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Research Article

Toxicological Assessment of Cassava Wastewater and the Ameliorative Effect of Activated Charcoal on Fingerlings of *Clarias Gariepinus*

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Abstract

Cassava processing generates substantial volumes of wastewater containing cyanogenic glucosides and organic pollutants that pose significant threats to aquatic ecosystems in Nigeria. This study evaluated the acute toxicity of cassava wastewater from three processing locations and assessed the ameliorative potential of activated charcoal on *Clarias gariepinus* fingerlings. Cassava wastewater samples were collected from Ilesha (CWIL), Ijebu (CWIJ), and Epe (CWEP) processing facilities in southwestern Nigeria. Physicochemical characterization followed standard methods, and 96-hour static bioassays determined median lethal concentrations (LC_{50}) in *C. gariepinus* fingerlings (mean weight: 2.5±0.3 g, mean length: 5.2±0.4 cm). Joint action studies examined activated charcoal's protective effects at various concentrations. Probit analysis determined LC_{50} values and synergistic ratios. Physicochemical analysis revealed CWEP exhibited the highest pollution loads, with total dissolved solids (8,094 mg/L), total suspended solids (578 mg/L), and turbidity (474.8 NTU) substantially exceeding National Environmental Standards and Regulations Enforcement Agency (NESREA) limits. The 96- hour LC_{50} values demonstrated concentration-dependent toxicity: CWIL (7.531mg/L), CWIJ (8.097mg/L), and CWEP (10.14mg/L). Co-exposure with activated charcoal significantly increased LC_{50} values to 18.542mg/L, 17.674mg/L, and 12.092mg/L for CWIL, CWIJ, and CWEP respectively, indicating protective effects. Synergistic ratios of 0.68, 0.65, and 0.44 confirmed antagonistic interactions, demonstrating activated charcoal's capacity to mitigate cassava wastewater toxicity. Cassava wastewater from all three locations exhibited acute toxicity to *C. gariepinus* fingerlings, with CWEP showing the highest toxicity correlating with elevated pollutant concentrations. Activated charcoal demonstrated significant protective effects through antagonistic mechanisms, suggesting its potential as a cost-effective remediation strategy for cassava processing effluents in Nigerian aquaculture systems.

Keywords

Cassava wastewater; *Clarias gariepinus*; activated charcoal; LC_{50}; acute toxicity; joint action; whole-effluent toxicity; Nigeria

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Introduction

1.1 Background

Cassava (*Manihot esculenta* Crantz) is a staple crop across sub-Saharan Africa. Nigeria is the world's largest producer, accounting for roughly 20% of global output (FAO, 2023). Processing cassava tubers into garri, fufu, lafun and starch generates large volumes of effluent. This effluent carries cyanogenic glucosides, principally linamarin and lotaustralin, together with organic matter and suspended solids (Oboh and Akindahunsi, 2003; Akintonwa *et al.*, 2009). Processing releases an estimated 300–400 litres of wastewater per tonne of cassava, and most of it enters Nigerian waterways without treatment (Igbinosa and Okoh, 2011).

The environmental burden of this effluent is not limited to its cyanide content. Cassava wastewater is characterised by high biochemical oxygen demand (BOD), high chemical oxygen demand (COD), high total dissolved solids (TDS) and low pH. These act together as multiple stressors on receiving waters (Igbinosa and Okoh, 2011). On discharge, the effluent drives oxygen depletion and pH depression, and both threaten aquatic biodiversity and fisheries productivity (Ogundiran and Osibanjo, 2009).

1.2 Toxicity pathways

Cassava wastewater acts on aquatic organisms through more than one pathway. Cyanogenic glucosides are hydrolysed by linamarase to release hydrogen cyanide (HCN). HCN inhibits cytochrome c oxidase in the mitochondrial electron transport chain and so blocks cellular respiration (Padmaja, 1995). The result is tissue hypoxia, metabolic acidosis and, at sufficient exposure, death (Nweke and Okpokwasili, 2003). Separately, the organic load depletes dissolved oxygen and creates hypoxic conditions that impose respiratory stress independently of any specific toxicant (Adeyemo, 2005). Low pH adds a third pathway, disrupting ionoregulation at the gill and increasing the proportion of cyanide present as the membrane-permeant molecular species, HCN.

