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Acute toxicity of produced water on selected organisms in the aquatic environment of the niger delta

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ABSTRACT

The discharge of produced water (PW) and drill cuttings from oil operations may elicit varied toxicity in fresh, brackish and marine organisms when exposed. The Niger Delta region which is the oil and gas province of Nigeria have incessantly been recipients of produced water. The study was aimed at investigating the toxic effects of produced water on freshwater fish- *Oreochromis niloticus* and brackish water shrimp- *Palaemonetes africanus* in the Niger Delta region of Nigeria. Probit toxicity tests were conducted on the organisms through a 96 h bioassay using produced water (PW) obtained from Mobil, Qua Iboe Terminal (QIT) in Eket, Akwa Ibom State, Nigeria. The organisms were tested in separate tanks containing produced water of 0.0, 100, 200, 400, 800 and 1000 mL, mixed with 2 L of habitat water (fresh/ brackish water). The physicochemical properties of the mixtures were determined by multiparameter hand held probe and atomic absorption photometer (AAS). The physico-chemical parameters, particularly the pH, and conductivity of the PW at 26.5 °C were significantly higher ($p < 0.05$) than those of the freshwater and brackish water samples. *O. niloticus* at the end of the 96 h test were 35%, 45%, 60%, 70% and 85%; while *P. africanus* had percentage mortalities of 25%, 35%, 45%, 60% and 80% at produced water (PW) of 5%, 10%, 20%, 40% and 50% respectively. Mortality rates of both test organisms being directly proportional to the percentages of PW and zero mortality recorded in the controls suggest that PW inflicted significant acute toxicity on the tested species. Higher mortality rates recorded in *O. niloticus* than *P.* ***africanus*** **could be attributed to the increase of salinity following the introduction of the PW in the fresh water test media.** The concentrations of PW administered in the study which were below the LC_{50} were are 49,500 and 99,000 ppm, while 198,000, 396,000, and 495,000 ppm were above the $LC_{50.}$ Results showed that the PW collected from the Mobil QIT effluent point source was toxic to the aquatic organisms, particularly the freshwater *Oreochromis niloticus*. We therefore speculate that 99,000 ppm of PW may be considered as the "no observed effect concentration" (NOEC).

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Introduction

Exploratory and exploitative discharges of produced water (PW) and drill cuttings from the downstream sector are an incessant source of contaminants to continental shelf ecosystems. The Niger Delta region is the oil and gas province of Nigeria, where about 90% of the country's oil and gas reserves are produced. The region comprises of Rivers, Bayelsa, Cross-Rivers, Akwa-Ibom, Delta, Edo, Imo and Ondo States [25]. Crude oil exploration and production activities have had devastating impacts on the environment of the Niger Delta [9,10]. Oil spills, effluents and cuttings discharged into the environment have resulted in despoliation of mangrove forests, death of aquatic organisms, destruction of farm lands and pollution of fresh water bodies in the affected region [18,23]. As a result of these devastations, there have been agitations for environmental protection and resource control by the affected populace [14].

Waste water released by crude oil-processing and petrochemical industries are characterized by the presence of large crude oil pollutants and by-products such as polycyclic aromatic hydrocarbons (PAHs), phenols, metal derivatives, surface-active substances (surfactants), sulfides, naphthylenic acids and other chemicals. Due to ineffective purification systems, waste water may become seriously dangerous, leading to the accumulation of toxic products in the receiving water bodies with potentially hazardous consequences on the ecosystem [7].

Production activities in the oil industry involve the use of different types of chemicals for the purpose of treating fluid and gas in order to separate oil from water. The gaseous by-product of these reactions is either flared through stacks or channelled in pipes for other purposes [21]. The constituent chemicals are typically complex mixtures of various molecular compounds, such as corrosion inhibitors, oxygen scavengers, scale inhibitors, biocides, demulsifiers, coagulants, water clarifiers, flocculants, solvents etc. [22]. Improperly treated PW also contains some minerals from its geological formation, which may also be toxic to aquatic organisms even at very low concentrations.

Toxic units (TUs) are used in estimating effluent toxicities. For examples, an effluent sample is said to be non-toxic when more than 100% concentration of the effluent sample is required to cause 50% mortality of the test organisms [13]. Various studies have shown positive correlation between pollutions from refinery effluents and the effect on physicochemical properties of water bodies and the aquatic organisms [5,12].

