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Microbial and Physicochemical Characteristics of Cassava Mill Effluents Receiving Soil in Abraka and Environs, Delta State

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Abstract
The microbial and physicochemical characteristics of soils receiving cassava mill waste water in Abraka and Oria, Delta State, Nigeria were assessed. The isolation and enumeration of microbial population was carried out using standard culture based methods while standard analytical methods were used for assessing the physicochemical characteristics. The mean bacterial count for contaminated soils were $1.53 \times 10^5 \pm 0.03$ cfu/g and $1.90 \times 10^5 \pm 0.10$ cfu/g and $2.10 \times 10^5 \pm 0.15$ cfu/g and $8.80 \times 10^5 \pm 0.23$ cfu/g , for control soils. The mean fungal counts were $3.0 \times 10^3 \pm 0.10$ cfu/g and $2.0 \times 10^3 \pm 0.50$ cfu/g for contaminated soil while $5.5 \times 10^5 \pm 0.50$ cfu/g and $2.5 \times 10^3 \pm 0.10$ cfu/g for control soils. The microorganisms isolated from contaminated and control soils were *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus* spp., *Klebsiella* spp., *Proteus* spp., *Aspergillus spp.*, *Penicillium spp.*, *Rhizopus.,* and *Mucor* spp. Results obtained from physicochemical parameters assessment varied, however soils receiving cassava effluents showed increase in various parameters determined as compared with control soils. The study revealed that cassava mill effluent may have some deleterious effect on soil structure, soil microorganisms and soil characteristics compared with control soil. Cassava mill waste water should be treated before released into the environment to prevent pollution of soil and water bodies.
Keywords: Cassava mill effluents, Microbial, Physicochemical, Soil, Abraka

1. Introduction
Cassava *(Manihot esculenta* Crantz) belongs to the family Euphoriobiaceae. It is one of the major sources of carbohydrate in the tropics, after maize and rice. It is a main staple food in the developing world providing nutrient for over half a billion people (Franquet and Dennis, 1992). Reports show that cassava originated from West Central Brazil, and then spread throughout the South American Continent. The plant was spread by Portuguese and Spanish adventurers to Africa, West Indies, Asia, Philippines and Taiwan, but now grown globally. The cassava root is long and tapered with a firm, homogenous flesh encase in a detachable rind, about 1mm thick, rough and brown in the outside. A woody cordon runs through the root axis, its flesh can be yellow or whitish. The root contain much starch and also significant quantity of phosphorus, calcium and vitamin C. The root however, lacks protein and some other nutrients. The leaves contain protein especially lysine (Nassar and Margnues, 2006) but deficient in the amino acid methionine (Velmerugu, 1992).

Though different species of cassava have been reported, but generally cassava is differentiated as sweet non-poisonous and bitter poisonous types (Essers, 1995). The poisonous effect of cassava is due to hydrocyanic acid present in parts of the bitter variety while only present on the skin of the non-poisonous one (Vetter, 2000). The glycoside which impacts the poisonous effect can be destroyed by removing the peel of sweet variety and boiling the biter variety in water (Balagopalan and Rajalakshmy, 1998). Mature plant can grow up to 0.7m high, possessing a single to few stems, and scanty branches

Cassava is one of the major sources of stable food for Nigeria population. Garri and fufu and other cassava products are obtained from cassava. It is a source of livelihood for to rural farmers where small/local production dominate. The processing of Cassava into edible forms involve series of steps. Cassava processing into garri involves peeling, cleaning of tubers through washing, grinding into paste and the de watering and frying (Afuye and Mogaji, 2015). These steps help to detoxify the tuber to edible products. These processing steps generate various wastes. Izah *et al*. (2018) citing other reports mentioned the following as by products from cassava processing. Peelings from cassava (21.8%), sieviates (7.5 %), air emission (19.8%), high quality cassava flour (25.0%) and cassava mill effluents (CME) (16.2%). These by-products have environmental consequence on the ecosystem. Soil fertility, air and water quality could be impacted negatively which could be detrimental to man and animals.