African catfish (*Clarias gariepinus* Burchell, 1822) is ecologically and economically important in Nigerian freshwaters. As a benthic omnivore with wide environmental tolerance, it is widely used as a bioindicator for anthropogenic pollution in tropical freshwater systems (Ezemonye and Kadiri, 2000). Its commercial weight in Nigerian aquaculture makes its response to cassava wastewater a matter of both ecological and food-security relevance (Federal Department of Fisheries, 2020).

1.3 Activated charcoal as a mitigation option

Activated charcoal is a widely used adsorbent in water treatment. It combines high surface area, typically 500–3,000 m²/g, with a pore structure and surface chemistry that favour organic compounds and some metals (Marsh and Rodríguez-Reinoso, 2006). Adsorption proceeds through van der Waals forces and electrostatic interaction between the charcoal surface and the sorbate (Bansal and Goyal, 2005). Activated charcoal has been shown to remove cyanide from industrial wastewaters, principally by physisorption and by surface complex formation (Dash *et al.*, 2009). It also removes dissolved organic matter and so reduces oxygen demand.

Its limitations matter as much as its capacity. Activated charcoal does not remove dissolved inorganic salts, does not neutralise acidity, and has limited affinity for small, highly hydrated ions. Any protective effect it exerts should therefore depend on which stressor in a given effluent is driving toxicity a prediction this study is positioned to test.

Materials and methods

2.1 Study design

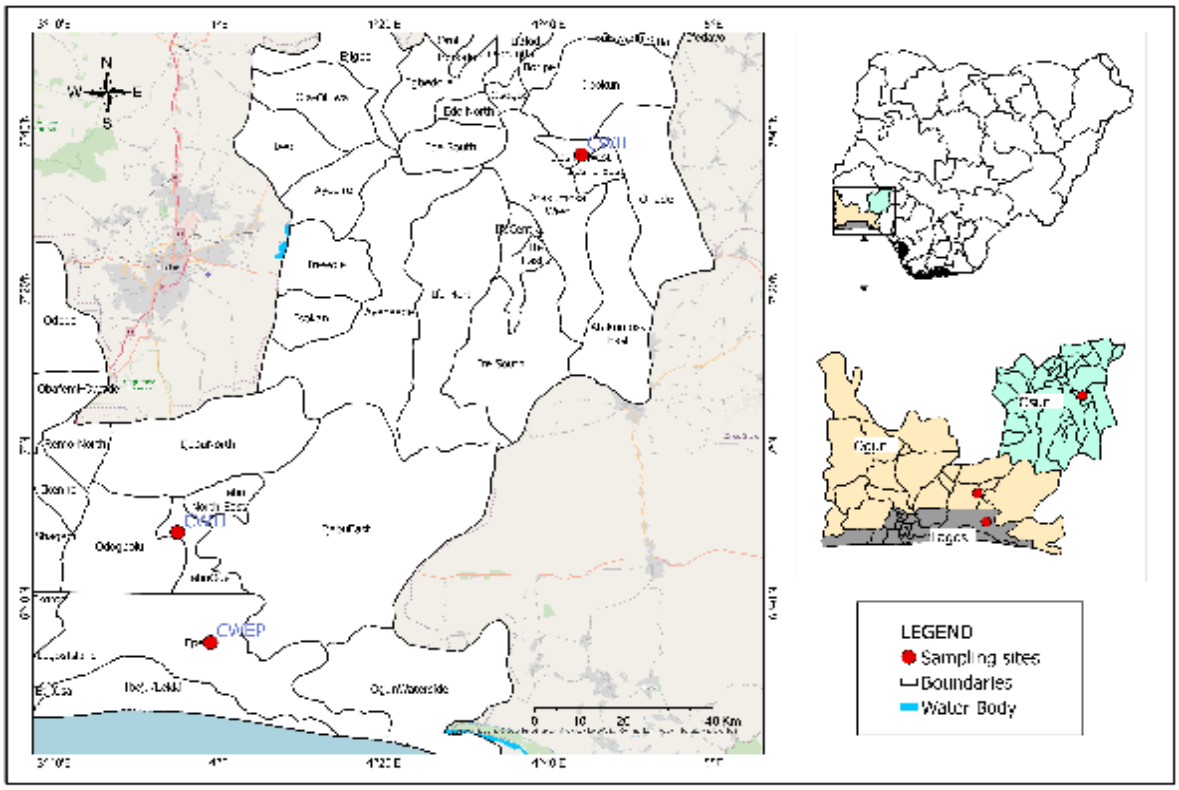
The study combined physicochemical characterisation of three cassava wastewater sources with 96-h static acute toxicity bioassays conducted under OECD Guideline 203 (OECD, 2019). Bioassays were run in two series: each effluent alone, and each effluent in binary combination with activated charcoal.

