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Research Article

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Fertility Index and Microbial Population of Acid Sand Receiving Cassava Mill Effluent in of Uyo, Nigeria

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Abstract
A study was conducted to examine the changes in fertility and microbial indices of acid sand, under cassava effluent discharge at varying periods. Three cassava mill sites at Uyo discharging cassava effluent within three periods: less than 5 years discharge, 10 years discharge and 15 years discharges were used. Adjacent plots to each of the sites served as control. Soil samples were collected on the surface along the discharge strides in the morning, afternoon and evening. Measured soil nutrients were fitted into fertility index to determine the fertility status while total bacterial count, total fungal count for mold were the parameters used to assed the microbial count as influenced by cassava mill effluent discharge. Data were analyzed using descriptive statistics and analysis of variance at 5% probability level. Soil fertility index was greater in discharge site of 15 years (25) compared to 10 years (10), less than 5 years (7) and control (12). Total bacterial count was significantly high in site with 15 years discharge period (6.36cfug^{-1}) compared to 10 years (2.88cfug^{-1}), less than 5 years (2.72cfug^{-1}) and control (3.83cfug^{-1}). Total fungal count for mold was significantly high in site with 15 years discharge period (4.32cfug^{-1}) compared to 10 years (3.27cfug^{-1}), less than 5 years (2.18cfug^{-1}) and control (3.77cfug^{-1}). Calcium was significantly highest at noon (4.7cmol.kg^{-1}) under less than 5 years period and in the morning (5.1cmol.kg^{-1}) at site with 10 years period of discharge whereas Ca was highest in the evening (6.00 cmol.kg^{-1}) in location that received effluent for 15 years. High soil fertility due to increase in population of microbes status was seen in the site receiving effluent for 15 years compared to other periods.

Keywords: Microbial Count, Bacteria, Effluents, Cassava, Fertility Index

Introduction
Soil is the important part of the geosphere that provides support and nutrient. There are often alterations in soil chemical, physical, biological properties due to land misuse, mismanagement and indiscriminate disposal of pollutants [1, 2]. Soil quality can be monitored by a set of measurable attributes termed indicators. These indicators can be grouped as physical, chemical and biological indicators, and one can assess overall soil quality by measuring changes in these indicators [3, 4, 5] and transforming them into a single value known as the soil quality /fertility index. It is imperative to compare the changes in soil properties to understand the influence of changes in soil quality, biodiversity and global climatic systems natural resources and ecological processes.

Microorganisms are involved in numerous processes controlling the flow of nutrients through specific enzymes located inside the cell [6, 7, 8] and their activity is dependent, among others, on the temperature. Soil microorganisms influence aboveground ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility [9]. The researches that have been done on the effect of cassava effluent have shown that there are always some physio-chemical changes in the soil properties when cassava effluents are discharged on it. One of the researches showed that there was increase in soil acidity, potassium, sodium, phosphorous, and organic carbon and decrease in calcium nitrogen and magnesium [10]. Reports have also shown that the cassava effluent contains harmful cyanides, copper, mercury and nickel which have the capacity to affect native micro-biota [11]. According to 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 [12]. Cyanide released from the cassava effluents are highly lethal, it is fairly mobile in the soil and destroy microbes [12].

Discharging 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 [13, 14]. The study aimed at assessing changes in soil fertility status and microbial population under cassava mill effluent discharge at varying periods.

Materials and Methods

Sampling Procedure

Soils from three cassava processing mills of different processing periods (viz, 15 years, 10 years, less than 5 years) were used and a non-effluent site to serve as control. Auger and core samples were taken at surface depth of 0-15 cm. Sampling was done fortnightly within the hours of 6am and 7am, 12pm and 1pm and 5pm and 6pm. A total of 36 core and 36 auger samples were collected for routine and microbial analyses. The samples for microbial analysis were collected in sterilized polythene bags and taken in ice-packed coolers. All samples were transported to the laboratory for analyses.

Chemical properties

Soil pH: Soil pH was determined using pH meter. 20g of air dried composite sample was stirred is distilled water and allowed to stand for 30 minutes. The glass electrode of the pH meter was introduced into the suspension to measure the soil pH at the ratio of 1:2.5 [15].

Electrical Conductivity: Electrical conductivity was determined using electrical conductivity meter inserted into a suspension where 20g of composite sample was stirred in distilled water, allowed to stand for 30 minutes at a soil/water ratio of 1:2.5 [15].

Organic Carbon: Organic carbon content was determined by wet oxidation method using an acid dichromate, as described by [16].