There is neither any defined method for treating cassava waste water which is majorly made of cyanide nor government policy guidelines to this effect (Okuande and Adekaline, 2013; Ukaegbu-Obi *et al*., 2018). Knowing that cassava effluent constitute environmental problem, it is therefore pertinent to ascertain the level of pollution on soil receiving cassava effluent and that soil not receiving cassava effluent. The purpose of study therefore is to investigate microbial and physicochemical characteristics of soil receiving cassava effluent and compare same with the one that do not receive cassava effluent in Abraka and Oria.

2. Materials and Methods

2.1 Sample Collection

Soil samples were collected into sterile containers and transported to the laboratory for analyses. Test soils were collected around the mills while control soils were collected 100m away from the cassava mill (Obueh and Odesiri-Eruteyan 2016). Soils were collected from Abraka and Oria. Abraka is located in Ethiope East Local government Area of Delta State, and lies within latitude 5º 48 N and longitude 6º06 E (Osakwe, 2012), while Oria is a suburb of Abraka town.

2.2 Microbiological Analysis

One gramme (1g) of each soil sample was introduced into 9 mls of sterile distilled water and serially diluted (10^1-10^{10}) One millilitre (1mL) of various samples were plated out into Nutrient agar and Potato dextrose agar after adding 0.05 W/V chloramphenicol for total viable counts for bacterial and fungal respectively. Nutrient agar plates were incubated at 28±2ºC for 48hrs and potato dextrose agar, 28±2ºC for 72hrs. Colonies obtained were expressed as colony forming units (CFU/g). Pure isolates were preserved in slants. Identification of bacterial isolates were based on cultural and biochemical analysis for bacteria (Stanley *et*

al., 1989) and fungi, according to methods described by Taibot, (1978).

Physicochemical Properties Analysis

Soils subjected to physicochemical analysis were air dried for seven days in the laboratory and sieved through a 2-mm stainless –steel sieve and kept in a sealed polythene bag at room temperature (28±2^{o}C) for 24 hrs.

The pH of various soils samples were measured using pH meter (Model Hanna H16107). The meter was introduced into sample and result taken after stable reading was obtained. Another reading was taken after rinsing the electrode with sterile water (AOAC, 1995). Conductivity was measured using conductivity meter (model: Searchtech DDS-307). Sulphate, nitrate, phosphate were determined using barium Chloride (Turbidimetric). The Calcium, Magnesium, lead, Cadmium, Chromium and Nickel were estimated using Atomic Adsorption Spectrometer (Model Agilent 280Z) by method of AOAC, (1995). Sodium, and potassium contents were determined by flame photometer (model: FP-640). Analysis of particle size of the soil was determined by hydrometer method (Bouyoucos, 1962). The oil content was determined using ASTM

For oil content, 10 grammes of air dried and sieved soils were weighed into glass bottles and 20ml of tetrachloroethylene was poured into the glass bottles. The bottles were placed into a shaker maintained at room temperature. The content was drained into glass bottles using a glass funnel stuffed with cotton wool containing anhydrous sodium sulphate. Analysis of sample was determined using Spectrophotometer (Obueh and Odesiri-Eruteyan 2016).

Statistical Analysis

Data obtained was analysed using T test for independent variable s using Statistical Package for Social Science software (SPSS), Inc, Chicago, Il, U S A Version 23. 0. Significant level with 0.05.

3. Results and Discussion

3.1 Results Presentation

The results of the microbial and physicochemical analysis of soil receiving cassava mill effluent in Oria and Abraka, Delta State are presented in Table 1-Table 3.

Table 1: The Microbial Count of Test and Control Soils.