2.2 Sample collection and preparation

2.2.1 Cassava wastewater

Wastewater was collected from three sites across southwestern Nigeria, chosen to span a range of processing environments: Ilesha, Osun State (7°37′N, 4°44′E), designated CWIL; Ijebu-Ode, Ogun State (6°49′N, 3°55′E), designated CWIJ; and Epe, Lagos State (6°35′N, 3°59′E), designated CWEP

Samples of 20 litres were taken in pre-cleaned high-density polyethylene containers directly from the discharge points of operating processing facilities, during peak production hours (0800–1000 h). Containers were rinsed three times with sample before filling and were filled with no headspace to limit loss of volatile cyanide. Samples were transported to the laboratory in ice-cooled containers at 4 °C and held at 4 °C in the dark until analysis, for no longer than 24 h. Before use in bioassays, samples were brought to ambient laboratory temperature (25 ± 2 °C). Effluent was used as collected, without filtration, so



that the test material represented the discharge as received by the environment.

Figure 1: Map of sampling sites

2.2.2 Activated charcoal

Commercial food-grade powdered activated charcoal of particle size 200–400 mesh was used as supplied, without further modification. A stock suspension of 10 g/L was prepared in dechlorinated tap water and stirred continuously for 30 min before use to ensure homogeneity. Stock was prepared fresh on each test day. Working concentrations for the joint-action series were prepared by volumetric dilution of this stock.

2.3 Physicochemical analysis

Parameters were determined in triplicate following standard methods (APHA, 2017). The following were measured: pH (electrometric); dissolved oxygen (Winkler); BOD (5-day incubation at 20 °C); COD (closed reflux, colorimetric); temperature; oil and grease (hexane extraction); turbidity (nephelometric); electrical conductivity; total dissolved solids, total solids and total suspended solids (gravimetric); total hardness, total alkalinity and total acidity (titrimetric); major ions (atomic absorption spectrophotometry and ion chromatography); and cyanide (colorimetric, following distillation).

2.4 Test organism

2.4.1 Source and acclimation

Clarias gariepinus fingerlings were obtained from a certified hatchery (Lagos State Fisheries Department) with documented health certification. Fish were transported in oxygenated containers and acclimated for 7 days in 500-litre fibreglass tanks holding dechlorinated tap water. Acclimation conditions were 26 ± 1 °C, pH 7.2 ± 0.3, dissolved oxygen above 5.0 ml/L, and a 12:12 h light:dark photoperiod. Fish were fed a commercial pelleted diet of crude protein daily. Feeding was suspended 24 h before testing.

