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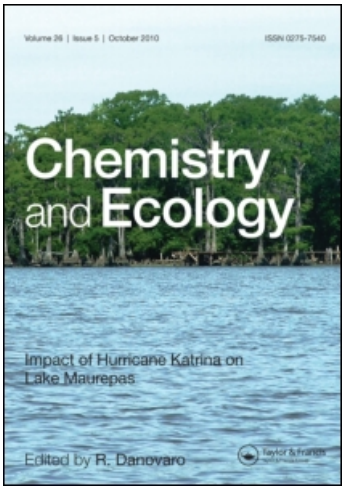

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
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To cite this Article Adekunle, I. M. , Arowolo, T. A. , Omoniyi, I. T. and Olubambi, O. T.(2007) 'Risk assessment in Nile tilapia (*Oreochromis niloticus*) and African mud catfish (*Clarias gariepinus*) exposed to cassava effluent', Chemistry and Ecology, 23: 5, 383 — 392
To link to this Article: DOI: 10.1080/02757540701587699
URL: http://dx.doi.org/10.1080/02757540701587699

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Chemistry and Ecology
Vol. 23, No. 5, October 2007, 383–392

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Risk assessment in Nile tilapia (*Oreochromis niloticus*) and African mud catfish (*Clarias gariepinus*) exposed to cassava effluent

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(Received 11 April 2007; in final form 2 July 2007)

Toxicity of cassava effluent in water on aquatic lives was examined via acute and chronic bioassay experiments on *Clarias gariepinus* (I) and *Oreochromis niloticus* (II) under laboratory conditions, using standard procedures. The effluent cyanide concentration exceeded the WHO limit for wastewater. Fish body weights and haematological parameters (HMP) significantly decreased with increasing effluent concentration at $p \leq 0.05$. Toxicity on HMP for the respective (I) and (II) varied from 5.4 to 52.8; 4.8 to 51.9% for packed cell volume, 4.1 to 43.9; 5.3 to 64.0% for red blood count, 0.0 to 15.7; 0.0 to 61.4% for white blood count, 3.6 to 45.9; 5.2 to 49.5% for haemoglobin, 11.6 to 71.9; 28.4 to 63.8% for total protein, 11.5 to 75.5; 15.0 to 58.2% for albumin and 11.8 to 75.0; 46 to 83.9% for globulin. Acute exposure yielded 96-h LC_{50} values of 0.45% for (I) and 0.25% for (II) and chronic exposure caused reduced growth and poor blood quality.

Keywords: Bioassay; Cassava effluent; Mud catfish; Tilapia; Toxicity

1. Introduction

Cassava (*Manihot esculanta Cranzt*) is an important staple food widely consumed in various forms (chips, flour, grain, and starch) in developing countries such as Indonesia, Malayasia, Thailand, Brazil, Ghana, and Nigeria [1]. It is also extensively utilized as a raw material in the industrial production of food additives, adhesives, textiles, and pharmaceuticals [2]. The cassava-processing industry is a fast-growing industry in Nigeria due to the recent National campaign to increase cassava export capacity of the country. Consequently, large volumes of untreated cassava effluents are discharged into streams, lagoons, drainage channels, and agricultural land. This practice interferes with the natural functioning of the ecosystem, impairing water quality and posing hazards to aquatic life and man [3].

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Chemistry and Ecology
ISSN 0275-7540 print/ISSN 1029-0370 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/02757540701587699

During cassava processing, large amounts of cyanoglycosides are released from the tubers to the effluent, and the cyanoglycosides rapidly decay to cyanides (CN^-) via enzymatic hydrolysis. Cyanides are harmful compounds, readily absorbed by inhalation, and in oral and dermal routes of exposure, and in the aquatic environment, they constitute stress factors for biota, including benthic organisms and fishes [4]. The central nervous system is the primary target organ of CN^- toxicity. Acute exposure to high concentrations results in immediate collapse, respiratory effects, growth retardation, and death [5]. The present study examines and compares the effect of untreated cassava effluent, released into water, on two fish species, Nile tilapia (*Oreochromis niloticus*) and African mud catfish (*Clarias gariepinus*) under laboratory conditions.

2. Materials and methods

2.1 *Fish sample collection*

Fish specimens were procured from the fisheries farm of the Federal University of Technology, Akure, Ondo State, Nigeria. The fishes were stocked and reared in large storage tanks from which samples needed for tolerance, acute, and chronic bioassay tests were removed. Juveniles of two fish species, of body weight in the range of 52.32–53.03 g for *C. gariepinus* and 45.90–46.76 g for O. *niloticus* were used for the study.

