ISSN:
Print - 2277 - 0593
Online - 2315 - 7461

Journal of Natural Science, Engineering and Technology

MICROBIAL LOAD, AFLATOXIGENIC PROPERTY AND HEAVY METALS IN SOIL CONTAMINATED WITH CASSAVA WASTEWATER IN ODEDA LOCAL GOVERNMENT AREA, OGUN STATE

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ABSTRACT

Environmental degradation poses a formidable challenge to sustainable development, emerging as one of the most significant threats. Cassava processing, a widespread human activity in both rural and urban centers in Nigeria and other regions, stands out as a potential contributor to environmental degradation. The study aimed to determine the microbial load, aflatoxigenicity and heavy metals in soils contaminated with cassava wastewater. Cassava wastewater-contaminated soil samples were collected from five sources in Odeda Local Government. The samples were collected in a sterile zip-lock bag and transferred to the laboratory. Standard microbiological procedures were employed for microbiological analysis; the screening for aflatoxigenicity in fungal isolates was performed on yeast extract agar and examined using an Ultraviolet (UV) spectrophotometer. Metallic tests were conducted as part of the assessment. Statistical analysis was conducted using the SPSS software. Results revealed varying degrees of microbial contamination in the samples. Fungi isolated from the samples were: *Aspergillus niger, Aspergillus flavus, Fusarium sp,* and *Rhizopus sp.* Bacteria isolated were: *Bacillus cereus, Staphylococcus sp, and Pseudomonas*. Fungi and bacteria counts in the soil samples exceeded a feasible counting limit. Aflatoxigenicity screening affirmed that about 40% of the isolates were toxigenic. The study underscores the significance of implementing effective post-harvest handling practices for cassava to reduce contamination, thereby preventing environmental pollution and degradation.

Keywords: *Manihot esculenta;* effluent; mycotoxins; soil; toxigenicity,

INTRODUCTION

Cassava (*Manihot esculenta*) is one of the major staple food plants in the World, utilized and consumed by nearly a billion people in 105 countries from tropical Africa, Latin America, and Asia (FAO, 2004). Africa produces more cassava than the rest of the world, with production hitting 230 million tonnes in 2010 (FAO, 2012). Cassava, recognized for its resilience in arid conditions, boasts substantial nutritional benefits. It is rich in essential elements such as carbohydrates, proteins, salt, lipids and a diverse range of minerals, including magnesium, calcium, and iron. Cassava is majorly processed into flour by farmers in Southern Nigeria. During processing, a large volume of effluent is produced in the pressing stage of cassava tuber processing and the effluents are discharged into the soil and drain into nearby

water bodies, and canals without treatment. Cassava mill effluents are known to cause environmental pollution by altering the receiving soil and water characteristics and adversely impacting the biota in such environments such as fishes (water), vegetation (soil), and domestic animals, consequently posing risks to human health (Izah *et al*., 2017). Cassava wastewater pollution arises from the discharge of effluents during cassava processing, these effluents contain high levels of organic matter, and cyanogenic compounds that contain substances that are lethal, mobile in soil, impact biodiversity, and lead to the extinction of macroinvertebrates, making it difficult for marine life to survive, prevent the germination of cereal seed, and kill microbes (Eziegbo *et al*., 2014).

The occurrence of bacteria load and aflatoxin contamination have been found in raw and processed cassava flour (*Manihot esculenta*), since they are natural products and all parts of the plants can be degraded by bacteria and fungi especially molds (Alwakeel, 2009). Aflatoxins are amongst the most poisonous mycotoxins and are produced by certain molds (*Aspergillus flavus and Aspergillus parasiticus*) which grow in the soil, decaying vegetations and grains (James, 2005; Lewis *et al*., 2005).

Mitigating the environmental impact of cassava wastewater necessitates the implementation of effective management strategies. In Nigeria, there is an increasing need to prioritize cassava's proper processing and waste management, given its escalating importance for food security, agricultural sustainability, and economic development (Okunade and Adekalu 2013).

The study was conducted to determine the microbial load, aflatoxigenicity and heavy metals in soils contaminated with cassava wastewater.

