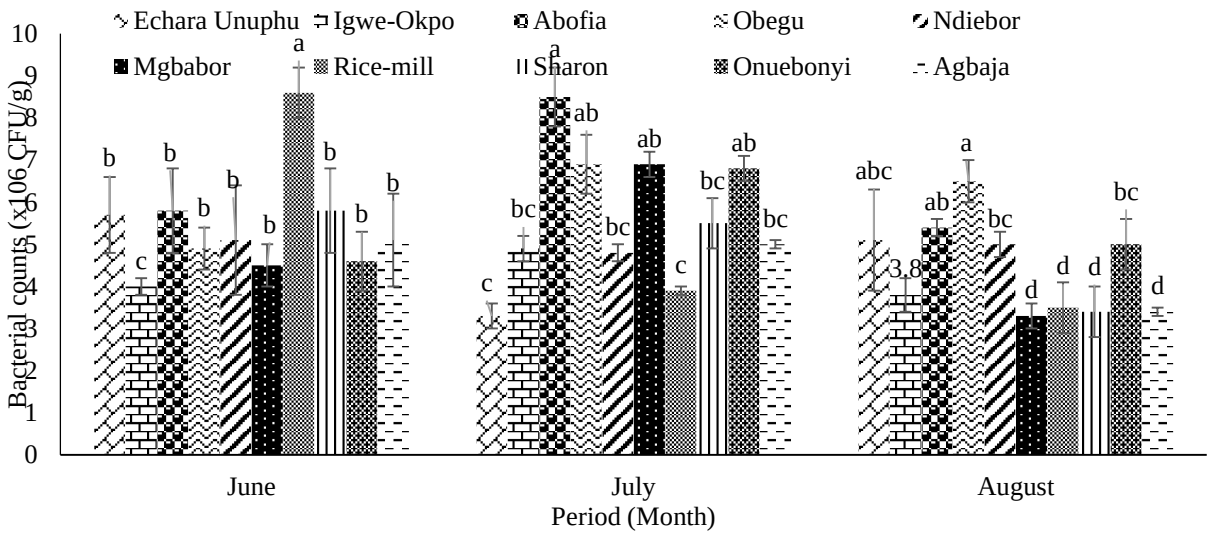

Figure 1: Bacterial population of cassava effluent polluted soil across the sampling locations and study period

Fungal population across the sampling locations

The fungal colony count in the month of June was significantly higher in Obegu ($3.4\pm0.3x10^6$ CFU/g), followed by Mgbabor ($3.2\pm0.8x10^6$ CFU/g) and Abofia ($3.0\pm0.1x10^6$ CFU/g) but lowest in Rice-mill ($1.7\pm0.1x10^6$ CFU/g). Meanwhile, in the month of July, the fungal count was highest in Echara Unuphu polluted site ($2.6\pm0.5x10^6$ CFU/g) followed by Agbaja and Ndiebor polluted sites ($2.3\pm0.5x10^6$ CFU/g and $2.3\pm0.2x10^6$ CFU/g) respectively, but lowest fungal population for the month was observed in Sharon cassava-mill effluent contaminated site ($1.7\pm0.2x10^6$ CFU/g). However, in the month of August, Abofia recorded the highest fungal count ($2.9\pm0.1x10^6$ CFU/g) followed by Echara Unuphu contaminated site ($2.2\pm0.4x10^6$ CFU/g), but lowest in Sharon ($1.2\pm0.3x10^6$ CFU/g) as shown in Figure 2. There was significant difference ($p<0.05$) in the fugal population across the study area.