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2.2 *Cassava effluent collection and characterization*

Cassava tubers harvested from a farm in Akure, Ondo state, Nigeria were peeled, washed, and ground using a cassava grinding mill. The white slurry was collected in a sack, allowed to stand for 96 h (time taken for fermentation of cassava for garri production in Nigeria), and the effluent squeezed into a receiving black plastic container. The supernatant was then separated from the white sediments and used for the experiments. The pH, NH_4^+, cyanide contents, and toxic metal (Cd, Cr, and Pb) concentrations in the effluent were determined using standard procedures [6].

The metal concentrations in 5 M HNO_3 sample digests were measured using an atomic absorption spectrophotometer (Buck Scientific, 200, Buck Scientific, Stamford, CT), equipped with an oxyacetylene burner, at 288, 282, and 357 nm spectral lines for Cd, Pb, and Cr, respectively. The water-culture medium for each fish type was analysed for relevant water-quality parameters, including pH, electrical conductivity, dissolved oxygen, and chemical oxygen demand, using standard procedures of AOAC [6] in order to investigate the effect of the cassava effluent on these parameters.

2.3 *Experimental design*

Two weeks' acclimatization of fish samples prior to the commencement of each test (tolerance, acute, and chronic tests) was carried out. Varying concentrations of the effluent were introduced into static water aquaria, each containing 10 fish samples. Twenty and thirty fish samples were used for the acute and chronic tests, respectively, at each cassava effluent concentration. Effluent concentrations used for the respective acute and chronic tests were 0.42, 0.46, 0.48, 0.50; 0.10, 0.15, 0.20, 0.25% for *C. gariepinus*; and 0.22, 0.24, 0.26, 0.28; 0.05, 0.075, 0.10, 0.125% for O. *niloticus*. These concentrations were derived from the cassava effluent tolerance tests and dilution series previously conducted on each of the fish species.

The fish were fed every other day throughout the experiments at 3% body weight with 'Copen' feed, and the culture media were renewed at 3-d intervals. Water-quality parameters such as dissolved oxygen (DO), chemical oxygen demand (COD), pH, temperature, and electrical conductivity were conducted on the change water for quality-assurance purposes. The initial and final body weights of fish were recorded at the commencement and termination of the experiments. Fish in the culture media without the addition of the cassava effluent served as control samples.

2.3.1 Determination of 96-h LC_{50}. Estimation of 96-h LC_{50} was achieved by the Probit method [7]. The percentage Probit was plotted against the percentage cassava effluent concentration transformed to the logarithmic function. The Probit mortality curve was subjected to regression analysis, from which the 96-h LC_{50} was deduced.

2.3.2 Bioassay experiments. Blood samples were collected from the two fish species by cardiac puncture with the aid of disposable sterile syringed needles and transferred to heparrinized vials. The blood samples were then taken to a haematology laboratory for immediate analysis using standard procedures [8, 9].

Packed cell volume was determined using the micro-haematocrit method. Sterile heparinized capillary tubes were used to collect blood directly for this determination. The direct readings were expressed as a percentage of packed red cells to the total volume.

Haemoglobin was measured by mixing the blood sample with 4 ml of Drabkins solution. The colour developed after 5 min, and the absorbance was measured at 550 nm on Corning series of a colorimeter (Model 253). Standards were also prepared using a blood sample of known haemoglobin concentration. The results were expressed as $g\,dl^{-1}$. The blood sample dilution for the determination of red cell count was 1:200 (v/v) via an Olympus microscope (39204/h 52601). The white blood count was similarly determined but at a dilution ratio of 1:20, and the results were expressed as mm^3.

The total protein was measured by the Biuret method using a protein haematological kit (Biosystems reagent and instruments). After 10 min, the absorbance of the resulting mixture was taken at 545 nm. Albumin was measured using Bromocresol Green, and using the haematological kit, and the absorbance was recorded at 630 nm. Standards were prepared for both determinations. Globulin was calculated from the difference in total protein and albumin concentrations (total protein–albumin). These results were expressed as $g\,dl^{-1}$.

2.3.3 Statistics. Analysis of variance (ANOVA), Duncan's multiple range test, Probit analysis, regression, and inter-element correlations were performed on the data using SPSS 10.0 software for Windows to test for significant differences in means, for prediction of LC_{50} and significant linear relationships among factors. Results were generally expressed as mean $\pm$ standard deviation.