Total Nitrogen: This was determined using the micro-keldahl digestion and distillation method was described by [17].

Available Phosphorus: Available phosphorus was determined by Bray P-1 method as described by [18]. Available phosphorus was then determined calorimetrically using atomic absorption spectrophotometer [15].

Exchangeable Bases (Calcium, Magnesium, Potassium and sodium): This was carried out using the Ethylamine Tetra Acetic Acid (EDTA) titration and flame photometric method as modified by [15].

Exchangeable Acidity: Exchangeable acidity was done by extracting the soil with IN neutral potassium chloride (KCl) solution and titrating with O.IN sodium hydroxide (NaOH) solution, using phenolphthalein indicator, from colourless to sharp pink end-point, as described by [19].

Effective Cation Exchange Capacity (ECEC): The ECEC was derived by summation of total exchangeable cations or bases (TEB) and exchangeable acidity (EA) as given in [15].

Computation of Soil Fertility Index (SFI) and Soil Evaluation Factor (SEF)

Values of soil fertility index was calculated according to method proposed by [20]. While soil evaluation factor was computed according to [21] model. SFI= pH + organic matter (g/kg, dry soil basis) + available P (mg/kg, dry soil +exch. K (cmol/kg, dry soil + exch.Ca (cmol/kg, dry soil) + exch. Mg (cmol/kg, dry soil) – exch. Al (cmol/kg, dry soil), SEF= [exch. K (cmol/kg, dry soil + exch. Ca (cmol/kg,dry soil) + exch. Mg (cmol/kg, dry soil) – log (1+ exch. Al (cmol/kg, dry soil)] × organic matter (g/kg, dry soil) + 5

Microbiological Analysis

Serial Dilution

The microbiological analysis was carried out based on the methods described by [21, 22]. One gramm of the soil sample collected from the cassava mill effluent was serially diluted in tenfold up to 10^6 tubes. Then 1ml from 10^6 tubes was aseptically inoculated unto already prepared plates of nutrient agar and potatodextrose agar using the pour plate method of inoculation. All plates were inverted and the nutrient agar plate (for bacteria) were incubated at 37°C. After the incubation, the total bacterial and fungal colonies on plate that contain 30-250 colonies were counted using a colony counter. The number of colonies on a plate were multiplied by the dilution factor to give the plate count per gram of the soil sample and recorded as $cfug^{-1}$. Example if the counts for 2 plates of the 10^6 dilution were 55 and 65, the average is 60. Therefore, the original soil sample contains 6×10^7 $cfug^{-1}$. The colonies were repeated sub-cultured unto fresh nutrient and potato dextrose agar media to obtain pure isolates.

Characterization and Identification

The bacterial isolates were characterized and identified using cultural, morphological and standard tests as described by [23]. The tests that were employed include Gram Stain, Motility, Catalase, Methyl red test, Voges-proskaaeur test, Indole production, Urease activity, Bile solubility test, Slide test, Coagulase test, Citrate test, Carbohydrate fermentation tests, Oxidase test and Spore test. The fungal isolates were identified according to the method described based on their colour of aerial hyphase and substrate mycelium, arrangement of hyphae and conidial arrangement [24].

Method of Data Analysis

Genstat (discovery edition 3) statistical software was used to analysed the data. Analysis of variance (ANOVA) using randomized complete block design (RCBD) was employed to assess the significant of treatment effect on data collected. Significant averages were separated by LSD at 5% probability level. Correlation analysis was used to assess relationship between changes in soil properties with soil fertility indices and microbial properties

Results

Soil Chemical Properties as affected by CME

Soil pH varied from 5.7 (slightly acidic) discharge period of 15 years to 4.3 (strongly acidic) in discharge period of less than 5 years. The Organic matter content was higher (47.38g/kg) in discharge period of 15 years, followed by control (46.87g/kg) and least (42.71g/kg) in discharge period of less than 5 years.