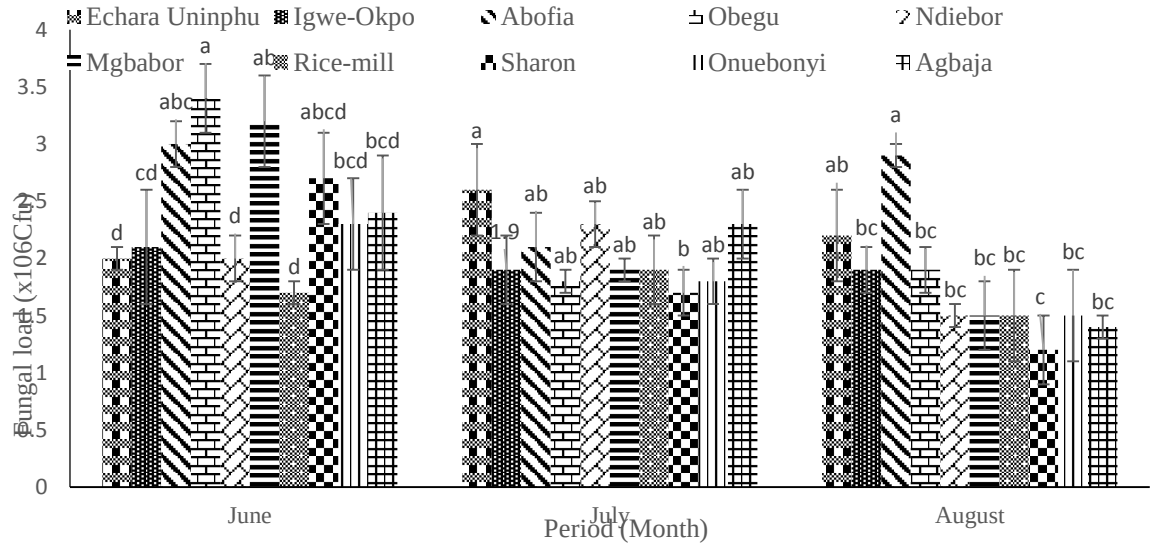

Figure 2: Fungal population across the sampling locations

Bacterial distribution across different location contaminated with cassava processing effluent

The bacterial species identified in both cassava effluent polluted and unpolluted sites across the locations are presented in Table 2. The result showed that in Echara unuphu, *E. coli* and *Staphylococcus aureus* were isolated from the polluted sites while only *E. coli* was isolated from the unpolluted sites in the month of June. In same month, *Staphylococcus* spp. were isolated from Igwe-Okpo polluted site while *E. coli* and *Staphylococcus aureus* were identified from the unpolluted site. In Abofia, *Klebsiella pneumonia* was isolated from both the contaminated and unpolluted sites while *E. coli* was the only isolate obtained from Obegu polluted and unpolluted sites. However, only *Shigella* spp. were identified from both sites in Mgbabor. On the other hand, Ndiebor sites gave two bacterial species: *E. coli* (from the polluted site) and *Klebsiella pneumonia* (from the unpolluted site). *Escherichia coli* was also identified in Rice-mill unpolluted sites while *Staphylococcus aureus* and *Klebsiella* spp. were isolated from the polluted site. In Sharon samples, *Shigella* spp. was obtained from the polluted site while *E. coli* was obtained from the unpolluted site. Similarly, in Onuebonyi, *E. coli* was isolated from the polluted site while *Pseudomonas aeroginosa* was obtained from the unpolluted site. But in Agbaja, *Shigella* spp. was isolated from polluted site while *Proteus* spp. was the only isolate obtained from the unpolluted site.

Meanwhile, similar bacterial species found in the month of June were identified in the July. Echara Unuphu sample gave *Staphylococcus aureus* (in the polluted site) and *E. coli* was isolated from the unpolluted site, Igwe-Okpo showed only *Klebsiella* spp. in polluted soil, while but *Staphylococcus aureus* and *Shigella* spp. were isolated from the unpolluted site. In Abofia, *Proteus* spp. and *Staphylococcus* spp. were identified in the polluted and unpolluted sites respectively, but in Obegu, only *Klebsiella* spp. was identified in both locations while Ndiebor samples gave yielded *E. coli* in both sites. However, Mgbabor samples gave *E. coli* (in the polluted soil) and *Klebsiella* spp. and *E. coli* (from the unpolluted soil). Also, only *Staphylococcus aureus* was isolated from both sites in Rice-mill, but Sharon sample gave *E. coli* in polluted location and *Proteus* spp. in unpolluted site. In Onuebonyi, *Pseudomonas* spp. was isolated from the polluted site while *E. coli* was isolated from the unpolluted sites, but in Agbaja, *Klebsiella pneumoniae* was isolated from the polluted site and *Pseudomonas* spp. from the unpolluted site (Table 2).

Moreso, in August, only *Staphylococcus* spp. were identified from both sites in Echara Unuphu, while *E. coli* was the solely isolated from both site in Igwe-Okpo. In Abofia and Obegu, *Shigella* spp. and *E. coli* were isolated from the polluted and unpolluted sites respectively; while *Staphylococcus* spp. were the sole isolates from both sites in Ndiebor. Additionally, *E. coli* was isolated from Mgbabor polluted site, whereas, *Staphylococcus* and *Shigella* spps. were obtained from the unpolluted site. Rice-mill samples yielded *E. coli* and *Klebsiella* spp. in cassava effluent contaminated location while only *Klebsiella* spp. was identified in the unpolluted site. In Sharon sample, *Staphylococcus* spp. and *E. coli* were isolated from the polluted site while *Klebsiella* spp. was identified from the unpolluted site. Onuebonyi sample yielded *E. coli* (in the polluted site), *E. coli* and *Staphylococcus* spp. were isolated from the unpolluted site. Conversely Agbaja indicated the presence of *Klebsiella* spp. in the polluted site and *E. coli* from the unpolluted site as shown in Table 2.