3. Results

3.1 *Effluent effect on fish water culture media*

The cassava effluent contained $190.62 \pm 1.49\,mg\,l^{-1}$ of CN^-, $4.26 \pm 0.04\,mg\,l^{-1}$ of NH_4^+, $0.162 \pm 0.001\,mg\,l^{-1}$ of Cr, and $0.054 \pm 0.001\,mg\,l^{-1}$ of Pb, but Cd was not detected. Changes in water-quality parameters for the fish water culture media are shown in table 1.

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Table 1. Effect of cassava effluent on mud catfish (*C. gariepinus*) and tilapia (*O. niloticus*) culture media.

Parameter	Control	Effluent concentration (%)			
		0.1	0.15	0.2	0.25
Catfish					
Temperature (°C)	24.1 ± 0.3a (23.8–24.3)	24.0 ± 0.3a (23.8–24.3)	23.9 ± 0.3a (23.7–24.3)	24.0 ± 0.3a (23.8–24.3)	24.0 ± 0.3a (23.7–24.3)
pH	7.3 ± 0.1a (7.2–7.3)	7.1 ± 0.1b (7.0–7.2)	6.9.0 ± 0.0c (6.8–7.0)	6.6 ± 0.1d (6.5–6.7)	6.3 ± 0.1e (6.2–6.4)
Conductivity ($\mu S\,cm^{-1}$)	118 ± 2e (116–120)	137 ± 2d (135–139)	142 ± 2c (140–143)	147 ± 2b (145–149)	151 ± 1a (150–151)
Dissolved oxygen ($mg\,l^{-1}$)	6.0 ± 0.2a (5.9–6.2)	5.9 ± 0.6a (5.4-6.5)	5.6 ± 0.4ab (5.3–5.9)	5.5 ± 0.4b (5.1–5.8)	5.4 ± 0.4b (5.1–5.7)
Chemical oxygen demand ($mg\,l^{-1}$)	17.5 ± 0.5e (17.2–17.9)	24.2 ± 0.6d (23.6–24.7)	27.3 ± 0.6c (26.8–27.8)	27.3 ± 0.6b (26.8–27.8)	29.3 ± 0.4a (28.9–29.6)
Tilapia		0.05	0.075	0.1	0.125
Temperature (°C)	23.9 ± 0.3a (23.8-24.2)	23.9 ± 0.3a (23.8–24.2)	24.0 ± 0.3a (23.8–24.3)	24.0 ± 0.3a (23.8-24.3)	24.0 ± 0.3a (23.7–24.3)
pH	7.3 ± 0.1a (7.3–7.4)	7.1 ± 0.1b (7.1–7.2)	7.0 ± 0.1c (7.0–7.1)	6.8 ± 0.1d (6.8–6.9)	6.5 ± 0.1e (6.4-6.6)
Conductivity ($\mu S\,cm^{-1}$)	118 ± 2d (116–120)	132 ± 5c (127–136)	137 ± 6b (132–142)	142 ± 6a (137–147)	147 ± 5a (143–150)
Dissolved oxygen ($mg\,l^{-1}$)	5.6 ± 0.2a (5.5–5.8)	5.4 ± 0.1b (5.3–5.4)	5.2 ± 0.2b (5.1–5.4)	5.0 ± 0.2c (4.9–5.1)	4.9 ± 0.1d (4.7–4.9)
Chemical oxygen demand ($mg\,l^{-1}$)	17.3 ± 0.9d (16.5–18.0)	23.2 ± 0.6c (22.7–23.7)	23.7 ± 0.7bc (23.2–24.2)	24.2 ± 0.4b (23.8–24.6)	24.9 ± 0.5a (24.5–25.3)

Note: Values in parentheses: range: sample population for each mean: 8; values in the same row with different letters following are significantly different at the 0.05 level (two-tailed).

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The water pH and dissolved oxygen decreased, respectively, from 7.1 ± 0.1 to 6.3 ± 0.1; 5.9 ± 0.6 to 5.4 ± 0.4 mg l^{-1} for *C. gariepinus* and 7.1 ± 0.1 to 6.5 ± 0.1; 5.4 ± 0.1 to 4.9 ± 0.1 mg l^{-1} for *O. niloticus*. By contrast, the electrical conductivity and chemical oxygen demand increased, respectively, from 137 ± 2 to 151 ± 1 $\mu S\,cm^{-1}$; 24.2 ± 0.6 to 29.3 ± 0.4 mg l^{-1} for *C. gariepinus* and 132 ± 5 to 147 ± 5 $\mu S\,cm^{-1}$; 23.2 ± 0.6 to 24.9 ± 0.5 mg l^{-1} for *O. niloticus*.

3.2 *Fish body growth and 96-h LC_{50}*

The effect of the cassava effluent on fish body weights before and after the experiments is presented in figure 1.