Table 1: Chemical Properties of Soils Affected by CME at Various Periods

		Effluent discharge period			
Properties	**Units**	**<5 years**	**10 years**	**15 years**	**Control**
pH		4.4b	4.3b	5.7a	4.4b
EC	$dS.m^{-1}$	0.08b	0.06b	0.23a	0.03c
Av.P	$mg.kg^{-1}$	57.14b	59.21b	78.11a	56.40b
OM	$g.kg^{-1}$	42.71b	43.28b	47.38a	46.87a
TN	$g.kg^{-1}$	0.11	0.11	0.12	0.13
EA	$cmol.kg^{-1}$	1.76a	0.85c	1.24b	1.32b
Ca	$cmol.kg^{-1}$	4.40c	4.17c	5.67a	4.80b
Mg	$cmol.kg^{-1}$	1.57c	1.48c	2.01a	1.84b
K	$cmol.kg^{-1}$	0.23a	0.17b	0.14c	0.15b
Na	$cmol.kg^{-1}$	0.07	0.06	0.07	0.06
ECEC	$cmol.kg^{-1}$	8.02c	6.73c	9.13a	8.95b
BS	%	78.06b	86.78a	86.78a	79.19b

EC = Electrical conductivity, Av.P = Available phosphorus, TN = Total Nitrogen, OM = Organic matter, EA = Exchangeable acidity, EB = Exchangeable bases, ECEC = Effective cation exchange capacity, BS = Base saturation
Means with the same letters are not significantly different (P > 0.05)

Table 2: Effect of Cassava Mill Effluent on Soil Exchangeable Cations at Different Time

	Effluent discharge period			
	<5 years	**10 years**	**15 years**	**Control**
Ca $cmol.kg^{-1}$				
Morning	4.60a	5.10a	5.60b	4.60a
Noon	4.70a	3.90b	5.40b	4.70a
Evening	3.90b	3.50b	6.00a	3.90b
LSD	**0.25**	**0.48**	**0.18**	**0.25**
Mg$cmol.kg^{-1}$				
Morning	1.64a	1.81a	1.99b	1.60b
Noon	1.67a	1.38b	1.92b	2.31a
Evening	1.39a	1.24b	2.13a	1.88b
LSD	**0.89**	**0.17**	**0.62**	**0.21**
K$cmol.kg^{-1}$				
Morning	0.26a	0.20a	0.14b	0.17a
Noon	0.24a	0.18a	0.21a	0.11b
Evening	0.19b	0.12b	0.06c	0.17a
LSD	**0.02**	**0.02**	**0.04**	**0.02**

Ca= calcium, Mg= magnesium, K= potassium
Means with the same letters are not significantly different (*p* > 0.05)

Soil Fertility Index and Soil Evaluation Factor under Different Discharge Periods

Soil fertility index and evaluation factor recorded highest in discharge period of 15 years with values (25.00 and 2.80) and this was closely followed by control site with values of 13.00 and 2.30, respectively.

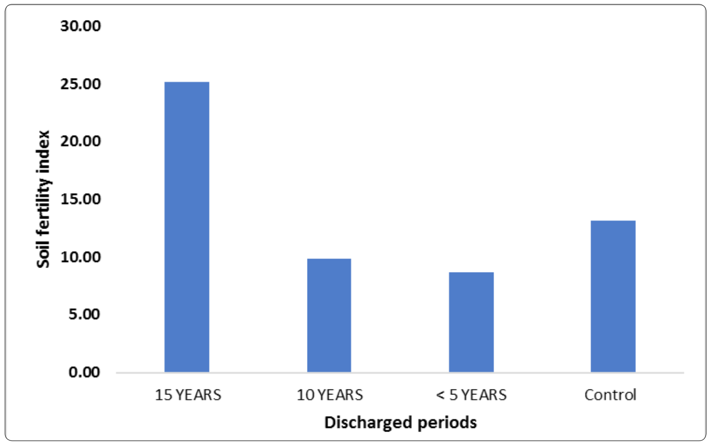

Figure 1: Soil fertility index under different discharge periods of CME

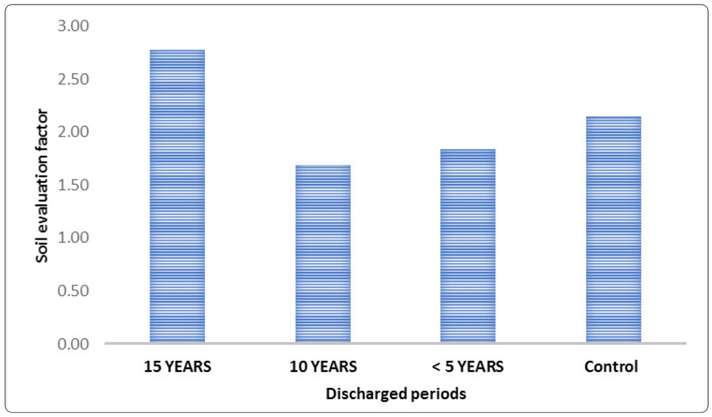

Figure 2: Soil evaluation factor under different discharge periods of CME

Discussions

Effects of Discharge Periods of Cassava Mill Effluent on Soil Chemical Properties

Soil pH varied from 5.7(slightly acidic) discharge period of 15 years to 4.3(strongly acidic) in discharge period of less than 5 years. This suggests that the effluent imparted acidic properties to the soil. The acidity could be attributed to the presence of hydrogen cyanide in the cassava mill effluent. The pH values recorded in this study are in the same range with those reported by [25-27]. The Electrical conductivity was found higher (0.23dS/m) in discharge period of 15 years and least (0.03dS/m) in control. The polluted site had higher salt content compare to the control. This could be attributed to the effect of the effluent discharge into the soil.