Microbial count (Cfu/g)	**Abraka**		**Oria**	
	Control	Contaminated soil	Control	Contaminated soil.
Bacterial counts	$2.10 \times 10^5 \pm 0.15$	$1.53 \times 10^5 \pm 0.03$	$8.80 \times 10^5 \pm 0.23$	$1.90 \times 10^5 \pm 0.10$
Fungi counts	$5.5 \times 10^5 \pm 0.50$	$3.0 \times 10^5 \pm 0.50$	$3.5 \times 10^3 \pm 0.10$	$2.0 \times 10^3 \pm 0.10$

Table 2: Microorganisms Isolated From Contaminated and Control Soil Samples and Their Percentage Occurrence

Microorganisms	Abraka		Oria		Total
	Control	Contaminate	Control	Contamination	
Bacillus spp	3 (20.0%)	1 (14.29%)	5 (29.41%)	3(21.43%)	12(85.13%)
Pseudomonas Aeruginosa	2(13.33%)	-	2 (11.76%)	2 (14.29%)	6 (32.38%)
Escherichia coli	1 (6.67%)	-	2 (11.76%)	2 (14.29%)	5(31.72%)
Klebsiella spp	2 (13.33%)	1 (14.29%)	2 (11.76%)	2(14.29%)	7 (53.69%)
Proteus spp	-	1 (14.29%)	1(5.88%)	1(7.14%)	3 (27.31%)
Staphylococus Aureus	2(13.33%)v	-	1 (5.88%)	1 (7.14%)	4(26.35%)
Aspergillus spp	2 (13.33%)	1 (14.29%)	1(5.88%)	1(7.14%)	5 (40.64%)
Penicillum spp	1 (6.67%)	1 (14.29%)	2 (11.76%)	1(7.14%)	5 (39.86%)
Rhizopus spp	2 (13.33%)	1 (14.29%)	1(5.88%)	1(7.14%)	5 (40.64%)
Mucor spp	-	1 (14.29%)	-	-	1(14.29%)
Total	15 (99.99%)	7(100%)	17 (99.97%)	14 (100%)	53 (399%)

Table 3: Physico-Chemical Characteristics of Samples

Parameters	Abraka		Oria	
	Control	Contaminated	Control	Contaminated
pH	6.50± 3.00	4.00±1.00	7.00± 1.00	3.50±1.00*
Moisture	27.50 ±1.75	38.60 ±2.30*	31.00 ±1.00	39.00 ±1.00*
Electric conductivity (µS/cm)	21.33 ± 2.10	24.00± 2.00*	20.00 ± 1.00	26.00± 1.00*
Cyanide (mg/kg)	0.00 ±0.00	11.47± 0.95*	0.00 ±0.00	10.82± 1.00*
Nitrate (mg/kg)	1.40 ±0.20	1.86 ±0.10*	1.20 ±0.40	1.94 ±0.10*
Phosphorus (mg/kg)	0.32± 0.10	0.48 ±0.1*	0.37± 0.10	0.56±0.10*
Sulphate (mg/kg)	3.40 ±1.00	5.80± 0.10*	3.60 ±1.00	6.32.00± 0.00*
Lead (mg/kg)	0.02 ±0.02	0.48± 0.02*	0.03 ±0.01	0.34± 0.10*
Cadmium (mg/kg)	0.04 ±0.04	0.08± 0.02	0.02 ±0.00	0.03±0.00
Chromium (mg/kg)	0.05 ±0.12	0.14±0.03*	0.03 ± 0.00	0.09 ± 0.00
Nickel (mg/kg)	1.16 ±0.16	2.02± 0.02*	1.08 ± 0.01	2.17 ± 1.00
Sodium (mg/kg)	31.00± 2.00	48.00± 3.00*	49.00 ± 0.100	29.67± 0.58
Oil content (mg/kg)	0.2 ± 0.10	1.60 ±0.60*	27.67 ± 0.58	49.00 ±1.00
Potassium (mg/kg)	15.44 ±0.44	28.40± 0.40*	14.00 ± 2.00	31.37±1.15*
Calcium(mg/kg)	21.00 ±3.00	26.03 ± 3.00	19.00 ± 1.00	24.60 ±1.00*
Magnesium (mg/kg)	22.40± 0.10	24.05± 1.73*	20.02 ± 1.00	31.40±1.00*
Sand (%)	94.00± 2.00	91.00 ±1.00	93.30 ± 2.0	89.00±1.00
Clay (%)	2.40± 0.20	2.00 ±0.20	3.10 ± 0.10	10.60±1.00*
Slit (%)	3.60 ±0.20	7.00 ±0.00*	3.90 ± 0.10	0.40±0.10*