2.4.2 Test organism characteristics

Fingerlings used in bioassays had a mean weight of 2.5 ± 0.3 g and a mean total length of 5.2 ± 0.4 cm, were free of visible injury or deformity, and were actively swimming. Restricting size range keeps the surface-area-to-volume ratio consistent and limits variability in uptake.

2.5 Acute toxicity bioassay

2.5.1 Range-finding test

Range-finding tests established the concentration interval for the definitive test. Five fish per concentration were exposed for 96 h to a low, mid and high concentration (10ml/L, 20ml/L and 50ml/L respectively.

2.5.2 Definitive LC_{50} test

Definitive tests followed OECD Guideline 203. For each effluent, ten fingerlings were exposed to each of five nominal concentrations 3ml/L, 5ml/L, 7ml/L, 9ml/L and 11ml/L plus a dilution-water control, in duplicate. Fish were not fed during the 96-h exposure. The test was static, with no renewal of test solution.

Mortality was recorded at 24, 48, 72 and 96 h. Fish were recorded as dead when gill movement had ceased and tactile stimulation produced no response; dead fish were removed immediately.

2.6 Joint action bioassay

Joint-action tests paired each effluent with activated charcoal in binary mixtures. Mixtures were prepared on a toxic-unit basis, combining each component at equal fractions of its individual 96-h LC50, giving a 1:1 toxic-unit ratio. Activated charcoal only series was run in parallel to establish the LC50 of activated charcoal alone and to confirm that the suspension itself was not contributing appreciably to mortality at the concentrations used.

2.7 Statistical analysis

2.7.1 LC50 estimation

Median lethal concentrations at 96 h and their 95ml/L limits were estimated by probit analysis in SPSS version 20 (Finney, 1971

2.7.2 Interaction between wastewater and activated charcoal

The nature of the interaction was quantified by the synergistic ratio:

R = LC50 of the toxicant acting alone ÷ LC50 of the toxicant in mixture

SR below 1 indicates antagonism, SR of 1 indicates additivity, and SR above 1 indicates synergism (Marking, 1977).

2.8 Ethical considerations

Procedures followed the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH, 2011). The number of animals used was the minimum required for valid probit estimation under OECD Guideline 203.

Results

3.1 Physicochemical characteristics

The three effluents differed substantially in composition (Table 1). CWEP was the most heavily loaded on almost every determinand. Its total dissolved solids reached 8,094 ml/L, total suspended solids 578 ml/L and turbidity 474.8 NTU. Its conductivity of 5,330 µS/cm was approximately tenfold that of CWIL and CWIJ, and its chloride concentration of 12,017 ml/L was roughly fifty to a hundred times higher. Total acidity in CWEP was 5,980 ml/L against 38 ml/L in both other sources, and pH was 4.10 against 6.90 and 6.80.

Dissolved oxygen was low in all three samples, at 3.03, 2.64 and 2.71 ml/L for CWIL, CWIJ and CWEP respectively. Organic load followed the same ranking as the solids data, with BOD of 32.71, 23.75 and 53.77 ml/L and COD of 49.22, 37.42 and 79.48 ml/L. BOD:COD ratios were 0.66, 0.63 and 0.68, indicating moderately biodegradable organic matter in all three.

Cyanide was below detection in CWIL, present at 0.01 ml/L in CWIJ and at 1.22 ml/L in CWEP. Total hardness was 140, 180 and 770 ml/L, the elevated value in CWEP corresponding to calcium of 308.62 ml/L and magnesium of 112.12 ml/L. Metals were detected at low concentrations: iron 0.07–0.39 ml/L, zinc 1.19–2.70 ml/L, lead 0.021–0.082 ml/L and chromium 0.005–0.017 ml/L, in each case with the maximum in CWEP.