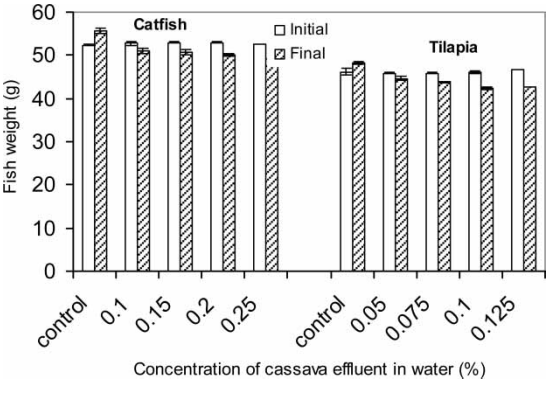

Figure 1. Effect of cassava effluent in water on the body weight of catfish and tilapia.

Fish body weights decreased by 3.1–6.7% for *C. garipienus* and 2.6–8.9% for *O. niloticus*, with increasing cassava effluent concentration. Strong, positive, and significant relationships at $p \leq 0.05$ were obtained for body weight losses vs. effluent concentrations for both fish species. The regression equations and the corresponding correlation coefficient (r) were $y = 27.033x; r = +0.97$ for *C. garipienus* and $y = 71.23x; r = +0.95$ for *O. niloticus*. However, weight gains of 4.7% (53.2 ± 0.2 to 55.7 ± 0.4 g) for *C. garipienus* and 4.8% (46.1 ± 0.4 to 48.3 ± 0.6 g) for *O. niloticus* were recorded in the control units. Probit mortality curves gave 96-h LC_{50} of 0.45% ($y = 139.87x + 53.046, r = 0.9751$) and 0.25% ($y = 23.424x + 19.011$, $r = 0.9779$) for *C. garipienus* and *O. niloticus*, respectively.

3.3 *Haematological changes*

Changes in the haematological parameters for both fish species in the presence of cassava effluent are shown in tables 2 and 3.

Toxicity on the haematological parameters for the respective *C. garipienus* and *O. niloticus* varied from 5.4 to 52.8; 4.8 to 51.9% for packed cell volume, 4.1 to 43.9; 5.3 to 64.0% for red blood count, 0.0 to 15.7; 0.0 to 61.4% for white blood count, 3.6 to 45.9; 5.2 to 49.5% for haemoglobin, 11.6 to 71.9; 28.4 to 63.8% for total protein, 11.5 to 75.5; 15.0 to 58.2% for albumin and 11.8 to 75.0; 46 to 83.9% for globulin. A steady increase in the induced toxicity on a particular haematological parameter with increasing cassava effluent

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Table 2. Quantitative changes in haematological parameters of mud catfish (*C. gariepinus*) with increasing effluent concentrations at 96-, 192-, and 288-h exposure.