The Organic matter content was higher (47.38g/kg) in discharge period of 15 years, followed by control (46.87g/kg) and least (42.71g/kg) in discharge period of less than 5 years. The values may be due to the discharge of the waste with some contents of organic matter and also suggests presence of degradable and compostable substances in the effluent [28]. It was observed that, the organic matter increases with increase in the discharge period. Nitrogen was higher (0.13g/kg) in control site and least (0.11g/kg) in all the polluted site. Nitrogen content was seen to be the same in the polluted site. These values are in the same range with the values reported by Osemwota (2009) [29].The greatest Phosphorus content was (78.11 g/kg) in discharge period of 15 years and least was (56.40 g/kg) in control. On average, the polluted site had the highest Phosphorus and was low in the control. The high value of phosphorus in cassava mill effluent soils is not surprising since cassava tuber is a rich source of phosphorus [29-30]. Phosphorus content tended to increase with increase in processing period.

For exchangeable nutrient cations (Ca, Mg, K), the content of Ca was higher (5.67cmol/kg) in discharge period of 15 years and least (4.17cmol/kg) in discharge period of 10 years. The soils had calcium levels above the critical level of 2 cmol/kg [31]. The Mg content was greatest (2.01cmol/kg) in discharge period of 15 years and least (1.48cmol/kg) in discharge period of 10 years. The exchangeable K varied from (0.14 cmol/kg) in discharge period of 15 years to (0.23cmol/kg) in discharge period of less than 5 years. The increase in the cations in the impacted samples could have been caused by the cassava mill effluent. Such increase in the level of calcium in the impacted soil have been reported by Adewoye *et al*., [32, 33]. Exchangeable acidity was higher (1.76cmol/kg) in discharge period of less than 5 years and least (0.85cmol/kg) in discharge period of 10 years. Effective cation exchange capacity was higher (9.13cmol/kg) in discharge period of 15 years, followed by control site (8.95cmol/kg) and least (6.73cmol/kg) in discharge period of 10 years.

Effect of Cassava Mill Effluent on Exchangeable Nutrients at Different Time

Calcium was higher (6.00 cmol/kg) in the noon time at the discharge period of 15 years, this was closely followed by morning (5.60 cmol/kg) and least (3.50 cmol/kg) in the evening at the discharge period of 10 years. Magnesium was greatest (2.30 cmol/kg) in the noon time at the control site and least (1.24 cmol/kg) in the evening at the discharge period of 10 years. Potassium was higher (0.26 cmol/kg) in the morning at discharge period of less than 5 years and least (0.06 cmol/kg) in the evening at discharge period of 15 years. The exchangeable nutrients were seen to be higher at noon compared to other time. This may be due to high mineralization rate at noon attributable to sunlight.

Soil Fertility Index and Soil Evaluation Factor under Different Discharge Periods

Soil fertility index (SFI) and soil evaluation factor (SEF) for the different discharge periods were calculated to find out the overall effect of the discharge period of the cassava effluent on soil quality. There was a sharp increase in fertility index in discharge period of 15 years and control site as compared to discharge period of less than 5 years. The greater fertility in the discharge period of 15 years may be attributed to the greatest accumulation of nutrients from the effluent. While calculating SFI for different discharge periods, it was found out that it decreases with decrease in discharge periods. Soil evaluation factor (SEF) was also calculated for the discharged periods. It was found that SEF was greater for discharge period of 15 years, followed by control and discharge period of 5 years and least for discharge period of 10 years.

Conclusion

It is evident from the study that there were changes in soil properties, fertility and microbial population due to cassava mill effluent discharge. Cassava effluent discharge for a longer period showed increased in organic matter content and microbial count in the soil. Exchangeable nutrients were higher at noon due to high rate of mineralization. Soils affected by cassava mill effluent for longer periods of time showed increase in soil fertility and microbial population.

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