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Evaluation of the effects of cassava mill effluent on the microbial populations and physicochemical parameters at different soil depths

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Abstract

The effect of cassava mill effluent on the microbial populations and physicochemical parameters of the soil at various depths was studied. The result revealed the bacterial isolates as *Proteus mirabilis, Proteus vulgaris, Bacillus* sp*, Staphylococcus saprophyticus,* and *Klebsiella* sp*, Streptococcus* sp, while the fungal isolates were *Aspergillus* sp*, Penicillium* sp*, Mucor* sp and *Rhizopus* sp. In addition, total aerobic bacterial count results at P<0.05 revealed no statistical difference between surface ($4.2x10^6$ ±2.01 cfu/g) and subsurface counts ($3.9x10^6$ ±1.46 cfu/g) while both were significantly higher than the deeper sample counts ($2.3x10^6$ ±0.76cfu/g). Total fungal results showed significant difference at P<0.05 between surface ($4.2x10^6$±2.38 cfu/g) and subsurface (2.1x106±1.36 cfu/g) sample counts, as well as between surface and deeper sample counts (0.2x106±0.44 cfu/g), while no significant difference was observed between subsurface and deeper sample counts at P>0.05. The pH, cyanogenic glycosides, and C/N ratio all increased with depths while %OC, %N, %OM and ECEC decreased with depth. Cassava mill effluent has negatively affected the microbial populations and physicochemical parameters at various depths.

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Introduction

Nigeria, the World's largest producer of cassava *Mannihot esculanta* (Crunz) very unfortunately, has consistently generated so much waste from cassava mills which are usually discharged on land or water indiscriminately and this in turn, affects the biota especially in Southern part of the country where most of the mills are located (FAO,2004 and Olorunfemi *et al*., 2008). In Nigeria, cassava can be converted to diverse traditional delicacies which include; garri, fufu, lafun flour etc, 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, sieving, roasting and drying (Okafor, 2008). Traditional garri production is associated with the discharge of large amounts of water, hydrocyanic acid and organic matter in the form of peels and sieves from the pulp as waste products. When these waste products are improperly disposed, they are left in mounds which generate offensive odours and unsightly scenarios (FAO, 2004). According (2006), the deleterious effects of cassava mill effluent on soil can be traced to the high levels of cyanogenic glucosides, biochemical oxygen demand and soluble carbohydrates and proteins in the effluent. Cyanide released from the cassava effluents are highly lethal, it is fairly mobile in the soil and destroy microbes (Akani, 2006). Dumping of cassava mill effluent on land results in sand reduction, and the textural composition of the soil becomes more of clay, and the cyanide concentration increases with depth (Okwu and Nwosu, 1999; Okafor, 2008 and Uzoije *et al*., 2011). Awka- North Local Government Area is an agrarian area in Anambra State, South-Eastern Nigeria. Cassava is one of the crops produced in large quantities, most of which are processed to gari. Many cassava mills are therefore established in the area, even neat residential homes, producing much effluent. These large volumes of waste water from cassava processing are indiscriminately discharged onto the surrounding soil, where they accumulate and sink, thereby posing serious health and environmental hazard.

This work therefore aims at evaluating the effect of cassava mill effluent on the microbial populations and physicochemical parameters of the soil at various depths.

Materials and methods

Sample collection

Soil samples were collected from a cassava mill at Amanuke, Awka-North L.G.A. Anambra State. These samples were collected 10m away from the mill at three different depths of 0-20cm, 20-40cm and 40-60cm, termed surface, subsurface and deeper soil samples respectively.

Sample preparations

The samples were air dried, crushed to fine particles and sieved using 2mm sieve and then stored in fresh clean polyethylene bags in the refrigerator at 2^{o}C between 7-14 days to maintain the stability of the samples without significant alteration in their biological properties (Clark, 1965).

Particle size and texture class

Hydrometer method as described by Bouyoucous (1951) and Agbenin (1995) was used in this test. After, the values for silt and clay were determined, the value of sand was obtained by subtracting the values of silt and clay from 100.

Soil pH

Soil pH was determined using potentiometric method as described by Brady and Weil (1990). A glass electrode Testronic digital pH meter (Model 511) was used.

Organic matter and organic carbon

These were determined according to the wet oxidation method of Walkey and Black (1945).

Total nitrogen

Total nitrogen assay was determined using Kjeldahl method as described by Bremner and Mulvaney (1982).

Available phosphorus

Available phosphorus was determined by the methods described by I.I.T.A. (1979) and Olsen and Sommers (1982).

Table 1. Morphological and biochemical characteristics of bacterial isolates.