Periwinkle, *Tympanotonus fuscatus*- epifauna, and polychaete, *Capitella capitata*- infauna were the only organism present in the aquatic habitat owing to their opportunistic nature. The researcher observed total absence of other aquatic organisms and mangroves. [2] demonstrated the accumulation of heavy metals with accompanying histopathological alterations in *Oreochromis niloticus* exposed to treated petroleum refinery effluent from the Nigerian National Petroleum Corporation (NNPC), Kaduna. Previous studies have also suggested a correlation between contamination of water and sediments with hydrocarbons from refinery effluents, and compromised fish health [1].

However, not so much work has been done on produced water and the effects on aquatic organisms [16]. The present study is therefore aimed at investigating the toxic effects of produced water on aquatic organisms in fresh and brackish water bodies in the Niger Delta region using freshwater- *O. niloticus* and brackish water- *P. africanus*.

Materials and methods

Description of the study area

Physicochemistry and trace metal analyses

Physicochemical parameters such as pH, dissolved oxygen and temperature were measured in-situ with portable meters at the point source of the Mobil Producing Nigeria Unlimited (MPNU) Qua Iboe Terminal (QIT) in Eket, Akwa Ibom State (06° 21.063′N and 004°22.396′E). Conductivity, salinity, TDS, TSS, turbidity measurements were carried out in the laboratory with a multimeter while gravimetric method was used for the determination of density and total suspended solids (TSS). A 5-day BOD test was used to determine biochemical oxygen demand (BOD) as described by APHA 5210B and the closed reflux colorimetric method was used for the determination of chemical oxygen demand (COD).

Oil and grease was analyzed in accordance with EPA 413.2 and 9070. It involved extraction, followed by analysis with an Infrared Analyzer. Heavy metal**s** were conducted with an Atomic Absorption Spectrophotometer (AAS) in accordance with APHA 3111B, 3030B/3114B and 3111C.

Aquatic toxicity test analysis

Test organisms. The species used for the bioassay were juveniles *P. africanus* (a brackish water shrimp) obtained from an extension of the Lagos Lagoon at Majidun, Ikorodu, and *O. niloticus* (a freshwater tilapia) obtained from a fish pond in Ipaja, Lagos. These organisms were chosen because they are readily available, sentinel and culturable.

Acclimatization of organisms. The animals were acclimatized to ambient laboratory conditions using sieved habitat water (HW), with the quality variables monitored daily for 10 d The tilapia and shrimps were acclimatized in separate glass tanks (60 × 45 × 30 cm) for 14 d, prior to the commencement of the bioassay experiments. The organisms were fed once daily with normal commercial fish feed. The feed was withdrawn 2 d prior to the bioassays to empty the fish stomach. The habitat water (HW) was renewed every 2 d Dead individuals were immediately removed from the tanks to prevent contamination.

Mortality recorded was less than 1% in each tank. The temperature of the HW was maintained at 28 °C, the animals were exposed to a light/ dark (12/ 12 h) photoperiod cycle and DC- and AC-powered aquarium pumps, connected in tandem were used to constantly aerate the tanks.

Preparation of test media. Two liters (2 L) each of the HW was put in labeled bioassay tanks. Five (5) of the test tanks served as a set of concentration treatments for the produced water. The 6th tank served as control, and contained only HW and the test organisms. The produced water was introduced into the test tanks at predetermined concentrations. Actual concentration of the produced water in each tank was determined thus:

$$\text{Concentration in test tank (mg/L)} = \frac{(1000 \text{ x } \rho \text{ x} V_t)}{V_f}$$

Where;

V_t = volume (mL) of the produced water in each test tank

V_f = total volume (litres) of the media in each test tank

ρ = specific gravity (or relative density) of the produced water.

10 mL of produced water (ρ = 0.990 g/mL) is equivalent to 9.9 g. Thus, 10.0 mL in 100 mL media (10% solution) is equivalent to100 mL/L HW, or 100 × 0.99 × 1000 = 99,000.0 mL PW/ L (or 198,000.0 mg/L).