Measured against the gazetted NESREA (2009) effluent limits for the food, beverages and tobacco sector, all three effluents exceeded the limits for total suspended solids, total dissolved solids and turbidity. CWEP additionally exceeded the limits for pH, BOD, COD, chloride, sulphate, nitrate, zinc and lead. CWIJ exceeded the limits for sulphate and nitrate, and CWIL exceeded the limit for BOD. Cyanide is not assigned an effluent limit for this sector, but the CWEP concentration of 1.22 ml/L exceeds the USEPA ambient water quality criterion for the protection of freshwater aquatic life, 0.022 ml/L as free cyanide, by approximately 55-fold (USEPA, 1985).

Table 1: Physicochemical characteristics of cassava wastewater from three processing sites, with regulatory and ecological benchmarks

Parameter	CWIL	CWIJ	CWEP	NESREA (2011)
pH	6.90	6.80	4.10	6.5–8.5
Dissolved oxygen (ml/L)	3.03	2.64	2.71	6.0
BOD (ml/L)	32.71	23.75	53.77	30
COD (ml/L)	49.22	37.42	79.48	30
Temperature (°C)	28.05	28.00	27.50	40
Oil and grease (ml/L)	BDL	BDL	BDL	10
Turbidity (NTU)	23.7	22.5	474.8	5.0
Conductivity (µS/cm)	480	520	5,330	NS
Total dissolved solids (ml/L)	714	786	8,094	500
Total solids (ml/L)	762	810	8,672	NS
Total suspended solids (ml/L)	48	34	578	25
Total hardness (ml/L)	140	180	770	NS
Total alkalinity (ml/L)	140	40	100	150
Total acidity (ml/L)	38	38	5,980	NS
Calcium (ml/L)	56.11	72.14	308.62	180
Magnesium (ml/L)	20.39	26.21	112.12	40
Chloride (ml/L)	124	236	12,017	250
Nitrate (ml/L)	3.99	13.90	21.95	10
Sulphate (ml/L)	90.6	314.5	494.1	250
Cyanide (ml/L)	BDL	0.01	1.22	0.001
Iron (ml/L)	0.07	0.09	0.39	0.05
Zinc (ml/L)	1.22	1.19	2.70	2.0
Lead (ml/L)	0.021	0.031	0.082	0.05
Chromium (ml/L)	0.011	0.005	0.017	1.0

3.2 Acute toxicity to *Clarias gariepinus*

Mortality was concentration-dependent for all three effluents, and no mortality occurred in any control vessel. (Table 2). The 96-h $LC5_0$ was 7.531ml/L for CWIL, 8.097ml/L for CWIJ and 10.14ml/L for CWEP. CWIL was therefore the most acutely toxic of the three and CWEP the least, with tolerance factors of 1.346, 1.252 and 1.000 respec-

tively. The ranking is the inverse of the ranking by pollutant load: CWEP carried the highest concentrations of dissolved solids, suspended solids, acidity, chloride and cyanide, yet required the greatest dilution volume to kill half the exposed fish. It is also the inverse of the ranking by cyanide, since CWIL contained no detectable cyanide but was the most toxic of the three.

Table 2: 96-h acute toxicity of cassava wastewater to *Clarias gariepinus* fingerlings

Effluent	LC50 (ml/L)	95% Confidence limits	Probit line equation	df	TF
CWIL	7.531	5.224	Y = −3.296 + 3.759x	3	1.346
CWIJ	8.097	9.926	Y = −2.701 + 2.973x	3	1.252
CWEP	10.140	11.626	Y = −4.536 + 4.509x	3	1.000

CWIL = cassava wastewater, Ilesha; CWIJ = cassava wastewater, Ijebu-Ode; CWEP = cassava wastewater, Epe. Toxicity factor is the ratio of the highest LC_{50} to each LC_{50}.

3.3 Effect of activated charcoal

Activated charcoal raised the 96-h LC50 of every effluent (Table 3). For CWIL the LC50 rose from 7.531ml/L to 18.542ml/L, a 2.46-fold reduction in toxicity. For CWIJ it rose from 8.097ml/L to 17.674ml/L, a 2.18-fold reduction. For CWEP it rose from 10.14ml/L to 12.092ml/L, a 1.19-fold reduction.