3.2 Discussion and Findings

The results of the microbial and physicochemical analysis of soil receiving cassava mill effluent in Oria and Abraka, Delta State are presented in Table 1-Table 3. Table 1 shows the microbial count from various soil receiving cassava waste water and control soil. The bacterial counts in both controls were $2.10\times10^5 \pm 0.15$ and $8.80 \times10^5\pm0.23$ CFU/g while the soil receiving cassava mill effluent were $1.53 \times 10^5 \pm 0.03$ CFU/g and $1.90 \times 10^5\pm 0.10$ CFU/g. The bacterial count for the control soils were more compared to the contaminated soils. Similar result was obtained for fungal counts contaminated soil had lesser fungal count than control soils. Table 2 shows the microbial organisms from the four sites. The isolated organisms included *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella* sp, *Proteus* sp, *Bacillus* sp*, Aspergillus Penicillium*, *Rhizopus* and *Mucor* species.

The physicochemical composition of samples are presented in Table 3. pH of samples for contaminated soils were lower (4.00±100 ; 3.50 ±1.00) compared with those obtained from contaminated samples (6.50± 3.00; 7.00±1.00). Cyanide was not detected in controls soils but detected in contaminated samples with soil obtained from Abraka being higher (11.47±0.95). Results of nitrate and phosphorus were higher in both contaminated soils compared with control soils. (1.86±0.10VS 1.40± 0.20) and (1.94±0.10 VS 1.20± 0.40) and phosphorus (0.48 ± 0.1 VS 0.32±0.10.) (0.56±0.10 VS 0.37±0.10). Similar trend was observed for sulphate, sodium, oil content, potassium, calcium and magnesium. In all these parameters, the contaminated samples showed higher concentration compared to the various controls. Concerning soil structure (sand, clay, slit), values obtained for controls were higher compared with contaminated samples. The sandy soil had higher values thus contrasting values obtained for slit (7.00% VS 3.60%) and clay (10.60% VS 3.10%) for contaminated samples for Abraka and Oria respectively. Lead, cadmium, chromium and nickel are all heavy metals. The concentration of heavy metals from contaminated soil samples from Abraka and Oria were higher compared with control soils.

The study was conducted to determine the microbial and physicochemical characteristics of soil receiving cassava effluent in Abraka and Oria. The bacterial population in control soils were more than the contaminated samples. This could be attributed to the effect of cyanide on the soil making it acidic and thus limiting the survival and growth of microorganisms in the soil environment. Similar result was obtained by Ukaegbu-Obi *et a*l (2018) and Obueh and Odesiri-Eruteyan, (2016). Similarly, population of fungal species were fewer compared to the bacterial species. The population of fungal in control soils were more than those on the soil contaminated with cassava effluent, even when fungi generally thrive in acidic environment compared to bacteria. One could have expected more population of fungi in the contaminated soil than the control soils. The neutral pH in control soils supported the growth of more population of microorganisms. The solubility of nutrients in the soil is influenced by soil pH. The activity of microorganisms are responsible for breaking down of organic matter and most chemical transformation in the soil and thus

the availability of plant nutrients are affected by the soil pH.

Microorganisms isolated included *Bacillus species, Pseudomonas aeruginosa, Escherichia coli, Klebsiella* species, *Proteus species, Staphylococcus aureus, Aspergillus species, Penicillium* species*, Rhizopus* species *and Mucor* species. These findings agree with the findings of Obueh and Odesiri- Eruteyan who isolated similar organism in their studies.

Bacillus species is most prevalent isolates. *Bacillus* spp is one of the indigenous bacteria in soil environment. Their presence in both contaminated and control soil attest to this. The presence of spore and being aerobic enables the organism to adapt to soil environment. Isolation of *Bacillus* from cassava effluent have also been reported by other researchers (obeuh and odesiri-Eruteyan , 2016; Igbinosa and Igiehon, 2015). Isolation of *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus* spp. fungi like *Aspergillus* spp., *Rhizopus* spp. *Mucor* spp *and Penicillium* spp also concur to previous researchers (Ukaegbu-Obi *et al*., 2018; Izah and Aigberua, 2017). The Physicochemical analysis of the soil samples are presented in Table 3. The soil receiving cassava effluent contained higher concentration of the parameters determined.