The data below represents the concentrations of produced water used for the bioassay:

The concentrations of the produced water in test tanks with either *P. africanus* or *O. niloticus* were 0.0 mL (control), 100 mL (tank 1), 200 mL (tank 2), 400 mL (tank 3), 800 mL (tank 4) and 1000 mL (tank 5) of the PW in 2 L HW; corresponding to 0.0, 5.0, 10.0, 20.0, 40.0 and 50.0% of the test sample in the test medium, respectively. Alternatively these concentrations are equivalent to 0.0, 49,500, 99,000, 198,000, 396,000 and 495,000 ppm (v/v) respectively in the test medium.

Introduction of test organisms into bioassay tanks. Twenty (20) juvenile shrimps (length; 1.26 ± 0.11 cm and weight; 0.52 ± 0.13 g; n = 20) were carefully introduced into the first set of test tanks, using a small elliptical net. Another twenty (20) juvenile fish (2.85 ± 0.16 cm, 1.69 ± 0.20 g; n = 20) were placed into the second set of test tanks. Control tests were set up, in which pairs of test tanks contained the habitat water and 20 test organisms per pair. Each test medium, including the controls, was renewed every 24 h, until 96 h.

Data transformation and statistical analysis

The statistical analysis was conducted using the probit analysis software, InStat, from GraphPad Inc. to determine the 96 h LC_{50}. Microsoft Excel formula NORMINV (A1/100,5,1) was used to transform the% mortality to probits. The concentration term was transformed by taking the logarithms.

Probits were plotted against the log of treatment concentrations in a linear graph showing the toxicity of the test chemical by a linear equation; $y = bx + a$. The vertical axis (y) was the mortality (transformed to probits), while the horizontal axis (x) was the logarithm of the toxicant concentration. The slope of the line (b), and the intercept (a) were determined.

The log of the concentration was determined by calculating **x:**

$$x = (y - a)/b; \text{ when } y = 5 \text{ (the probit value for 50\% mortality)}$$

Back-transformation (antilog) of the resulting value (log x) determined the concentration term (LC_{50}) in mg/L.

The statistical tests for variance and the significance of the test at 95% confidence limits were determined. The confidence limits and the error (residuals) around the estimation of the LC_{50} were plotted.

Results

The pH, particularly conductivity ($p < 0.05$) of PW at 26.5 °C was significantly higher than those recorded in the freshwater and brackish water samples (Table 1). The TDS recorded in the PW was also significantly higher than the values recorded for the habitat water samples ($p < 0.05$). The salinity, COD, BOD, and TSS of the PW samples were significantly higher than the values recorded in the habitat water, while the DO of the former was significantly lower than the latter.

The total suspended solids, total oil and grease, sulfate, iron, and barium

Aquatic toxicity bioassay

Water quality of bioassay tanks

Freshwater and produced water were mixed in varied proportions and the resulting water qualities were analyzed (Table 2). The levels of nitrate and BOD in Tank 5 (50% PW) were significantly higher than the level in control and other tanks ($p<0.05$). Sulfate was significantly higher in tank 5> tank 4> tank 3, which was higher than the control and other treatments. The trend of phosphate was tank5> tank 3 > other tanks. Iron was tank 5> tanks 1 and 3> other tanks. The trend in levels of COD and TSS was tank 5> tank 4> other tanks. No significant difference was recorded in other assessed parameters.

Table 1
Physico-chemical properties of 100% PW compared with habitat water samples.

Parameter	Produced water	Freshwater	Brackish water
Colour	Light brownish	–	–
Odour	Unpleasant	–	–
Solubility in water (at 26 °C)	Soluble	–	–
Relative Density (g/mL, at 26 °C)	0.9900	–	–
pH (at 26.5 °C)	8.65	7.0	7.5
Conductivity (µS/cm, at 26.0 °C)	23,860*	473	997.5
Total Dissolved Solids (mg/L)	14,850*	303	618
Salinity (ppt, as NaCl)	13.4*	0.2	0.5
Chemical Oxygen Demand (mg/L)	2300*	32	54
Biochemical Oxygen Demand (mg/L)	32*	2	9
Dissolved Oxygen (mg/L)	2.3*	5.0	5.2
Turbidity (NTU)	90	–	–
Total Suspended Solids (mg/L)	86*	8	23
Total Oil and Grease (mg/L)	14.0*	<0.5	<0.5
Nitrate (mg/L)	3.1	0.82	0.60
Sulfate (mg/L)	40.0*	1.22	1.35
Phosphate (mg/L)	0.08	1.15	0.90
Iron (mg/L)	83.2*	0.16	0.28
Chromium (mg/L)	<0.001	<0.001	<0.001
Copper (mg/L)	0.067	0.0012	0.004
Zinc (mg/L)	0.038	0.0024	0.008
Nickel(mg/L)	0.601	<0.005	0.010
Manganese (mg/L)	0.058	0.003	0.011
Barium (mg/L)	0.820*	<0.01	<0.01

Asterisked numbers are significantly higher at $p<0.05$.