MATERIALS AND METHODS

Study Area

Soil samples contaminated with cassava wastewater were collected from five villages across Odeda Local Government, Abeokuta, Ogun State: Apa-Kila 1, Apa-Kila 2, Olufowora, Ayetoro and Olodo. From each village, two samples were collected, making a total of ten samples. At each location, soil samples were randomly collected from different points within the contaminated area, using a sterile soil auger, ensuring representative sampling. The samples were thereafter placed into sterile Ziploc bags, properly labelled and transported to the laboratory under aseptic conditions for further analysis. Each sampling point had two replicates to enhance reliability.

Isolation of Microorganisms from Cassava Wastewater Contaminated Soil

Serial dilution and culturing

One milliliter (1 ml) of each soil sample was aseptically transferred into 9 ml of sterile distilled water and thoroughly mixed. From the resulting suspension, 0.1 ml of the 10−3 and 10−5 dilutions was pipetted onto sterile Petri dishes. Subsequently, 20 ml of freshly prepared Nutrient Agar (for bacterial isolation) and Malt Extract Agar (for fungal isolation), cooled to 40°C was poured into the plates and allowed to solidify. The Nutrient Agar plates were incubated at 37°C for 24 hours to support bacterial growth, while the Malt Extract Agar plates were incubated at 25°C (room temperature) for five days to promote fungal growth. After incubation, developed colonies were counted using a colony counter to determine the total viable count for both bacteria and fungi. Mixed col-

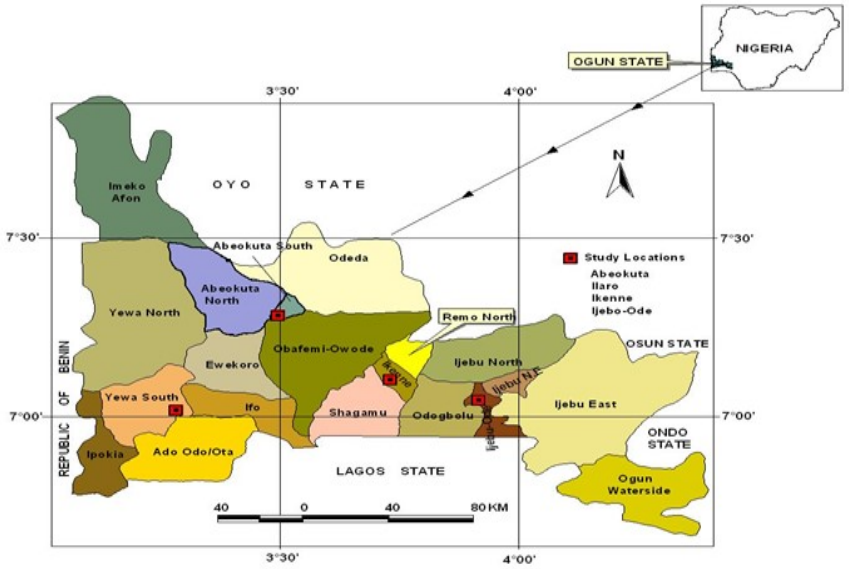

Figure 1: Specific Locations Used From Odeda Local Government Area (6.2°N and 7.8° N and Longitude 3.0°E and 5.0°E.

ony plates were sub-cultured to obtain pure isolates.

Phenotypic Identification of Bacterial and Fungi Isolates:

The bacteria isolates were identified using microscopic, macroscopic, and various biochemical tests. Fungal isolates were stained with lactophenol cotton blue and observed under a microscope using x40 and x100 objective lenses. Identification of fungi depended largely on morphological characteristics such as the type and arrangement of spores produced as well as the mycelial type (Adetunji *et al*., 2018).

Figure 2: Cassava wastewater contaminated soil

Determination of the Aflatoxigenic Potential of the Fungi Isolates

Media Preparation and Fungal Cultivation

The fungal isolates were sub-cultured on Yeast Extract Agar (YEA). To prepare the medium, 11.5 g of Yeast Extract Agar was weighed and dissolved in distilled water inside a 500 ml conical flask. The solution was stirred vigorously for even distribution and dissolution. The flask was then covered with a cotton plug to prevent contamination and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the medium was allowed to cool before being poured into sterile Petri dishes. The fungal isolates were then inoculated onto the plates and incubated at room temperature for 4 days to allow proper growth.

