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Original Research Article

The Effect of Cassava Mill Effluent on Soil Microorganisms in Aba, Nigeria

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Abstract

The impact of cassava mill effluent on total aerobic bioload of soil samples in Aba was determined between the months of August and October, 2013. The soil samples designated A, B, C, D, E were collected from cassava processing mill effluent dump sites with sterile containers and subjected to standard microbiological analysis. The results revealed a decrease in microbial load of soil as distance approaches the dump sites. The total bacterial and fungal counts of soil samples collected 50m away from the dump sites ranged from 3.0 $\times 10^6$ to 3.4 $\times$ 10^6 cfu/g and 4.1 $\times$ 10^6 to 4.6 $\times$ 10^6 cfu/g respectively, while those at the dump sites ranged from 1.2 $\times$ 10^6 to 1.5 $\times$ 10^6 cfu/g for total aerobic bacterial count and 2.3 $\times$ 10^6 to 2.9 $\times$ 10^6 cfu/g for the total aerobic fungal count. The various bacteria isolated from the dump site include *Bacillus* species, *Pseudomonas* species, *Klebsiella* species, *Escherichia coli*, *Micrococcus* species and *Chromobacterium* species while the fungal isolates include *Aspergillus* species, *Penicillum* species, *Mucor* species, *Rhizopus* species and yeast. The above results indicated an adverse effect of cassava mill effluent on soil microorganisms and calls for regulations on the disposal of the effluent to avoid environmental degradation.

Keywords

Bioload
Cassava mill effluent
Dump sites
Soil microorganisms
Soil samples

Introduction

Cassava (*Manihot esculenta* Crantz) is a root tuber crop that is widely cultivated in the tropical regions of the world (Oboh and Akindahunsi, 2003). It is mainly a food crop whose tubers are harvested between 7-13 months based on the cultivars planted. The tubers are quite rich in carbohydrates (85.9%) with very small amount of protein (1.3%), in addition to cyanogenic glucoside (Nwabueze and Odunsi, 2007). This high carbohydrate content makes cassava a major food item especially for the low income earners in most tropical countries especially Africa and Asia (Desse and Taye, 2001).

Cassava is believed to have originated from South America. In Africa, cassava was introduced in the 16th century around the Congo River basins (Cock, 1985). In sub-Saharan Africa, cassava is a major stable food that is consumed in processed forms in many areas. In West Africa and Nigeria in particular, the crop is most commonly consumed as garri, a dry granulated meal made from fermented cassava (IITA, 1990).

Currently, Nigeria is the highest producer of cassava in the world with growth and processing of more cassava for domestic and international needs. In Nigeria, many cassava processing industries have been established, even around residential homes; many people are involved in garri processing, particularly among the rural farmers. The raw materials are fresh cassava which is very abundant and cheap. The production and consequent consumption of cassava have increased extensively in recent times. Traditional garri production is associated with discharge of large amounts of water, hydrocyanic acid and organic matter in the form of peels and sieves from the pulp as waste products. Around the cassava mills, this liquid waste is indiscriminately discharged and allowed to accumulate, even near residential homes, producing offensive odours and unsightly scenarios (FAO, 2004; Okafor, 2008; Ehiadgonare et al., 2009). Effluent from grating mills running freely along surfaces contaminates agricultural farmlands and streams, as it percolates into underground water and subsoil. Studies have shown that at toxic concentrations of the effluent, germination of cereal seeds are prevented (Olorunfemi et al., 2008). The high cyanide content from the effluent equally poses significant threat to humans and the environment, which calls for regulations in the discharge of the waste generated (Akani et al., 2006; Adewoye et al., 2005). Hence the contemporary issue of investigating the extent to which the environment is adversely affected cannot be overemphasized. This work therefore intends to ascertain the impact of cassava mill effluent on soil microbial load.

Materials and methods

Sample collection

Soil samples were aseptically collected from five different cassava mill dumps (coded A to E) in Aba, Nigeria. The soil samples from the cassava mill dumps were collected at different distances: from the dump site, 5 m and 50 m away from the site using sterile plastic bags. The soil samples from the 50m distance served as control. All the samples were transported to the Microbiology Laboratory of Abia State Polytechnic, Aba for analysis in refrigerated coolers to arrest microbial growth.

Preparation of samples for analysis

The samples were air dried, crushed to fine particles and sieved using 2mm sieve and then stored in clean polyethylene bags in the refrigerator.

Serial dilution and inoculation

Ten-fold serial dilution of the samples was made as described by Cheesbrough (2005). One gram of each soil sample was homogenized in 9 ml distilled water. One ml of homogenized soil sample was transferred into the second tube containing 9 ml distilled water. This was continuously repeated until the tenth tube (10^{-10}).