Synergistic ratios were 0.41 for CWIL, 0.46 for CWIJ and 0.84 for CWEP. All three fall below unity, indicating antagonism in each case.

Activated charcoal alone gave a 96-h LC50 of 27.007ml/L, well above the concentrations present in any joint-action vessel. Direct toxicity of the charcoal suspension was therefore not an appreciable contributor to mortality in the mixture series.

Table 3: Joint action of cassava wastewater and activated charcoal on *Clarias gariepinus* fingerlings

Treatment	LC50 (ml/L)	95% confidence limits	Probit line equation	df	Fold change	SR
CWIL : AC	18.542	12.662	Y = −6.466 + 5.099x	3	2.46	0.41
CWIJ : AC	17.674	11.626	Y = −4.956 + 3.974x	3	2.18	0.46
CWEP : AC	12.092	8.587	Y = −4.203 + 3.882x	3	1.19	0.84
AC alone	27.007	15.759	Y = −4.018 + 2.807x	3	—	—

Discussion

The physicochemical characterization revealed that cassava wastewater from all three processing locations exhibited substantial pollution loads, with CWEP demonstrating the most severe contamination profile. The elevated total dissolved solids and total suspended solids in CWEP, exceeding FEPA limits respectively, reflect intensive processing practices and potentially inadequate effluent management at the Epe facility. These findings align with previous studies documenting high TDS and TSS in cassava processing effluents, attributed to starch granules, cell debris, and dissolved organics released during cassava grating and pressing operations (Oboh and Akindahunsi, 2003; Akintonwa *et al.,* 2009).

The acidic pH observed in CWEP results from organic acid accumulation during cassava fermentation, particularly lactic, acetic, and butyric acids produced by microbial metabolis (Padonou *et al.,* 2010). This pH depression poses dual threats to aquatic ecosystems: direct physiological stress on fish through disruption of osmoregulatory function and acid-base balance, and mobilization of toxic heavy metals from sediments in receiving waters (Ezemonye and Kadiri, 2000). The circumneutral pH in CWIL and CWIJ suggests different processing methodologies or shorter detention times before discharge, limiting organic acid accumulation. Dissolved oxygen depletion observed across all samples below FEPA's standards reflects the high organic load characteristic of cassava wastewater. The elevated BOD and COD quantify this oxygen-demanding potential, which upon discharge into receiving waters, triggers microbial decomposition, consuming dissolved oxygen and creating hypoxic conditions lethal to aquatic fauna (Anhwange *et al.,* 2009).

The detection of cyanide in CWEP and CWIJ, though below FEPA's limit, confirms the presence of the primary toxic principle derived from cassava's cyanogenic glucosides. Linamarin and lotaustralin undergo enzymatic or spontaneous hydrolysis to release hydrogen cyanide, which exhibits high acute toxicity to aquatic organisms through inhibition of cellular respiration (Padmaja, 1995). The below-detection cyanide in CWIL may reflect analytical detection limits or rapid volatilization/degradation of HCN in the sample. Heavy metal contamination, particularly zinc exceedances exceeding FEPA's <1.0 mg/L limit), likely originates from metal processing equipment (zinc-galvanized graters) used in cassava processing facilities (Ogundiran and Osibanjo, 2009). Chronic zinc exposure disrupts fish osmoregulation, gill function, and reproductive processes, posing sublethal threats even at concentrations below acute toxicity thresholds (Jezierska *et al.,* 2009).

The stark contrast in pollution severity between CWEP and the other two sources (CWIL, CWIJ) suggests geographic variation in processing practices, cassava varieties processed, production volumes, or effluent management systems. Epe, located in Lagos State's peri-urban zone, may experience higher processing intensities to meet urban market demands, generating more concentrated effluents. Alternatively, differences in cassava cultivar selection (sweet vs. bitter varieties with varying cyanogenic glucoside content) may contribute to toxicity variation (Cardoso *et al.,* 2005).