Conductivity, cyanide, nitrate, phosphorus sulphate were higher in contaminated soils than the control soils. Similar report was obtained from findings of Eze and Onyilide, (2015). The values for potassium, calcium, magnesium and sodium were significantly higher than controls for the two soils. The reason for increase being that the continuous seeping of hydrogen cyanide into the soil, from cassava tuber during processing into paste and dewatering, may have contributed to increased concentration of the minerals. Acidic pH support the release of the minerals to the soil. Cyanide interacts with soil water to produce a weak acid (Shape, 1976). With increase in concentration of minerals in these soils, one expects the microorganisms in these environment to proliferate in the soils. But the presence of the heavy metals may have countered the effect of these minerals and make them unavailable to the organism for growth. Similar values were obtained by previous researcher in Abraka and Oria soils compared to control; Abraka soil pH was 4.60, Oria 3.89 and control 6.96 for top soils (Osakwe 2014). Reduction in pH may allow the release of toxic metals instead of adsorption to soil or sediment (Mouvet and Borg, 1983). Obueh and Odesiri-Eruteyan (2016) reported that microorganisms are unable to convert cyanogenic glycoside completely due to few enzymes present in cassava fibre. Contrasting result was obtained, however, by Ukegbu-Obi *et al* (2018). In their study, all the values for minerals were lower than control with exception of phosphorus.

Continued release of the cassava mill effluent into the environment can alter the physical and chemical properties of the soil. This can affect the soil health, and in the long run affect human and animal lives. Soil is the site for plants and is the source of nutrients to the growing plant. If soil for agricultural produce is polluted, then the human population and livestock would be affected with attending harmful effects. If the cassava effluents are not treated before release to the environments, the consequence is that the soil, surface water and underground water may be polluted.

Critical look on the table also showed that the contaminated soil contained

higher concentration of heavy metals (lead, cadmium, chromium and Nickel). The high concentrations of these metals is worrisome. Concentrations of cadmium in soils were far above the FAO/WHO and EC standard but within the set standard of USA, Germany, and Poland. In similar view, Nickel values for contaminated soils were well above the FAO/WHO and EC standard of (1.5mg/kg). However, Chromium and lead were within the set standards by FAO/WHO, United States, Poland, Germany and Austrialia but higher than EC standard (Ali *et al*., 2017). Nickel may be carcinogenic, can also react with DNA to cause DNA damage Musa *et al*., (2017)

Similar result was reported by Osakwe, (2012). Lower pH cause release of toxic metals in to solution and can move downward with water through the soil. The presence of these heavy metals may have contributed to lesser population of microorganisms compared with control soils. Heavy metals interacted with metabolism of microorganisms. The cellular components of the cells are disrupted. Proteins are denature, leading to destruction of integrity of cell membranes (Obueh and Odesiri-Eruteyan (2016). The soil structure was made of sand, clay and slit. The percentage slit and clay were low. Similar findings were reported by previous researcher (Igbinosa and Igiehon, 2015).

Conclusion

The study showed that cassava effluent water have capacity to significant change microbial and physicochemical properties of the soil. Results showed that soil contaminated with cassava waste water had lower population of microorganisms. The physicochemical characteristics of soil contaminated with CME had higher values than control with higher concentration of cadmium and nickel well above FAO/WHO and EC standards. Chromium and lead were within set standards by FAO/WHO and EC. The need to treat cassava waste water before release to the environment to conserve healthy soil for agricultural produce and human health cannot be overemphasized.

References

Zarie, K.A. and Al Mugren, K.S., 2010. Measurement of natural radioactivity and assessment of radiation hazard in soil samples from Tayma area (KSA). Isotope and Rad. Res. 42(1):1- 9

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 Research International* 3(3): 37-43.