Inoculation and enumeration

Two different media were used for analysis. The media (Nutrient agar and Potato dextrose agar) were prepared according to the manufactures' procedures. 0.1 ml of appropriate ten-fold serial dilution (10^{-6}) of the soil sample was inoculated into each of the nutrient media in triplicate using pour plate method (Cheesbrough, 2005). The inoculated plates were incubated at 37°C for 24 h for the enumeration of the total heterotrophic bacteria. Inoculated plates of Potato dextrose agar were kept at room temperature for 5-7 days. Visible discrete colonies on inoculated plates were counted using colony counter and expressed as colony forming units per gram (cfu/g) of the soil samples. Each discrete colony was counted using colony counter and recorded as total microbial count and expressed as colony forming units per gram of soil sample (cfu/g).

Characterization and identification of microbial isolates

Discrete colonies were purified by sub-culturing into appropriate agar media. Pure cultures of microbial isolates were identified based on cultural parameters. The isolates were identified using microscopic and biochemical analysis (Fawole and Oso, 2004; Cheesbrough, 2005).

Results

The total aerobic bacterial count at various distances from the cassava mill dump site is shown on Table 1.The result revealed an increase in total aerobic bacterial count as distance increased away from the dump site. The total aerobic bacterial count at the dump site ranged from 1.2×10^6 to 1.5×10^6 cfu/g while the bacterial count at 50m away from the dump ranged from 3.0×10^6 to 3.4×10^6 cfu/g. Similarly, the total fungal count also increased away from the dump site. At the dump site the total fungal count ranged from 2.3×10^6 to 2.9×10^6 cfu/g while it ranged from 4.1×10^6 to 4.6×10^6 cfu/g 50 m away from the dump site.

Table 1. Total aerobic bacterial and fungal counts at various distances from the dump site.

Sample	Distance	Total Aerobic Bacterial Count (cfu/g)	Total Fungal Count (cfu/g)
A	At the dump site	1.3×10^6	2.3×10^6
	5m away from the mill	2.3×10^6	3.9×10^6
	50m away from the mill	3.1×10^6	4.5×10^6
B	At the dump site	1.5×10^6	2.5×10^6
	5m away from the mill	2.1×10^6	3.6×10^6
	50m away from the mill	3.3×10^6	4.2×10^6
C	At the dump site	1.2×10^6	2.9×10^6
	5m away from the mill	2.5×10^6	3.7×10^6
	50m away from the mill	3.0×10^6	4.6×10^6
D	At the dump site	1.4×10^6	2.7×10^6
	5m away from the mill	2.7×10^6	3.5×10^6
	50m away from the mill	3.4×10^6	4.1×10^6
E	At the dump site	1.5×10^6	2.5×10^6
	5m away from the mill	2.6×10^6	3.8×10^6
	50m away from the mill	3.3×10^6	4.6×10^6
(Key: A-E represent different sampled cassava mile dump sites)			

Table 2. The morphological and biochemical characteristics of the bacterial isolates.

Tests	*Escherichia coli*	*Pseudomonas* species	*Bacillus* species	*Klebsiella* species	*Micrococcus* species	*Chromobacterium* species
Morphology	Rod	Rod	Rod	Rod	Cocci	Rod
Gram reaction	-	-	+	-	+	-
Spore	-	-	-	+	-	
Catalase	+	+	+	+	+	-
Oxidase	-	-	-	-	-	-
Coagulase	-	-	-	-	+	
Indole	+	-	-	-	-	
Methyl red	-	-	+	+	+	-
Voges-Proskauer	-	-	-	+	-	-
Urease	-	-	-	+	-	
Citrate	+	-	-	+	+	-
Lactose	A/G	-	A/G	A	A	-
Glucose	A/G	A/-	A/-	A/G	A	-
Sucrose	A/-	-	A/-	A	-	-
Maltose	A/G	-	A/G	A/G	A	-
Mannitol	A/-	A/-	A/-	A/-	A/-	-
(Key: A/G= Acid and gas production; A= Gas production; - = Negative; + = Positive)						

The morphological and biochemical characteristics of the bacterial isolates are shown in Table 2. A total of six bacterial isolates were identified, which include *Escherichia coli, Pseudomonas* species, *Bacillus* species, *Klebsiella* species, *Micrococcus* species and

Chromobacterium species. The morphological characteristics for the identification of fungal isolates are shown in Table 3. Fungal isolates identified include *Aspergillus* species, *Penicillum* species, *Mucor* species, *Rhizopus* species and yeast. Table 4 shows the percentage occurrence of the different isolates. *Bacillus* species, *Pseudomonas* species, *Klebsiella* species, *Aspergillus* species, *Penicillum* species, *Mucor* species and *Rhizopus* species had each 100% occurrence; *Micrococcus* species had 80% occurrence; *Escherichia coli* had 20% while *Chromobacterium* species and yeast had 10% occurrence respectively.