Table 2
Quality of freshwater in test tanks with PW and *Oreochromis niloticus* before test.

Parameter		Percentage PW in test tanks				
(10%)	Control	1 (5%)	2	3(20%)	4(40%)	5(50%)
Nitrate (mg/L)	0.82^{b}	0.1^{b}	0.16^{b}	0.30^{b}	0.8^{b}	1.6^{a}
Sulfate (mg/L)	1.22	1.1	1.9	3.0^{ab}	6.1^{b}	12.2^{a}
Phosphate (mg/L)	1.15^{ab}	0.16^{ab}	0.08^{ab}	5.0^{b}	0.16^{ab}	9.1^{a}
Iron (mg/L)	0.28^{ab}	15.8^{b}	0.68^{ab}	28.1^{b}	1.33^{ab}	58.2^{a}
COD (mg/L)	32^{ab}	35^{ab}	45^{ab}	77^{c}	160^{b}	323^{a}
BOD (mg/L)	2	1	1	3^{b}	5^{b}	13^{a}
TSS (mg/L)	8	10	18	30	58^{b}	103^{a}
TOG (mg/L)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Chromium (mg/L)	<0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Copper (mg/L)	0.0012	0.003	0.008	0.016	0.018	0.031
Zinc (mg/L)	0.0024	0.003	0.006	0.009	0.012	0.018
Nickel (mg/L)	<0.005	0.040	0.072	0.180	0.149	0.298
Manganese (mg/L)	0.003	0.004	0.009	0.011	0.017	0.026
Barium (mg/L)	<0.01	0.067	0.133	0.260	0.243	0.392

Percentages in parenthesis are actual percentages of PW. Values with same superscripts, in same column are not significantly different, while those with different superscripts are significantly different ($p<0.05$).

The similar trend also occurred in the resulting physicochemical properties of mixture of brackish water and PW in which the *P. africanus* were experimented. The trends of nitrate, sulfate, phosphate, and iron were tank 5> tank 4> tank 3> tank 2 > tank 1> control (Table 3). However, COD, BOD, and TSS were in the order of tank 5> tank 4> tank 3> tank 2 > control > tank 1.

All physicochemical parameters of the freshwater (Table 4) and brackish water (Table 5) tested using *O. niloticus* and *P. africanus* respectively exhibited no significant difference before and after the bioassay experiment, except for the dissolved oxygen which dropped significantly ($p<0.05$) at the end of both experiments. Dissolved oxygen level dropped in all treatments of both experimental setups, except the control.

Concentration-response data transformations and statistics

Percentage mortality of *O. niloticus* in tank 1 was 10 (Fig. 1) at 24 h and increased to 15, 20, and 35% at 48 h, 72 h, and 96 h respectively (Figs. 2–4, Table 6). Percentage mortalityof the freshwater fish in tank 2 was 20 at 24 h, 30 at 48 h and 72 h, and 45 at 96 h (Figs. 1- 4, Table 6). In tank 3, the fish had percentage mortality of 30 at 24 h, with subsequent trend

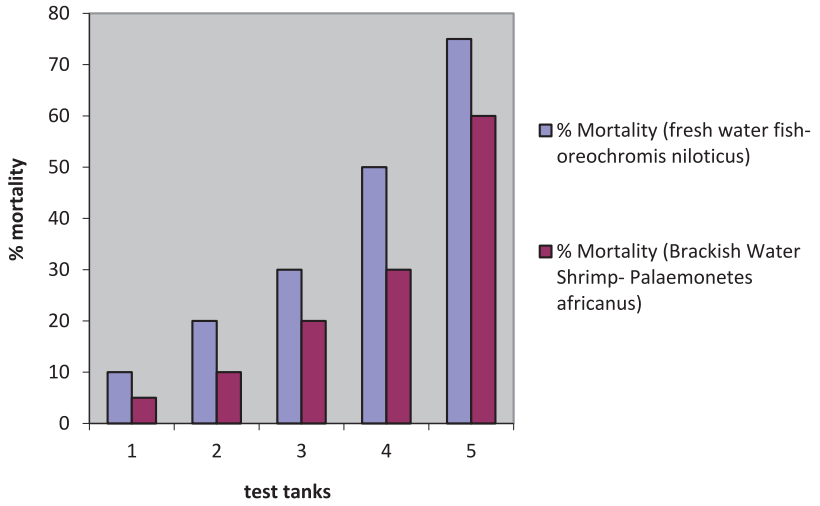

Fig. 1. 24h-% Mortality of fresh and brackish water species in each test tank.