Ali, Al. M., Ahmed, H. A., Emara, H. A., Jangua, M. A. and Alhafez, N., 2019. Estimation and Bioactivity of Toxic metals between soil and Plants. *Pol. J. Environ. Stud*. 28(1): 15-24.

Association of Official Analytical Chemist (AOAC), 1995. Official methods of Analysis 16th Edition Virginia.

American standard for testing and materials (ASTM), 2003. Standard methods for the Examination of water and wastewater, Washington DC

Bouyoucos G.H., 1962. Improved hydrometer method for making particles size analysis. *Agron. J*., 43: 434-438 A recalibration of thr

hydrometer for making mechanical analysis of soil.

Essers, A.J.A., 1995. Removal of cyanogens from cassava roots; Studies on domestic sundering and soil substrate fermentation in rural africa. PhD Thesis Wageningen Agricultural University, Netherlands.

European Union (EU), 2006. Commission regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of European Union L364/5.

Eze, V. C. and Onyillide, D. M., 2015. Microbiological and Physicochemical characteristics of soil receiving cassava effluents in Elele, River State Nigeria, Journal of Applied and Environmental Microbiology 3(1): 20-24.

FAO/WHO, 2011. Joint FAO/WHO Food Standards Programme, Codex Committee on Contaminants in Foods. 2011.

Franquet C. and Denis F., 1990. African cassava mosaic virus: Etiology, Epidemiology and control. *Plant Disease,* 74(6): 404-11.

Igbinosa, E.O. and Igiehon, O.Z., 2015. The impact of cassava Effluent on the Microbial and physicochemical characteristics on soil Dynamics and Structure. *Jordan Journal of Biological Sciences* 8(2): 107-112.

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

Mouvet, C. and Borg, A.C.M., 1983. "Speciation (including adsorbing species) of copper, lead, nickel and zinc on the Meuse River". *Water Research* 6: 641-649.

Musa, J.J., Mustapha, H.I., Bala, J.D., Ibrahim, Y.Y., Akos, M.P.., Daniel, E.S., Oguche, F.M. and Kuti, I.A., 2017 Heavy metals in Agricultural soils in Nigeria: A review. Arid Zone Journal of Engineering, Technology and Environment. 13(5): 593-603.

Obueh, H.O. and Odesiri- Erutayan, E., 2016. A study on effects of cassava processing wastes on the soil environment of a local cassava mill. Journal of pollution effects and control 4(4): 24-32

Okuande, O.A. abnd Adekalu, K.O., 2013. Physicochemical analysis of contaminated water resources due to cassava wastewater effluent disposal. Eur. J. Sci. Techol. 2:75-82.

Osakwe, S.A., 2012. Effect of cassava processing mil effluent on physical and chemical properties of soils in Abraka and Environs, Delta State, Nigeria, Chemistry and Metals Research 27:27-40.

Shape, A.G., 1976. *The chemistry of cyanocomplexes of the transition metals*. Academic press, London.

Stanley, J. T., Bryabt, M.R, Pfenny, N. and Holt, G. G. (ed), 1989. Bergy's Manual of Systematic Bacteriology. Vol. 3. Baltimore, Williams and Wilkins.

Tailbot, P.H.B., 1978. Principle of fungal Taxonomy. MacMillan Press Ltd London.

Ukaegbu-Obi, K.M. Appah, O.G., itaman, O.V. and Nwosu, P.C., 2018. Microbial and physiocochemical analysis of soil receiving cassava mill waste water in Umudike, Abia State, Nigeria. *Nigerian Journal of Biotechnology*. 35 (2):99-107.

Velmerugu, R., 1992. Preparation of cassava leaf products and their uses as animal feed. Fao and production and health paper (Rome, Italy): *Food and Agricultural Organization of the United Nations,* 95: 11-25.

Vetter, J., 2000. Plant Cyanogenic Glycosides. *Toxicon,* 38 (1): 11-36.

WHO/FAO. 2007. Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission 13th Session. Report of the Thirty Eight Session of the Codex Committee on Food Hygiene. Houston, United States of America, ALINORM 07/30/13.