Aflatoxigenicity Screening

To assess the aflatoxigenic potential of the fungal isolates, a screening was conducted based on the fluorescence emitted under ultraviolet (UV) light at 365 nm. After a 4-day incubation period at room temperature on Yeast Extract Agar (YEA), the cultures were examined under UV light. Aflatoxin-producing isolates exhibited characteristic blue or green fluorescence on the undersides of the colonies, while non-producing isolates did not display any fluorescence. This method aligns with the procedures described by Ezekiel *et al.* (2012) and Atanda *et al.* (2011), where the presence of fluorescence under UV light at 365 nm indicates aflatoxin production.

Molecular Characterization of Fungal Isolates

The genomic DNA of each pure fungal isolate was extracted following the manufacturer's instructions for the PrepMan™ Ultra DNA Isolation Kit (Thermo Fisher Scientific, USA).

DNA Extraction Procedure

The extraction procedure was performed as described by Thermo Fisher Scientific (2023) with slight modifications.

Fungal Cell Lysis

A loopful of each pure fungal culture was suspended in 100 µl PrepMan™ Ultra reagent in a sterile microcentrifuge tube (Thermo Fisher Scientific, 2023).

Heat Treatment

The suspension was heated at 100°C for 10 minutes in a heat block to lyse the cells and release DNA (Smith *et al.*, 2020).

Centrifugation

The lysate was centrifuged at 12,000 rpm for 2 minutes to separate cell debris from the DNA-containing supernatant.

Supernatant Collection

The clear supernatant containing genomic DNA was carefully transferred into a fresh sterile tube and stored at -20°C until further analysis (Thermo Fisher Scientific, 2023).

Data Analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 28.0, and data were expressed as mean ± standard deviation (SD), with significance set at $p < 0.05$ (IBM Corp., 2022).

RESULTS

Most samples exhibited too numerous to count (TNC) colonies (Table 1), particularly at higher dilutions (10^{-3}). However, at 10^{-5} dilutions, countable colonies were observed, with bacterial counts on Nutrient Agar (NA) ranging from 6.3 to 69.0 cfu/g (Table 1A) and fungal counts on Malt Extract Agar

(MEA) ranging from 4.8 to 9.4 cfu/g (Table1B). Soil Sample 5 showed the highest bacterial count (69.0 cfu/g) at 10^{-5} dilution, while Soil Sample 4 had the highest fungal count (11.0 cfu/g) at 10^{-3} dilution (Table 1B). The TNC values suggest a high microbial presence in most soil samples, indicating significant microbial activity in cassava wastewater-contaminated environments.

Table 1A: Bacterial Count (cfu/g) on Nutrient Agar (NA)

Soil Sample	No. of Colonies (10^{-3}) NA	No. of Colonies (10^{-5}) NA
1	TNTC	TNTC
2	TNTC	8.1 ± 0.5
3	TNTC	7.4 ± 0.6
4	7.8 ± 1.2	6.3 ± 0.9
5	TNTC	69.0 ± 2.3

Table 1B: Fungal Count (cfu/g) on Malt Extract Agar (MEA)

Soil Sample	No. of Colonies (10^{-3}) MEA	No. of Colonies (10^{-5}) MEA
1	TNTC	TNTC
2	TNTC	6.9 ± 0.3
3	TNTC	5.2 ± 0.4
4	11.0 ± 0.8	4.8 ± 0.5
5	12.0 ± 1.1	9.4 ± 0.7

KEY:
- **TNTC**: Too Numerous to Count
- **NA**: Nutrient Agar (for bacterial growth)
- **MEA**: Malt Extract Agar (for fungal growth)

Staphylococcus sp. (Gram-positive cocci) and *Bacillus sp.* (Gram-positive rods), all of which tested positive for citrate, catalase, and oxidase. *Pseudomonas sp.* (Gram-negative rod) was isolated from one sample, differing in its negative oxidase reaction (Table 2). Fungal analysis revealed the presence of *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum,* and *Rhizopus sp.* across the samples. Nutrient Agar (NA) cultures exhibited creamy yellow to whitish-yellow colonies with flat or raised elevations, while Malt Extract Agar (MEA) cultures displayed diverse pigmentation, including black, yellow-green, white, and grayish-white colonies.