		Effluent concentration (%)			
Parameter	Control	0.10	0.15	0.20	0.25
96 h					
PCV (%)	31.7 ± 0.10a	30.00 ± 1.00b	29.00 ± 1.00b	24.00 ± 1.00c	18.30 ± 1.50d
RBC ($\times 10^6$ mm^3)	2.23 ± 0.03a	2.13 ± 0.01b	1.78 ± 0.02c	1.62 ± 0.02d	1.25 ± 0.01e
WBC ($\times 10^3$ mm^3)	4.97 ± 0.06c	$5.53 \pm 0.0.3$b	5.92 ± 0.12a	6.08 ± 0.13a	6.15 ± 0.11a
HB (g dl^{-1})	13.80 ± 0.2a	13.30 ± 0.10b	13.13 ± 0.20	12.10 ± 0.20c	9.63 ± 0.30d
TP (g dl^{-1})	4.92 ± 0.20a	4.35 ± 0.30b	3.23 ± 0.20c	2.80 ± 0.20d	2.30 ± 0.10e
Alb (g dl^{-1})	3.90 ± 0.10a	3.45 ± 0.20b	2.42 ± 0.20c	2.07 ± 0.10d	1.63 ± 0.10e
Glb (g dl^{-1})	1.02 ± 0.10a	0.90 ± 0.10b	0.81 ± 0.10bc	0.73 ± 0.30cd	0.67 ± 0.09d
192 h					
PCV (%)	30.3 ± 1.30a	26.00 ± 1.6b	23.70 ± 1.30c	19.00 ± 0.80c	14.70 ± 0.50e
RBC ($\times 10^6$ mm^3)	2.15 ± 0.40a	2.04 ± 0.30b	1.40 ± 0.10c	1.32 ± 0.09d	1.25 ± 0.01e
WBC($\times 10^3$ mm^3)	5.45 ± 0.04d	5.65 ± 0.08c	5.77 ± 0.06bc	5.85 ± 0.10c	5.95 ± 0.04e
HB (g dl^{-1})	13.43 ± 0.42a	10.87 ± 0.40b	10.2 ± 0.42bc	9.63 ± 1.20d	8.20 ± 0.14e
TP (g dl^{-1})	4.67 ± 0.24a	3.83 ± 0.10b	2.49 ± 0.21c	2.57 ± 0.17d	2.28 ± 0.25e
Alb (g dl^{-1})	3.70 ± 0.24a	3.03 ± 0.10b	2.49 ± 0.21c	2.14 ± 0.21d	1.93 ± 0.22e
Glb (g dl^{-1})	0.97 ± 0.13a	0.80 ± 0.19b	0.53 ± 0.05c	0.43 ± 0.17d	0.36 ± 0.26e
288 h					
PCV (%)	29.0 ± 0.80a	22.3 ± 0.90b	18.3 ± 0.90b	13.7 ± 0.90d	13.7 ± 0.90c
RBC ($\times 10^6$ mm^3)	2.22 ± 0.20a	2.13 ± 0.01b	1.78 ± 0.02c	1.62 ± 0.02d	1.25 ± 0.01e
WBC ($\times 10^3$ mm^3)	5.75 ± 0.08a	5.50 ± 0.8b	5.28 ± 0.11c	5.15 ± 0.11c	4.85 ± 0.11de
HB (g/dl)	13.13 ± 0.15a	11.00 ± 0.30b	10.40 ± 0.35c	9.33 ± 0.31d	7.10 ± 0.17e
TP (g/dl)	4.27 ± 0.25a	3.43 ± 0.17b	2.63 ± 0.12c	2.06 ± 0.08d	1.20 ± 0.04e
Alb (g/dl)	3.47 ± 0.14a	2.76 ± 0.12b	2.13 ± 0.08c	1.67 ± 0.15d	0.85 ± 0.05e
Glb (g/dl)	0.80 ± 0.31a	0.67 ± 0.26b	0.50 ± 0.09c	0.39 ± 0.22d	0.20 ± 0.14e

*PCV: packed cell volume; RBC: red-blood-cell count; WBC: white-blood-cell count; HB: haemoglobin; TP: total protein; ALB: albumin; GLB: globulin; values in the same row with different letters following are significantly different from each other at $p < 0.05$ (two-tailed).

Table 3. Quantitative changes in haematological parameters of tilapia (*O. niloticus*) with increasing effluent concentrations at 96 -, 192-, and 288-h exposure.