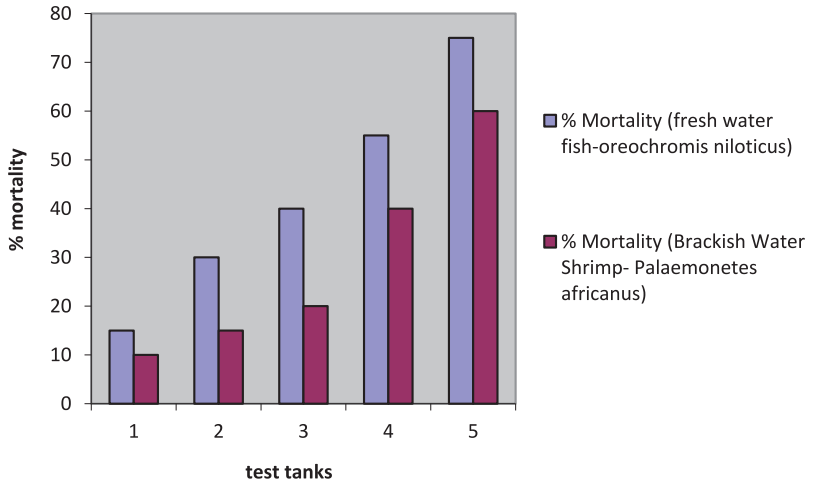

Fig. 2. 48h-% Mortality of fresh and brackish water species in each test tank.

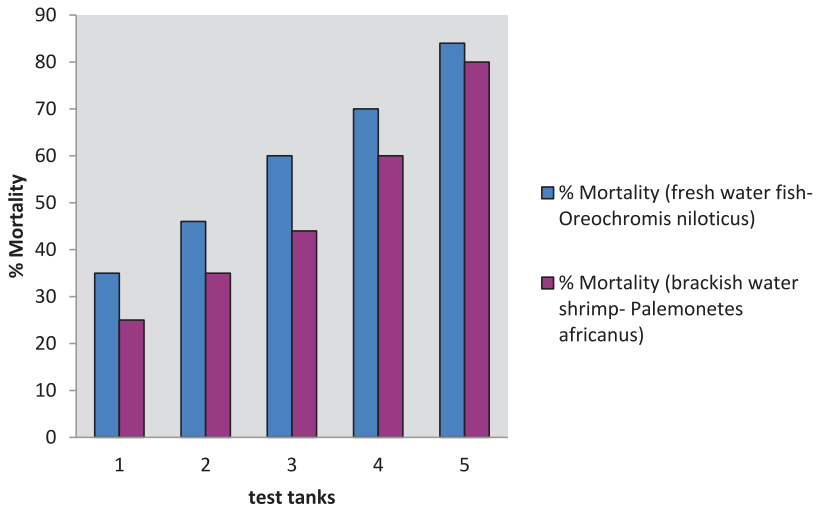

Fig. 3. 72h-% Mortality of fresh and brackish water species in each test tank.

Table 3
Quality of brackish water in test tanks with PW and *Palaemonetes africanus* before test.

Parameter		Levels in test tanks				
(5%)	Control	1	2 (10%)	3(20%)	4(40%)	5(50%)
Nitrate (mg/L)	0.60	0.2	0.54	0.74	1.44 *	2.02*
Sulfate (mg/L)	1.35	2.5	4.3	7.2	12.9*	17.3*
Phosphate (mg/L)	0.90	0.2	0.37	0.6	1.11*	1.69*
Iron (mg/L)	0.28	6.4*	12.8*	22.9*	46.4**	72.6**
COD (mg/L)	54	38	43	70	113*	133*
BOD (mg/L)	9	2	3	6	11*	17*
TSS (mg/L)	23	14	22	30	58	63
TOG (mg/L)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Chromium (mg/L)	< 0.001	<0.001	< 0.001	< 0.001	< 0.001	< 0.001
Copper (mg/L)	0.004	0.005	0.011	0.020	0.027	0.040
Zinc (mg/L)	0.008	0.003	0.008	0.012	0.015	0.021
Nickel (mg/L)	<0.010	0.055	0.124	0.166	0.250	0.310
Manganese (mg/L)	0.011	0.007	0.010	0.015	0.022	0.030
Barium (mg/L)	<0.01	0.080	0.169	0.212	0.299	0.400