The concentration-dependent acute toxicity observed across all cassava wastewater sources confirms their potential to cause fish mortality upon discharge into aquatic ecosystems. The 96-hour LC_{50} values indicate that relatively small dilutions (approximately 1:10 to 1:13 wastewater: receiving water ratios) are required to reach median lethal concentrations, posing significant risks to fish populations in receiving streams with limited dilution capacity. The substantial increases in LC_{50} values observed upon co-exposure with activated charcoal (2.46-fold for CWIL, 2.18-fold for CWIJ, 1.19-fold for CWEP) provide compelling evidence for activated charcoal's capacity to mitigate cassava wastewater toxicity. The consistent synergistic ratios <0.8 (CWIL: 0.68, CWIJ: 0.65, CWEP: 0.44) confirm antagonistic interactions, ruling out simple dilution effects and indicating active detoxification mechanisms.

Activated charcoal's protective effects operate through multiple interconnected mechanisms that collectively reduce cassava wastewater toxicity to exposed fish. The primary mechanism involves adsorptive removal of cyanide, whereby activated carbon exhibits high affinity for hydrogen cyanide through both physisorption driven by van der Waals forces and chemisorption involving surface complex formation (Dash *et al.,* 2009). By sequestering dissolved HCN from the water column, activated charcoal reduces bioavailable cyanide concentrations, preventing uptake across fish gill membranes and subsequent cytochrome c oxidase inhibition that would otherwise lead to cellular hypoxia and mortality. The demonstrated acute toxicity of cassava wastewater to *C. gariepinus* fingerlings, combined with widespread effluent discharge into Nigerian freshwater systems, poses substantial threats to aquatic biodiversity and fisheries productivity. Clarias gariepinus serves as both an ecological indicator species and a commercial aquaculture species, making these findings relevant to both ecosystem health and food security concerns.

Nweke and Okpokwasili (2003) reported 96-hour LC50 values of 8.5-12.3mg/L for cassava mill effluent tested against Tilapia guineensis, similar to our *C. gariepinus* results. Anhwange et al. (2009) documented 96-hour LC_{50} of

6.2mg/L for cassava wastewater against juvenile *Clarias gariepinus,* slightly more toxic than our findings, potentially reflecting differences in effluent source or fish size/age. The physicochemical characterization revealed higher pollution loads than some previous studies (Oboh and Akindahunsi, 2003: TDS 1,200-2,400 mg/L; Akintonwa *et al.,* 2009: BOD 150-450 mg/L), but lower than others (Ogundiran and Osibanjo, 2009: TDS up to 12,000 mg/L), reflecting the wide variability in cassava processing practices across Nigeria. The protective effect of activated charcoal demonstrated here appears effective in the Nigerian cassava wastewater literature. While activated carbon treatment of industrial effluents is well-established internationally (Bansal and Goyal, 2005), its specific application to cassava wastewater toxicity mitigation represents an underexplored area. Dahunsi et al. (2019) documented activated charcoal's capacity to reduce cyanide toxicity in synthetic solutions but did not evaluate complex cassava wastewater matrices or conduct joint action assessments with fish.

Conclusions

Cassava wastewater from all three processing sites was acutely lethal to *Clarias gariepinus* fingerlings. From an aquatic ecosystem management perspective, these findings underscore the urgent need for regulatory oversight of cassava processing discharges, coupled with technical assistance programs enabling processor compliance. Activated charcoal treatment represents one component of a comprehensive pollution prevention strategy that should also include process optimization, cultivar selection, effluent detention, and stakeholder education. The geographic variation in toxicity profiles among the three study sites highlights the importance of site-specific assessments rather than one-size-fits-all regulatory approaches. Future research should expand geographic coverage, assess temporal variation in effluent characteristics, evaluate chronic toxicity endpoints, and conduct field-scale demonstration of activated charcoal treatment systems to facilitate technology transfer to cassava processing communities.

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