Table 2: Biochemical and Morphological Characteristics of Bacterial and Fungal Isolates from Cassava Wastewater-Contaminated Soil

Soil Sample	Bacterial Biochemical Tests	Bacterial Suspected Organisms	Fungal Morphology on NA	Fungal Morphology on MEA	Fungal Suspected Organisms
1	Citrate (+), Catalase (+), Oxidase (+), Cocci, Gram (+)	*Staphylococcus sp.*	Whitish yellow, flat	Black, Yellow-green, White	*Aspergillus niger, Aspergillus flavus, Fusarium oxysporum*
2	Citrate (+), Catalase (+), Oxidase (+), Rod, Gram (+)	*Bacillus sp.*	Creamy yellow, flat	White, Black	*Fusarium oxysporum, Aspergillus niger*
3	Citrate (+), Catalase (+), Oxidase (+), Rod, Gram (+)	*Bacillus sp.*	Creamy yellow, raised	Black, Yellow-green	*Aspergillus niger, Aspergillus flavus*
4	Citrate (+), Catalase (+), Oxidase (+), Rod, Gram (+)	*Bacillus sp.*	Whitish yellow, raised	Yellow-green	*Aspergillus flavus*
5	Citrate (+), Catalase (+), Oxidase (-), Rod, Gram (-)	*Pseudomonas sp.*	Mucoid yellow, flat	Yellow-green, Grayish-white	*Aspergillus flavus, Rhizopus sp.*

The toxigenicity screening of fungal isolates from cassava wastewater-contaminated soil samples revealed that *Aspergillus flavus* exhibited aflatoxigenic potential only in Soil Samples 1 and 4 (Table 3). In contrast, *Aspergillus niger* tested negative for aflatoxin production across all samples (Table 3). These findings indicate that 40% of the soil samples contained aflatoxigenic A. flavus strains, raising concerns about potential aflatoxin contamination in the environment.

Table 3: Toxigenicity screening on yeast extract agar

Soil Sample		Aflatoxigenicity%
1	*Aflavus*	+
2	*A niger*	-
3	*A niger*	-
4	*Aflavus*	+
5	*Aflavus*	-

KEYS:
+ = Aflatoxin Present
- = Aflatoxin Absence

The heavy metal analysis of soil samples contaminated with cassava wastewater revealed varying concentrations of Cadmium (Cd), Lead (Pb), Manganese (Mn), Copper (Cu), and Zinc (Zn) – Table 4. Copper (Cu) showed the highest concentrations across all samples, ranging from 2.56 to 4.57 mg/kg, indicating potential environmental concern. Lead (Pb) levels varied significantly, with some samples showing relatively high concentrations (up to 2.03 mg/kg), while others had minimal traces. Manganese (Mn) and Zinc (Zn) were detected at lower concentrations, whereas Cadmium (Cd) was present in all samples but at minimal levels (Table 4).

Table 4: Heavy Metal Test on Cassava Wastewater-Contaminated Soil Samples

SAMPLES CODE	Cd (Mg/Kg)	Pb Mg/Kg	Mn Mg/Kg	Cu Mg/Kg	Zn Mg/Kg
1	0.0234	2.0217	0.3485	3.3646	0.4222
2	0.0134	1.0986	0.3125	2.8896	0.3870
3	0.0278	2.0342	0.9895	4.5674	0.9781
4	0.0134	0.0999	0.5643	2.5642	0.4550
5	0.0458	0.0823	0.3247	3.9723	0.3222

KEY:
Sample 1 – Soil from Apa-Kila 1; Sample 2 – Soil from Olufowora;
Sample 3 – Soil from Ayetoro; Sample 4 – Soil from Olodo;
Sample 5 – Soil from Apa kila 2;
Cd- Cadmium, Pb – Lead, Mn – Manganese, Cu – Copper, Zn – Zinc.

Fusarium clamydosporum was found major in soil sample 2, while *Pseudomonas mosselli* was major in soil sample 5 (Table 5).