Percentages in parenthesis are actual percentages of produced water. Asterisked values represent significantly higher values at $p<0.05$.

Table 4
Freshwater quality in 4 test tanks and control, before and after bioassay experiment.

Parameter	Time	Tank 1 (24 h)	Tank 2 (48 h)	Tank 3 (72 h)	Tank 4 (96 h)	Control
pH	Before assay	7.39 ± 0.02	7.61 ± 0.02	8.00 ± 0.02	7.77 ± 0.02	6.25
	End of assay	7.39 ± 0.02	7.51 ± 0.02	7.82 ± 0.02	7.78 ± 0.02	6.73
Temperature (°C)	Before assay	26.4 ± 0.1	26.4 ± 0.1	26.4 ± 0.1	26.4 ± 0.1	26.8
	End of assay	26.4 ± 0.1	26.4 ± 0.1	26.4 ± 0.1	26.4 ± 0.1	26.7
Dissolved Oxygen (mg/L)	Before assay	4.3* ± 0.1	4.4* ± 0.1	4.6* ± 0.1	4.7* ± 0.1	4.7
	End of assay	0.6 ± 0.1	0.7 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	3.1
Conductivity (µS/cm)	Before assay	3796.0 ± 0.1	3804.2 ± 0.1	3799.0 ± 0.1	3797.0 ± 0.1	476
	End of assay	3796.6 ± 0.1	3805.4 ± 0.1	3798.0 ± 0.1	3796.6 ± 0.1	477
Total Dissolved	Before assay	2467 ± 1	2473 ± 1	2469 ± 1	2468 ± 1	309
	End of assay	2468 ± 1	2474 ± 1	2469 ± 1	2468 ± 1	310
Solids (mg/L)	Before assay	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 .]l[=;p0.1	0.2
Salinity (ppt)	End of assay	2.0 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	0.2

Asterisked values before assay are significantly higher than values after assay ($p<0.05$). Number of replicates (N) = 20.

Table 5
Brackish water quality in 5 test tanks and control with PW and *P. africanus*.

Parameter	Time	24 h	48 h	72 h	96 h	Control
pH	Before assay	7.9 ± 0.02	7.90 ± 0.02	7.91 ± 0.02	7.85 ± 0.02	7.81
	End of assay	7.91 ± 0.02	7.92 ± 0.02	7.92 ± 0.02	6.46 ± 0.02	7.83
Temperature (°C)	Before assay	26.6 ± 0.1	26.6 ± 0.1	26.6 ± 0.1	26.6 ± 0.1	26.7
	End of assay	26.8 ± 0.1	26.8 ± 0.1	26.8 ± 0.1	26.8 ± 0.1	26.8
Dissolved Oxygen (mg/L)	Before assay	4.8* ± 0.1	4.7* ± 0.1	4.8* ± 0.1	4.7* ± 0.1	4.8
	End of assay	0.5 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	2.6
Conductivity (µS/cm)	Before assay	3845.8 ± 0.1	3833.6 ± 0.1	3846.4 ± 0.1	3848.8 ± 0.1	995
	End of assay	3844.8 ± 0.1	3834.6 ± 0.1	3845.4 ± 0.1	3849.2 ± 0.1	997
Total Dissolved Solids (mg/L)	Before assay	2500 ± 1	2492 ± 1	2500 ± 1	2502 ± 1	647
	End of assay	2499 ± 1	2492 ± 1	2500 ± 1	2502 ± 1	648
Salinity (ppt)	Before assay	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	0.5
	End of assay	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	0.5

Asterisked values before assay are significantly higher than values after assay ($p<0.05$). Number of replicates (N) = 20.

of 40, 55, and 60% at 48 h, 72 h, and 96 h respectively. Percentage mortality recorded in tank 4 was 50, 55, 60, and 70%, while tank 5 was 75, 75, 80, and 85% at 24 h, 48 h, 72 h, and 96 h respectively. No mortality was recorded in the control throughout the bioassay experiment (Fig.S 1–4, Table 6).