Parameter	Control	Effluent concentration (%)			
		0.05	0.075	0.10	0.125
96 h					
PCV (%)	29.00 ± 0.80a	26.70 ± 0.50b	23.00 ± 0.80c	20.00 ± 1.60d	17.00 ± 0.80e
RBC ($\times 10^6$ mm^3)	1.83 ± 0.05a	1.41 ± 0.01b	1.26 ± 0.02c	0.73 ± 0.03c	0.68 ± 0.01c
WBC ($\times 10^3$ mm^3)	4.82 ± 0.02d	6.00 ± 0.03c	6.12 ± 0.10b	6.20 ± 0.03a	6.27 ± 0.09a
HB (g dl^{-1})	9.67 ± 0.30a	8.90 ± 0.14b	7.23 ± 0.26c	6.10 ± 0.29d	5.00 ± 0.21e
TP (g dl^{-1})	4.80 ± 0.16a	3.43 ± 0.17b	2.83 ± 0.16c	2.2 ± 0.16c	2.0 ± 0.12d
Alb (g dl^{-1})	2.94 ± 0.09a	2.50 ± 0.43b	2.37 ± 0.26b	1.8 ± 0.12c	1.7 ± 0.19c
Glb (g dl^{-1})	1.86 ± 0.09a	0.93 ± 0.55b	0.43 ± 0.12c	0.4 ± 0.17cd	0.3 ± 0.12d
192 h					
PCV (%)	26.70 ± 0.94a	25.00 ± 0.85a	20.70 ± 1.25b	17.30 ± 1.25c	13.70 ± 1.86d
RBC ($\times 10^3$ mm^3)	1.50 ± 0.21a	1.42 ± 0.37a	1.24 ± 0.37c	0.76 ± 0.34d	0.64 ± 0.21e
WBC ($\times 10^3$ mm^3)	5.07 ± 0.06a	5.0 ± 0.02b	4.95 ± 0.04a	4.63 ± 0.13b	4.37 ± 0.12c
HB (g dl^{-1})	9.26 ± 0.28a	8.78 ± 0.17b	7.07 ± 0.26b	6.66 ± 0.43d	5.13 ± 0.24e
TP (g dl^{-1})	4.30 ± 0.22a	3.00 ± 0.16b	2.33 ± 0.31c	1.86 ± 0.29d	1.57 ± 0.09e
Alb (g dl^{-1})	2.97 ± 0.09a	2.37 ± 0.12b	1.96 ± 0.31c	1.53 ± 0.36c	1.31 ± 0.12e
Glb (g dl^{-1})	1.33 ± 0.21a	0.63 ± 0.13b	0.37 ± 0.24c	0.33 ± 0.42c	0.26 ± 0.17c
288 h					
PCV (%)	26.70 ± 0.94a	25.00 ± 0.85a	20.70 ± 1.25b	17.30 ± 1.25c	13.70 ± 1.86d
RBC ($\times 10^3$ mm^3)	1.50 ± 0.21a	1.42 ± 0.37a	1.24 ± 0.37c	0.76 ± 0.34d	0.64 ± 0.21e
WBC ($\times 10^3$ mm^3)	5.07 ± 0.06a	5.0 ± 0.02b	4.95 ± 0.04a	4.63 ± 0.13b	4.37 ± 0.12c
HB (g dl^{-1})	9.26 ± 0.28a	8.78 ± 0.17b	7.07 ± 0.26b	6.66 ± 0.43d	5.13 ± 0.24e
TP (g dl^{-1})	4.30 ± 0.22a	3.00 ± 0.16b	2.33 ± 0.31c	1.86 ± 0.29d	1.57 ± 0.09e
Alb (g dl^{-1})	2.97 ± 0.09a	2.37 ± 0.12b	1.96 ± 0.31c	1.53 ± 0.36c	1.31 ± 0.12e
Glb (g dl^{-1})	1.33 ± 0.21a	0.63 ± 0.13b	0.37 ± 0.24c	0.33 ± 0.42c	0.26 ± 0.17c

*PCV: packed cell volume; RBC: red-blood-cell count; WBC: white-blood-cell count; HB: haemoglobin; TP: total protein; ALB: albumin; GLB: globulin; values in the same row with different letters following are significantly different from each other at $p < 0.05$.

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concentration was obtained at any given exposure period, 96 h, 192 h, or 288 h, for both fish species (figure 2).

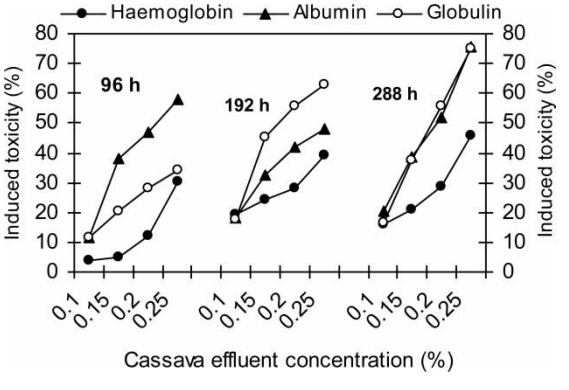

Figure 2. Toxicity on haemoglobin, albumin and globulin of mud catfish with increasing cassava effluent at 96-h, 192-h, and 288-h exposure periods.

3.4 *Correlations between induced toxicity on haematological parameters, effluent concentration and exposure period*

The coefficients for the inter-element correlations between the induced toxicity on the haematological parameters (I), correlations for the induced toxicity on the haematological parameters

Table 4. Pearson coefficients for inter-element correlations for induced toxicity on haematological parameters, exposure period and effluent concentration for catfish and tilapia.

	PCV	RBC	WBC	HB	TP	ALB	GLB
Catfish							
PCV	+1.0	+0.89***	+0.07	+0.92***	+0.93***	+0.90***	+0.90***
RBC		+1.0	−0.133***	+0.87***	+0.94***	+0.93***	+0.86***
WBC			+1.0	+0.07	−0.06	−0.09	+0.08
HB				+1.0	+0.88***	+0.85***	+0.91***
TP					+1.0	0.99***	+0.90***
ALB						+1.0	+0.84***
GBL							+1.0
Exposure time	−0.45**	−0.18	−0.44**	−0.45**	−0.32*	−0.25	−0.53***
Effluent concentration	−0.84***	−0.94***	−0.24	−0.82***	−0.92***	−0.93***	−0.78***
Tilapia							
PCV	+1.0	+0.91***	+0.47	+0.93***	+0.90***	+0.92***	+0.78***
RBC		+1.0	+0.44***	+0.93***	+0.90***	+0.89***	+0.83***
WBC			+1.0	+0.41**	+0.43**	+0.50***	+0.30*
HB				+1.0	+0.91***	+0.92***	+0.81***
TP					+1.0	0.97***	+0.95***
ALB						+1.0	+0.84***
GBL							+1.0
Exposure period	−0.20**	−0.28	−0.72**	−0.13**	−0.25*	−0.21	−0.27***
Effluent concentration	−0.94***	−0.91***	−0.32*	−0.97***	−0.92***	−0.94***	−0.82***