Table 5: Molecular Identification of the isolates from cassava wastewater contaminated soil samples

CODE	NAME	PERCENTAGE IDENTITY (%)	ACCESSION
Soil Sample 2	*Fusarium clamydosporum*	99.61	JX867235.1
Soil Sample 5	*Pseudomonas mosselli*	95.00	MT089928.1

DISCUSSION

Cassava flour, both in its raw and processed forms, can be prone to microbial and aflatoxin contamination (Lewis *et al.,* 2005). Results from this study revealed varying degrees of microbial contamination in cassava wastewater. *Aspergillus niger, Aspergillus flavus, Fusarium sp,* and *Rhizopus sp* were the fungi isolated from the samples, likewise *Bacillus* sp, *Staphylococcus* sp and *Pseudomonas sp* were the bacteria isolated from the samples which are in agreement with the findings of Lewis *et al.,* (2005). Also, a study on cassava roasted flour ("rale") in Mozambique identified microbial contaminants such as moulds, lactic acid bacteria, general aerobic bacteria, *Bacillus* spp., yeasts, and *Staphylococcus aureus* during different processing stages (Mohamed, 2023). Soil contaminated by cassava mill effluent had higher counts of bacteria and fungi than the control. (Ezeigbo *et al.,* 2014). Some microorganisms develop the skill to degrade nitriles (nitrilase, nitrile hydratase and amidase) and cyanides in contaminated environments, according to the biodegradation of cyanide under aerobic conditions causes the microorganisms to consume HCN and generate hydrogen cyanate (HCNO), being transformed into ammonia and carbon dioxide through hydrolysis. Anaerobic biodegradation of cyanide and HCNO only occurs in the presence of hydrogen sulfide (H2S) and at pH > 7.0 (dominant species) or pH < 7.0 (prevalent species), being slower (Bhalla *et al.,* 2012).

Cassava, being a susceptible crop, can become contaminated with aflatoxins during cultivation, harvest, and storage. Aflatoxins are highly carcinogenic and can lead to serious health issues when consumed in contaminated food products (Lewis *et al*., 2005). Similarly, a 2021 study in Zambia reported that aflatoxin levels in cassava flour samples were below detection limits, indicating minimal contamination.

These findings underscore the importance of monitoring and controlling microbial and aflatoxin contamination in cassava products to ensure food safety.

Aflatoxigenicity screening affirmed that 40 % of the soil samples contained toxigenic strains of fungi which raises concern about its health impact. This finding aligns with previous studies, such as Salano (2015), which reported a similar prevalence of aflatoxigenic fungi in agricultural soils, emphasizing the widespread nature of aflatoxin contamination in the environment. These results reinforce the need for continuous monitoring and effective mitigation strategies to reduce aflatoxin contamination in soil and food products. Aflatoxin contamination is a significant concern in cassava flour production, as it can occur if cassava is not properly handled and stored. Controlling aflatoxin contamination is crucial to ensure the safety of cassava flour and protect consumer health.

The results of heavy metals obtained from the analysis of cassava indicated that the concentrations of Cu are relatively high but lower than the WHO standard except for manganese which is higher than the WHO standard. Elevated copper levels in soil can have both beneficial and harmful effects. While Cu is an essential micronutrient for plant growth, excessive concentrations can lead to phytotoxicity, inhibiting root elongation and nutrient uptake. Furthermore, prolonged exposure to high Cu levels in food can pose health risks to humans, including liver and kidney damage, gastrointestinal distress, and neurological disorders Alloway,

(2013). This finding highlights the need for continuous monitoring and possible remediation strategies to prevent excessive heavy metal accumulation in the food chain. The cassava wastewater-contaminated soil demonstrated a substantial content of essential metals, including Iron, Cadmium, lead, Manganese and zinc. Notably, processing methods such as milling as a result from the milling machine, likewise run off surface water to the soil from heavy metals polluted areas influenced metal concentrations and this was also established by (Ajuzie *et al.* 2015).

The investigation found a wide variety of microbial isolates, with *Bacillus sp* being the most common among bacteria and *Aspergillus* being the most common among fungal species. Cassava mill effluent's biodegradability may account for the high prevalence of these isolates. However, some of the microorganisms reported in this study had earlier been documented by previous researchers Okechi *et al.,* (2012) and Omotioma *et al.,* (2013) where they reported *Staphylococcus saprophyticus, Bacillus sp, Aspergillus, Penicillium,* and *Rhizopus* species. These organisms: *Bacillus,* and *Pseudomonas* are considered as opportunistic pathogens that can cause wound infections leading to sepsis.

RECOMMENDATION

It is important to implement proper post-harvest handling and storage practices to minimize contamination and improve food safety. There is a dire need for public sensitization on the hazards, and implications of these contaminations and why we have to improve our food quality. There should be call for strict enforcement of aflatoxin legislations.

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(Manuscript received:13th February, 2025; accepted: 13th May, 2025).