Colony Morphology	Gram reaction	Spore	Motility	Catalase	Coagulase	Oxidase	Indole	MR	VP	Citrate	Urease	Glucose	Maltose	Mannitol	Lactose	Zylose	Genera
Swarmy, colourless, irregular and raised	-Rods	-	+	-	-	-	-	+	-	+	+	+	-	-	-	+	*Proteus mirabilie*
Translucent, swarmy, and creamy	-rods	-	+	+	-	-	+	+	+	-	+	+	+	-	-	-	*Proteus vulgaris*
Round, thick, irregular and opaque	+ rods	+	+	+	+	-	-	+	-	+	-	+	+	+	+	+	*Bacillus* sps
Abundant, opaque, and golden	+ rods	-	-	+	-	-	-	+	+	-	-	+	+	+	-	-	*Stapylococcus* sps
Mucoid, shiny and milkish	-rods	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	*Klebsiella* sp
Translucent, tiny and smooth	+ short chains	-	-	-	+	-	-	+	-	-	-	+	+	+	-	-	*Streptococcus* sps

Key: + = Positive and - = Negative

Table 2. Cultural characteristics of fungal isolates.

Colony Morphology	Microscopy	Suspected Organism
Black, powdery, myceliated, spreading and zonated colonies	Well branched mycelia with colourless but septate hyphae and black conidia borne on Starigmata.	*Aspergillus* sp
Greenish velvety mass of mycelia with some sporadic dark spots	Chains of conidia wit branched phialides and septate hyphae	*Penicillium* sp
Whitish woolly mass of mycelia, spreading and covering the petri dish within 2-3days	Filamentous, non-septate hyphae, with black spots at the tips	*Mucor* sp
Yellowish-grey hyphae with some black dots at the center	Filamentous and non septate hyphae with black dots at the tip of the hyphae	*Rhizopus* sp.

Exchangeable cations

These were determined according to the methods described by I.I.T.A. (1979) and Agbenin (1995).

Cation exchange capacity

This was determined by the summation of the cubic centimeter (cm^3) values of the exchangeable cations of each sample determined above.

Hydrocyanic acid content

This was determined using alkaline titration method as described by AOAC, (1984).

Microbial isolation

Approximately, 1g of each sample was placed in 9ml of sterile water in a labeled test tube as described by Fawole and Oso (1995). Ten-fold serial dilutions were then carried out to 10^{-6} dilution for each of the samples. Pour plate technique as described by Fawole and Oso (1995) was adopted for the study, using Oxoid Nutrient agar and Saboroud Dextrose agar prepared according to the manufacturer's instructions.

Identification of isolates

Discrete colonies on agar plates were carefully examined macroscopically for cultural characteristics such as shape, colour, size, and consistency. Wet mount, Gram staining as well as biochemical tests was carried out in agar slants as described by Fawole and Oso, (1995) and Cheesbrough, (2004).

Statistical analysis

The values obtained for the total aerobic counts were analyzed using ANOVA at $P<0.05$ and $P>0.05$ as described by Snedecor and Cochran (1987).

Results

Table 1 represents the morphological and biochemical characteristics of bacterial isolates. The isolates include *Proteus mirabilis, Proteus vulgaris, Bacillus* sp, *Staphylococcus saprophyticus, Klebsiella* sp and *Streptococcus* sp. Table 2 shows the cultural characteristics of the fungal isolates. These isolates include *Aspergillus* sp, *Penicillium* sp, *Mucor* sp *a*nd *Rhizopus* sp. Table 3 represents the total aerobic bacteria counts in cfu/g at various depths. The ANOVA results at $P>0.05$ showed that there was no significant difference in total aerobic bacterial counts between the surface ($4.2x10^6 \pm 2.01$) and subsurface sample ($3.9x10^6 \pm 1.46$). However, the total aerobic bacterial counts obtained from the surface and subsurface were statistically higher than those of the deeper sample ($2.3x10^6 \pm 0.76$) at $P<0.05$ and $P>0.01$ respectively. Table 4 represents the total fungal counts at various depths. The ANOVA results at $P<0.05$ showed significant difference between surface ($4.2x10^6 \pm 2.38$) and subsurface fungal counts ($2.1x10^6 \pm 1.36$), as well as between surface and deeper counts ($0.2x10^6 \pm 0.44$) at $P<0.01$, while no significant difference was observed between subsurface and deeper sample counts at $P>0.05$. Table 5 shows the physicochemical properties of the soil samples at various depths. The results showed that the pH, HCN, and C/N ratio, all increased down the depth, while %OC, %N, %OM, and ECEC decreased with depth.

Table 3, Total aerobic bacterial counts (cfu/g) at various depths.