Note: PCV: packed cell volume; RBC: red-blood-cell count; WBC: white-blood-cell count; HB: haemoglobin; TP: total protein; ALB: albumin; GLB: globulin; ***value is significant at the 0.001 level; **value is significant at the 0.01 level; *value is significant at the 0.05 level.

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vs. exposure periods (II), and correlations between the induced toxicity on the haematological parameters and cassava effluent concentration (III) are presented in table 4.

Positive coefficients, ranging from +0.84 to +0.99 for *C. garipienus* and + 0.30 to + 0.97 for *O. niloticus* were obtained for (I), but the white blood count vs. red blood count, total protein, and albumin for *C. garipienus* gave negative correlations. However, only negative coefficients were obtained for (II) and (III) in the range of −0.32 to −0.53; −0.78 to −0.94 for *C. garipienus* and −0.20 to −0.72; −0.32 to −0.94 for *O. niloticus*, respectively.

4. Discussion

The decrease in water pH and dissolved oxygen as well as the increase in the electrical conductivity and chemical oxygen demand by the cassava effluent indicates increased water acidity, oxygen depletion, electrolyte concentration, and organic matter contents, respectively. These are in agreement with literature reports on the effects of cassava effluent on water quality [2, 3].

The threshold toxicity limit for free cyanide in well-oxygenated waters is in the range of 0.03–0.05 mg l^{-1} and 0.1–1.0 mg l^{-1} for metal-cyano complexes [10]. The concentration of cyanide (190.62 ± 1.49 mg l^{-1}) in the cassava effluent therefore exceeded the threshold by several factors of up to 6000. By contrast, Pb, NH_4^+, and Cr, which exceeded the recommended values by considerably reduced factors, thus confirmed cyanide (CN^-) as the major pollutant in the cassava effluent.

Cyanide is a chemical compound that contains the cyano functional group with the carbon triple-bonded to a nitrogen atom (C≡N). It occurs in nature and is extremely toxic to both human and aquatic life. In plants, CN^- is usually bound to sugar molecules in the form of cyanogenic glycosides. Cassava contains cyanogens in the form of linamarin and

lotaustralin that induce cyanide production when hydrolysed during fermentation, and cyanide concentrations in cassava effluent as high as 200 mg l^{-1} have been reported [5, 11]. The low 96-h LC_{50} for both species was therefore attributed to the high cyanide concentration in the wastewater.

The primary target organs for CN^- are cardiovascular, respiratory, and central nervous systems. The cyanide toxic effect is due to its affinity for the ferric haem form of cytochrome, also known as cytochrome c, the terminal oxidase of the mitochondrial respiratory chain. Formation of a stable cytochrome c oxidase–CN complex in the mitochondria produces a blockage of electron transfer from cytochrome oxidase to molecular oxygen and cessation of cellular repiration, causing cytototoxic hypoxia in the presence of normal haemoglobin oxygenation.

The combination of cytotoxic hypoxia with lactate acidosis depresses the central nervous system, resulting in respiratory arrest and even death [12]. In addition, dissolved oxygen depletion of the water media by the cassava effluent probably resulted in insufficient oxygen supply to the mitochondrial cells of the fishes, causing death. Nonetheless, the contributory effects of the trace metals (Pb and Cr) in the water were not completely ruled out.

At regulated concentration, Cr plays a role in glucose and cholesterol metabolism and is thus an essential element to man and animals, but becomes toxic if the threshold limit for safety is exceeded. On the contrary, Pb is currently known only for its toxicological profile. Humans and animals localize Cr and Pb in the lung, liver, kidney, spleen, adrenals, plasma, bone marrow, and red blood cells, causing damage to the renal, central nervous system, and blood-formation process [13, 14].