Table 2: Bacterial spp distribution across locations contaminated with cassava processing effluent over three months duration

S/N	Location	June		July		August	
		Polluted soil	Unpolluted soil	Polluted soil	Unpolluted soil	Polluted soil	Unpolluted soil
1	Echara Unuphu	*E. coli*, *Staphylococcus* spp.	*E. coli*	*Staphylococcus aureus*	*E. coli*	*Staphylococcus* spp.	*Staphylococcus* spp.
2	Igwe-Okpo	*Staphylococcus aureus*	*E. coli*, *Staphylococcus* spp.	*Klebsiella* spp.	*Staphylococcus aureus*, *Shigella* spp.	*E. coli*	*E. coli*
3	Abofia	*Klebsiella pneumonia*	*Klebsiella pneumonia*	*Proteus* spp.	*Staphylococcus* spp.	*Shigella* spp.	*E. coli*
4	Obegu	*E. coli*	*E. coli*	*Klebsiella* spp.	*Klebsiella* spp.	*Shigella* spp.	*E. coli*
5	Ndiebor	*E. coli*	*Klebsiella pneumonia*	*E. coli*	*E. coli*	*Staphylococcus* spp.	*Staphylococcus spp.*
6	Mgbabor	*Shigella* spp.	*Shigella* spp.	*E. coli*	*Klebsiella* spp., *E. coli*	*E. coli*	*Staphylococcus spp.& Shigella* spp.
7	Rice-mill	*Staphylococcus aureus*, *Klebsiella* spp.	*E. coli*	*Staphylococcus* spp.	*Staphylococcus* spp.	*E. coli & Klebsiella* spp.	*Klebsiella* spp.
8	Sharon	*Shigella* spp.	*E. coli*	*E. coli*	*Proteus* spp.	*Staphylococus* spp. *& E. coli*	*Klebsiella* spp.
9	Onuebonyi	*E. coli*	*Pseudomonas aeroginosa*	*Pseudomonas* spp.	*E. coli*	*E. coli*	*E. coli & Staphylococcus* spp.
10	Agbaja	*Shigella* spp.	*Proteus* spp.	*Klebsiella pneumonia*	*Pseudomonas* spp.	*Klebsiella* spp.	*E. coli*

Fungal distribution across locations contaminated with cassava processing effluent

The result in Table 3 showed that only *Trichoderma spp*. was the fungal spp. isolated from both (the polluted and unpolluted site) sites in Echara Unuphu, Abofia, Mgbabor, Rice - mill and Agbaja in the month of June. Meanwhile, *Aspergillus nigar* was isolated from Igwe-Okpo, Obegu and Onuebonyi unpolluted sites and *Articulospora inflata* from Sharon unpolluted site. However, *Articulospora inflata* was isolated from Igwe-Okpo, Ndiebor and Sharon polluted sites *Aspergillus niger* was isolated from Obegu and Onuebonyi polluted samples (Table 3).

Similarly, in July, *Trichoderma* spp. and *Aspergillus* spp. were isolated from Echara unuphu and Agbaja polluted and unpolluted samples respectively, but only *Trichoderma* spp. was the fungal spp. isolated from both samples from Igwe-Okpo and Onuebonyi. Also, Abofia showed on *Aspergillus* spp. in both polluted and unpolluted sites, while Obegu showed *Aspergillus* spp. and *Penicillium* spp. (in polluted and unpolluted sites respectively). In Ndiebor and Rice-mill, *Aspergillus* spp. and *Trichoderma* spp. were identified in polluted and unpolluted samples respectively while in Mgbabor, *Penicilium* spp. and *Aspergillus* were identified (polluted and unpolluted sites). In Sharon samples, *Aspergillus* spp. were identified while *Trichoderma* spp. was the prevalent spp. in Onuebonyi samples, but in Agbaja *Trichoderma* spp. and *Aspergillus* spp. were isolated from the unpolluted and polluted sites respectively.