Table 3. Morphological characteristics of identified fungal isolates.

Colony Features	Morphological Characteristics	Probable Organisms
Moderate growth, dark branched colony and surfaces with numerous visible tiny black spots like spores.	Upright conidiophores, uniform hyaline, unsepted and terminating in a globose swollen head bearing phialids at the apex.	*Aspergillus* species
Moderate growth of mass of mycelia, greenish in colour with a velvety covering bearing visible dusty spores.	An erect conidiophores which is septed and branching midway giving rise to almost parallel branchlet ending in a group of phialids bearing conidia.	*Penicillum* species
Whitish colonies of limited growth with no visible mycelia, slightly evaluated colonies with serrated edge and dry surface.	Ovoid numerous cells and some bearing smaller attached ovoid cells or buds.	Yeast
Profuse growth of gray-brown woolly colonies.	Sporangiophore which does not produce rhizoid.	*Mucor* species
White colonies with cotton-like mycelia, some of which bear slightly dark spores on top.	Sporangiophores erect and uniform while others were horizontal (stolons). Each was unsepted and unbranched ending in a large globus.	*Rhizopus* species

Table 4. Percentage occurrence of the different microbial isolates.

Organisms isolated	A	B	C	D	E	Percentage occurrence (%)
Bacillus species	+	+	+	+	+	100
Pseudomonas species	+	+	+	+	+	100
Klebsiella species	+	+	+	+	+	100
Micrococcus species	+	+	+	-	+	80
Chromobacterium species	-	+	-	-	-	10
Escherichia coli	-	-	-	+	+	20
Aspergillus species	+	+	+	+	+	100
Penicillum species	+	+	+	+	+	100
Yeast	-	+	-	-	-	10
Mucor species	+	+	+	+	+	100
Rhizopus species	+	+	+	+	+	100

Discussion

According to Akani et al. (2006), there is increased utilization of processed cassava products which has equally increased the environmental pollution associated with disposal of cassava effluent. In most areas, cassava mills are mainly on small scale basis, owned and managed by individuals who have no basic knowledge of environmental protection (Adewoye et al., 2005). The results obtained from

this study revealed that samples obtained from the cassava dump sites showed at increase in microbial load as distance increases away from the dump sites. This could be attributed to the deleterious effects of the cassava mill effluent on the impacted soil samples. This lends credence to the report that the microbial load of all bacterial groups increases with distance away from the mill (waste pit), suggesting adverse growth conditions towards the pit (Nwaugo et al., 2008).

The high cyanogenic glycoside content of the effluent must have limited the growth of these microorganisms. Nwaugo et al. (2008) reported that when organic matter was added to the contaminated soil, in small concentrations, bacterial growth was encouraged. However the higher fungal count in this result could be attributed to the more robust nature of fungi which enables them to withstand the more acidic environment of the dump sites than bacteria. The ability of fungi to thrive better than bacteria in acid soils have been reported (Rangaswami and Bargyaraji, 1993).

A total of six bacterial genera were isolated, which include *Bacillus* species, *Pseudomonas* species, *Klebsiella* species, *Micrococcus* species, *Chromobacterium* species and *Escherichia coli*, while the fungal isolates include *Aspergillus* species, *Penicillum* species, yeast, *Mucor* species and *Rhizopus* species. This result corroborates with the findings of Ehiagbonare et al. (2009), Akpor et al. (2007), Okechi et al. (2012) and Ahanotu et al. (2013). These microorganisms may possess the genetic attributes that enable them to survive in such acidic environment. The results therefore indicate adverse effects of the cassava mill effluent on soil microorganisms and calls for regulations on the disposal of cassava mill effluent to avoid environmental degradation.

Conclusions

The results of this study have revealed adverse environmental effects of cassava mill effluents. It has impacted negatively on total aerobic microbial counts. This calls for serious rehabilitation, if such soil will be used for agriculture and other purposes as the necessary factors for soil health are negatively affected. This becomes more glaring as most of these cassava mills are sited near farms and residential areas. In addition, this study revealed the absence of proper regulation in the disposal of wastes (cassava mill effluent) and so calls for introduction of such regulations.

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