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The results are in agreement with previous studies [3, 15], which reported that the acute toxicity of cassava effluent directly administered to juvenile catfish gave rise to impaired tissue utilization of oxygen, which eventually resulted in death. Early symptoms of cyanide inhalation or ingestion include anxiety, weakness, headaches, nausea, gasping for air and difficulty in breathing, loss of consciousness, seizures, and cardiac arrest. Late signs of cyanide toxicity include hypotension, cardiovascular collapse, pulmonary oedema, and death [9, 16]. Most of these were observed in the study. The behavioural patterns exhibited by the fish upon effluent introduction were erratic movement, air gulping, loss of balance, and convulsion. During the acute tests, mortality set in for both fish species within 10 min.

The 96-h LC_{50} values, suggested that *C. gariepinus* (0.45%) was more resistant or tolerant to cassava effluent than *O. niloticus* (0.25%). This could be attributed to the sedimentary lungs of mud catfish (*C. gariepinus*) and the observed bimodal respiratory pattern (up and down movement), which could have enabled the utilization of both dissolved oxygen in water and atmospheric oxygen. The higher resistance of catfish over tilapia in the presence of other environmental toxicants such as tobacco, gammalin-20, and actellic 25 EC has been reported [17–19].

Resistance to toxicants is also dependent on age for a given fish type. For instance, the LC_{50} of 0.09% reported for tilapia fingerlings [3] was lower than the 0.25% obtained in the present study for tilapia juveniles. Other toxicity studies for cyanide ion on other organisms gave a 96-h LC_{50} of 0.018% for bluegill (*Leopnomis macrochinus*) and 0.028% for *pneumonate* snail. Oral LD_{50} values of 3 mg HCN kg^{-1} (body weight) in mice and 6.0 in rat and dermal toxicity of 6.8 mg HCN kg^{-1} in rabbit have been reported [17, 18, 20].

Reduced fish body weights on exposure to the effluent are an indication that effluent in water is a factor in inhibition of body growth in fish, with tilapia being more adversely affected than catfish. These are in agreement with previous studies [21, 22, 23] which showed a dose-dependent weight loss, increase in thyroid weight, and decrease in blood haemoglobin for rats fed diets containing 800–1600 mg CN kg^{-1} for 14 weeks, and their offspring also showed reduced foetal body weight.

Haematology is used as an index of fish health status to detect physiological changes following different stress conditions like exposure to pollutants such as cyanide [22, 24, 25]. The decrease in the quantitative values of the haematological parameters revealed an alteration in the physiological profiles of both fish species by cassava effluent. The response of white blood counts deviated from the regular pattern found for the other parameters by giving an initial increase before reduction in quantity. This phenomenon was attributed to the immune response or body defense mechanism.

Reductions in packed cell volume, haemoglobin, and red blood count implied haemodilution, indicating a probable anaemic condition fish. An anaemic response in the test fish was perhaps also due to the destruction of intestinal cells, which could have affected food intake and body weight [23, 24]. Other investigators reported similar trends in fish exposed to various toxicants [17, 25, 26].

The proteineous haematological parameters (total protein, albumin, and globulin) were most adversely affected. Proteins are the building blocks of all cells and body tissues. Albumin, being the most abundant protein in serum, is synthesized by the liver to maintain homeostatic levels when the protein intake is insufficient. Decreased albumin concentration is correlated with decreased protein concentration, reflecting disorders of the intestine, kidneys, and liver. Globulin is also an indicator of protein level. Its decrease indicates intestinal disease. Reduction of these parameters in fish by exposure to cassava effluent implied health disorder [27, 28]. Impaired health often leads to low food and water consumption and, consequently, reduced body weight.

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5. Conclusions

The cyanide levels exceeded the WHO limits for cassava wastewater. Acute and chronic exposures of tilapia (*O. niloticus*) and catfish (*C. gariepinus*) to cassava effluent adversely affected the body growth and haematological parameters. Chronic toxicity was dose-dependent, increasing with cassava effluent concentration. The study showed that the discharge of untreated cassava effluent into the aquatic environment is a potential health risk to aquatic lives and a threat to biodiversity conservation. Human populations residing near the riverine area, where fishing serves as main means of livelihood, such as the Niger-Delta region of Nigeria, are highly at risk of cyanide poisoning via consumption of contaminated fish. It is recommended that the discharge of cassava effluent directly into fields, ditches, ponds, lakes, streams, rivers, and agricultural land in Nigeria and other developing countries be strongly discouraged. The effluent should be subjected to adequate treatment processes to reduce the cyanide content and organic and inorganic contaminants before release into the aquatic environment.

Acknowledgements

The authors acknowledge the contributions of Agbon, A.O. of the Department of Aquaculture and Fisheries Management and the University of Agriculture, for assisting in probit analysis.

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