In August, however, *Aspergillus nigar* was identified from soil samples from Echara unuphu and Ndiebor, but *Trichoderma* spp. and *Penicillium* spp. were obtained from Igwe-Okpo and Sharon soil samples (unpolluted and polluted sites respectively).

Furthermore, *Trichoderma* spp. and *Articulospora inflata* were the fungal spp. isolated from Obegu samples (unpolluted and polluted sites respectively) but only *Trichoderma* spp. was isolated in mgbabor, Onuebonyi and Agbaja as shown in Table 3.

Table 3: Fungal spp. distribution across locations contaminated with cassava processing effluent

S/No.	Location	June		July		August	
		Unpolluted	Polluted	Unpolluted	Polluted	Unpolluted	Polluted
1	Echara Unuphu	*Trichoderma* spp.	*Trichoderma* spp.	*Trichoderma* spp.	*Aspergillus* spp.	*Aspergillus niger*	*Aspergillus niger*
2	Igwe-Okpo	*Aspergillus* spp.	*Articulospora inflate*	*Trichoderma* spp.	*Trichoderma* spp.	*Trichoderma* spp.	*Penicillium* spp.
3	Abofia	*Trichoderma* spp.	*Trichoderma* spp.	*Aspergillus* spp.	*Aspergillus* spp.	*Penicillium* spp.	*Trichoderma* spp.
4	Obegu	*Aspergillus* spp.	*Aspergillus* spp.	*Aspergillus* spp.	*Penicilium* spp.	*Trichoderma* spp.	*Articulospora* inflata
5	Ndiebor	*Trichoderma* spp.	*Articulospora inflata*	*Aspergillus* spp.	*Trichoderma* spp.	*Aspergillus niger*	*Aspergillus niger*
6	Mgbabor	*Trichoderma* spp.	*Trichoderma* spp.	*Penicilium* spp.	*Aspergillus* spp.	*Trichoderma* spp.	*Trichoderma* spp.
7	Rice-mill	*Trichoderma* spp.	*Trichoderma* spp.	*Aspergilus* spp.	*Trichoderma* spp.	*Aspergillus niger*	*Trichoderma* spp.
8	Sharon	*Articulospora inflata*	*Articulospora inflata*	*Aspergillus* spp.	*Aspergillus* spp.	*Trichoderma* spp.	*Penicillium* spp.
9	Onuebonyi	*Aspergillus* spp.	*Aspergillus* spp.	*Trichoderma* spp.	*Trichoderma* spp.	*Trichoderma* spp.	*Trichoderma* spp.
10	Agbaja	*Trichoderma* spp.	*Trichoderma* spp.	*Trichoderma* spp.	*Aspergillus* spp.	*Trichoderma* spp.	*Trichoderma* spp.

DISCUSSION

Cassava processing generates great volume of effluent (wastewater) which causes land pollution that create environmental nuisance in the region when discharged into the environment (Eze, 2015). This study observed the average total bacterial colony count of the cassava effluent polluted area to range from $4.0\pm0.2x10^6$ CFU/g (Igwe-Okpo) to $8.6\pm0.8x10^6$ CFU/g (rice mill) across the locations studied. Fungal colony count also ranged from 1.4 ± 0.1 to $3.4\pm0.3x10^6$ CFU/g across the locations studied within the three months study period. There was a significant difference ($p<0.05$) in the microbial load among the locations and between polluted and unpolluted sites. In a similar study by Akpan *et al.* (2011) on the effects of cassava mill effluent on some chemical and micro-biological properties of soils in Cross River State, Nigeria, it was reported that bacterial population ranged from $1.6x10^6$ to $4.8x10^6$CFU/g in unpolluted site and $3.4x10^6$ to $5.2x10^6$ CFU/g in polluted sites. While the fungal population ranged from $4.0x10^3$ to $9.0x10^3$ in both the unpolluted and polluted soils. The findings above is in line with the findings of this study which reported bacterial population of $5.8\pm1.3x10^6$ CFU/g and $5.8\pm1.2x10^6$ CFU/g in cassava wastewater (effluent) polluted soil at Abofia and Sharon respectively (Fig 1). The observation of higher bacterial population in polluted sites compared to the unpolluted locations across the study areas suggests that cassava wastewater (effluent) polluted soil has higher microbial activities. This can be attributed to the presence of biodegradable compounds as well as higher decomposable organic matter content impacted on the soil by the polluting cassava effluent.