DEPTH	AMANUKE
SURFACE	$4.2 \times 10^6 \pm 2.01$
SUBSURFACE	$3.9 \times 10^6 \pm 1.46$
DEEPER	$2.3 \times 10^6 \pm 0.76$

Table 4. Total fungal counts (cfu/g) at various depths.

DEPTH	AMANUKE
SURFACE	$4.2 \times 10^6 \pm 2.28$
SUBSURFACE	$2.1 \times 10^6 \pm 1.36$
DEEPER	$0.2 \times 10^6 \pm 0.44$

Discussion

The results revealed the isolation of such bacteria as *Proteus mirabilis, Proteus vulgaris, Bacillus* sp, *Staphylococcus saprophyticus, Klebsiella* sp and *Streptococcus* sp , while the fungal isolates include *Aspergillus* sp, *Penicillium* sp, *Mucor* sp and

Rhizopus sp. These isolates were among those isolated by previous authors (Towill et al., 1978; Knowles, 1988; Ehiagbonare et al., 2009 and Akpor et al., 2009). These microbes may possess or have acquired the genetic attributes that enable them to survive in such acidic environment. This ability to degrade cyanide has been reported to be widely distributed in natural ecosystems and have enzymatic systems that can be broadly described as oxidative, hydrolytic, and substitution/transfer in nature (Knowles, 1988; Silva-Avalos *et al.,* 1990; Knowles and Wyatt, 1992; Towill *et al.*, 1978; Cummings and Baxter, 2006 and Ubalua, 2010). In addition, the high organic matter and organic carbon contents of the mill effluent may have contributed to the proliferation of these aerobic microorganisms as reported by (Wood, 1977; Okwute *et al.*, 2007 and Nwaugo *et al.*, 2008). The aerobic bacterial counts observed at the surface and subsurface were significantly higher than those of the deeper samples. This could be attributed to the continuous sinking and accumulation of the effluent known to be high in cyanogenic glycosides content. Such great negative effects led to the greatest loss of microbes in the soil thus causing soil infertility for agricultural products as reported by (Ehiagbonase, 2009).

Table 5. Results of the physico-chemical properties of the soil samples from amanuke at various depths.

DEPTH	%SAND	%SILT	%CLAY	TEXTURE	pH	HCN/100g	Pmg	%N	%OC	C/N	%OM	Ca	Mg	K	Na	EA	ECEC
B_1	90.0	15.4	11.0	S	4.90	9.06	18.00	0.98	1.99	2.03	5.00	5.30	3.00	0.92	0.92	0.99	15.00
B_2	90.0	8.60	7.03	S	5.20	12.7	15.00	0.32	1.50	4.69	1.02	2.50	1.50	0.87	0.80	0.85	14.60
B_3	70.4	12.0	17.6	SL	5.16	18.0	15.11	0.12	1.15	9.43	1.20	2.00	1.80	0.70	0.56	0.56	8.84

Key: B_1 = Surface sample
B_2 = Subsurface sample
B_3 = Deeper sample

The fungal counts obtained at the surface were statistically higher than those of the subsurface and deeper samples. This could equally be attributed to the deleterious effects of the effluent on the microbes as the concentration increases with depth. The stratification in diversity and number of these organisms may also be associated with such factors as the gradient or regime in the availability of nutrients in the soil, biological and physicochemical factors of the soil and soil intrinsic and extrinsic parameters (Shakir, 1989; Schlesinger *et al,*. 1990 and Atlas *et al.*, 1998).

The texture of the soil was sandy at the surface and subsurface while sandy loam at the deeper depth. Similar results have been observed by (Uzoije *et al.*, 2011). The percentage clay contents were low, ranging from 7.03-17.6. This and the low pH of the soil could explain the increasing concentration of cyanogenic glycosides with depth. Such factors as low pH, high negative soil charges, and low clay

content have been reported as soil conditions that increase cyanide mobility (Alessi and Fuller, 1976 and Fuller, 1984). Increase in the cyanogenic glycoside with depth has been reported by previous authors (Okafor, 2008; Ebhaye and Dada, 2004 and Uzoije et al., 2011). The soil was equally water-logged thus creating anaerobic environment which has been reported to enhance cyanide leaching to ground water (Ubalua, 2010 and Fuller, 1984). The carbon-nitrogen ratio was found to have increased with depth. This could be as a result of percentage organic carbon and nitrogen, both of which decreased with depth. The effective cation exchange capacity of the samples also decreased with depth, as reflected by the values recorded by the cations. These could be as a result of the effects of the cassava mill effluent on the soil.

Conclusion

The microbial loads as well as most of the tested physicochemical parameters were negatively affected by the cassava mill effluent at the various depths.

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