The microbial spp. dynamics over the period of three months revealed the presence of *E. coli, S. aureus, K. pneumoniae, Shigella* spp.*, P. aeroginosa, Bacillus* spp. and *Proteus* spp. across the cassava wastewater effluent polluted sites although their population where significantly higher ($P<0.05$) in the polluted samples when compared to unpolluted sites in this study. Similarly, Obueh and Odesiri-Eruteyan (2016) who carried out a study on the effects of cassava processing wastes on the soil environment of a local cassava mill, and identified similar bacteria spp. such as *K. pneumoniae, P. aeroginosa, Lactobacillus plantarum, L. delbruekii* and *S. aureus* as well as the following fungal spp. *Fusarium solani, Aspergillus niger* and *Saccharomyces cerevisiae* from cassava effluent and soil samples polluted with cassava wastewater. Izah *et al.* (2018) who studied the impacts of cassava mill effluents in Nigeria, further reported microbial diversity comprises of several microbial genera which includes *Neisseria, Streptococcus, Staphylococcus, Bacillus, Entrobacter, Proteus, Lactobacillus, Pseudomonas, Micrococcus, Saccharomyces, Penicillium, Aspergillus* and *Mucor.* Another study also identified the presence of microbes which include *Lactococcus lactis* and *Bacillus subtilis;* as well as fungi (*Articulospora inflate, Trichoderma* spp.*, Aspergillus niger, Fusarium, Rhizopus* and *Penicillium* spp.) in cassava wastewater effluent polluted and unpolluted soils (Akpan *et al.,* 2011). Thus, the above report by previous researches is akin with the findings of this study which reported presence of similar bacteria and fungi (*Penicilium* spp. and *Aspergillus* spp., *Articulospora inflate,* and *Trichoderma spp.*) from both polluted and unpolluted sites. The result of this study suggests no significant difference ($p<0.05$) in the microbial (bacterial and fungal) population structure across the locations between polluted and unpolluted sites but inherent microbial spp. inhabiting an area also colonized the polluted site which lead to significant increase in their population as a result higher nutrient availability. The increase in bacteria and fungi in the polluted soils might help in the rapid decomposition of organic matter and the release of essential nutrients from the soils for plants growth. The hydrocarbon utilizing bacteria (HUB) including *Bacillus* spp. as observed in this study may help in the breakdown of the cassava waste effluent while other catalase positive organisms (including *E. coli, Pseudomonas* spp., *Klebsiella* spp., *Shigella* spp., *Staphylococcus aureus* etc.) may use the pollutants as substrate to catabolize glucose sugar to pyruvate and decarboxylate the pyruvate in the effluent to acetaldehyde which later reduces to ethanol by alcohol dehydrogenase with nicotinamide adenine dinucleotide phosphate (NADP) (Akpan *et al.,* 2011). This reaction affects crop production because during this process energy and CO_2 are liberated for effective respiration by the plants. Meanwhile, the hydrocarbon utilizing fungi (HUF) such as *Aspergillus niger* and *Articulospora inflata* are slow decomposers and secondary consumers which live on already prepared substrate.

CONCLUSION

This study has shown that bacterial population was higher in polluted soil samples when compared to the unpolluted sites, while fungal population showed only a mild increase in polluted site compared to the unpolluted sites. The bacteria spp. identified in the polluted soil samples include *E. coli, Bacillus* spp. *Staphylococcus* spp*., Klebsiella pneumonia, Shigella* spp*., Pseudomonas aeruginosa* and *Proteus* spp., while the fungal spp. identified were *Articulospora inflata, Trichoderma* spp., *Aspergillus* spp. and the *Penicillium* spp.. These organisms were also present in the unpolluted soil samples except for *Articulospora inflata* which was solely observed in polluted soil sample across some locations.

This study has also shown the dynamics in microbial population structure of soil exposed to cassava effluent pollution. There is need to adopt the report of this study as a guide to further investigate the specific roles of each spp. in toxic hydrocarbon degradation such as hydrogen cyanide. The isolates with promising result may then be adopted and utilized in cassava fermentation and processing to produce cyanide free cassava products such as garri.

REFERENCES

Afuye, G. G. and Mogaji, K. O. (2015). Effect of cassava effluents on domestic consumption of 'shallow well' water in Owo Local Government Area, Ondo State, Nigeria. *Physical Science and Research International Journal*, **3(3)**:37-43.

Agedah, E. C., Ineyougha, E.R., Izah, S.C. and Orutugu, L.A. (2015). Enumeration of total heterotrophic bacteria and some physicochemical characteristics of surface water used for drinking sources in Wilberforce Island, Nigeria. *Journal of Environmental Treatment Techniques*, **3(1)**:28–34.

Aiyegoro, O. A., Akinpelu, D. A., Igbinosa, E. O. and Ogunmwonyi, H. I. (2007). Effect of cassava effluent on the microbial population dynamic and physicochemical characteristic on soil community. *Journal of Science Focus*, **12:** 98-101.

Akpan, J. F., Solomon, M. G. and Bello, O. S. (2011). Effects of cassava mill effluent on some chemical and micro-biological properties of soils in Cross River State, Nigeria. *Global Journal of Agricultural Sciences*, **10**(2): 89-97

Cheesbrough, M. (2006). District laboratory practice in tropical countries, Microbiological tests. Chapter 7.**In**: Cheesbrough M, Ed. pt 2. 2nd Ed. Cambridge: Cambridge University Press. pp: 9-267.

Eze, V. C., and Onyilide, D. M. (2015). Microbiological and physiochemical characteristics of soil receiving cassava effluents in Elele, Rivers state, Nigeria. *Journal of Applied and Environmental Microbiology*, 3(1), 20 – 24.

Etta, A. B., Victor, O. A., Young, I. A., Asuquo, B. A. and Asiya, A. M. (2019). Assessment of the effects of cassava mill effluent on the soil and its microbiota in Biase local government area of Cross river state, Nigeria. *World Journal of Advanced Research and Reviews*, 1(2): 034-044.

Food and Agriculture Organization, FAO (2008). The global cassava development strategy. Published by Food and Agricultural Organization. http://www.fao.org/ag/agp/agpc/gcds/en/strategy.html (Assessed Jan.10, 2022).

Izah, S.C., Bassey, S.E. and Ohimain, E.I. (2018). Impacts of cassava mill effluents in Nigeria. *Journal of Plant and Animal Ecology*, **1** (1): 14-41.

Onuoha, S.C, Okafor C.O, Aduo, B.C and Nwaka. F.C (2016) Distribution of Antibiotic Resistant Bacteria from Abattoir Wastes and its Receiving Waters at Nkwo-ezzamgbo, Ebonyi State, Nigeria, *World Journal of Medical Sciences*, 13 (4): 242-250

Oti, E.E. (2002). Acute toxicity of cassava mill effluent to the African catfish fingerlings. *Journal of Aquatic Science*, **17**: 31- 34.

Okafor, J.O. (2011). Physico-chemical characteristics of effluents from garri processing industries in Bida, Niger State, Nigeria. *Bayero Journal of Pure Applied and Sciences*,**4:** 150-154.

Okafor, N. and Uzuegbu, J.O. (2008). Studies on the contribution of micro-organism on the organo-leptic properties of garri. A fermented food derived from cassava (*Manihot esculenta* Gantz). *Journal of Food Agriculture*,**2:** 99-105.

Olorunfemi, D.I., Emoefe, E.O. and Okieimen, F.E. **(**2008). Effect of Cassava Processing Effluent on Seedling Height, Biomass and Chlorophyll Content of Some Cereals. *Research Journal of Environmental Sciences*,**2(3):** 221-227.

Obueh, H.O. and Odesiri-Eruteyan, E. (2016). A Study on the effects of cassava processing wastes on the soil environment of a local cassavamill. *Journal of Pollution, Effect and Containment*,**4**: 177

Oboh, G. (2006). Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisae* and *Lactobacillus* spp. solid media fermentation. *Electronic Journal of Biotechnology*, **9**(1): 46–49.

Nwankwo, E.D., Anadu E. and Usoro, R. (2005). Cassava fermenting organisms. *World Journal of Microbiology and Biotechnology*, **5**: 169- 179

Ude, E.F. (2011). Evaluation of Seasonal Occurrence of Cat fish Families in Ebonyi River, Nigeria. *Nigerian Journal of Fisheries*. **8** (1):208-214.

Uzoije, A.P., Egwuonwu, N. and Onunkwo, A.A. (2011). Distribution of cyanide in acassava mill effluent polluted eutrictropofluvent soils. *African Journal of Natural Science*,**9:** 18-23.

Uhegbu, F.O., Akubugwo, E.I. and Iweala, E.E.J. (2012). Effect of garri processing effluents (waste water) on the cyanide level of some root tubers commonly consumed in the South East of Nigeria. *African Journal of Food, Agriculture, Nutrition and Development*, **12**(5): 6748 – 6758