P. africanus in tank 1 recorded percentage mortality of 5, 10, 10, and 25% at 24 h, 48 h, 72 h, and 96 h respectively (Figs. 1-4, Table 7). In tank 2, the percentage mortality recorded for the brackish water shrimp were 10, 15, 20, and 35% at 25 at 24 h, 48 h, 72 h, and 96 h respectively. The percentage mortality recorded in tank 2 was 20, 20, 30, and 45% at 25 at 24 h, 48 h, 72 h, and 96 h respectively. Mortalities of 20, 20, 30, and 45% (tank 3); 30, 40, 55, and 60% (tank 4); and 60, 60, 75, and 80% (tank 5) at 24 h, 48 h, 72 h, and 96 h respectively (Figs. 1-4, Table 7).

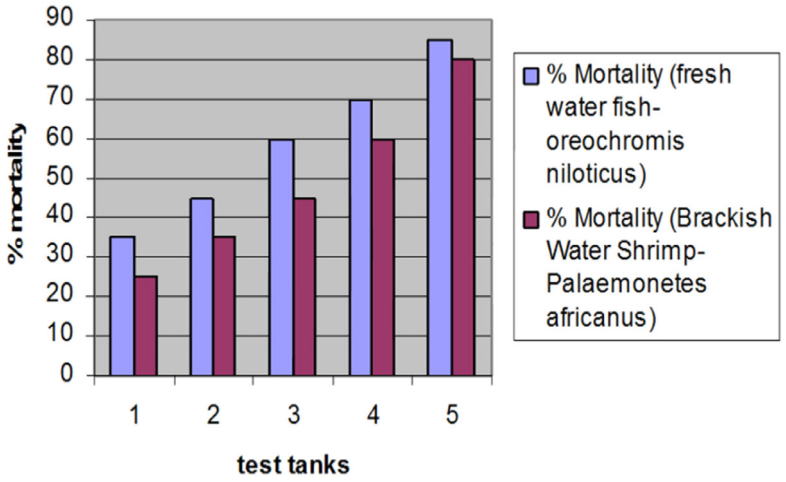

Fig. 4. 96 h-% Mortality of fresh and brackish water species in each test tank.

Table 6
Acute toxicity testing of PW against *Oreochromis niloticus*.

Test tank	Conc. of PW				Mortality							
	ppm (v/v)	Log Conc.	%	Log Conc.	24 h		48 h		72 h		96 h	
					%	probit	%	Probit	%	probit	%	probit
Control	0.0	-	0.0	-	0	-	0	-	0	-	0	-
1	49,500.0	4.69	5.0	0.70	10	3.72	15	3.96	20	4.16	35	4.61
2	99,000.0	5.00	10.0	1.00	20	4.16	30	4.48	30	4.48	45	4.87
3	198,000.0	5.30	20.0	1.30	30	4.48	40	4.75	55	5.13	60	5.25
4	396,000.0	5.60	40.0	1.60	50	5.00	55	5.13	60	5.25	70	5.52
5	495,000.0	5.69	50.0	1.70	75	5.67	75	5.67	80	5.84	85	6.04

Number of test organisms/ tank (N) = 20.

Table 7
Acute toxicity test of PW (ppm) against *Palaemonetes africanus*.

Test tank	Conc. of PW				Mortality							
	ppm (v/v)	Log Conc.	%	Log Conc.	24 h		48 h		72 h		96 h	
					%	probit	%	probit	%	probit	%	probit
control	0.0	-	0.0	-	0	–	0	–	0	–	0	–
1	49,500.0	4.69	5.0	0.70	5	3.36	10	3.72	10	3.72	25	4.33
2	99,000.0	5.00	10.0	1.00	10	3.72	15	3.96	20	4.16	35	4.61
3	198,000.0	5.30	20.0	1.30	20	4.16	20	4.16	30	4.48	45	4.87
4	396,000.0	5.60	40.0	1.60	30	4.48	40	4.75	55	5.13	60	5.25
5	495,000.0	5.69	50.0	1.70	60	5.25	60	5.25	75	5.67	80	5.84

Number of test organisms/ tank (N) = 20.

The freshwater fish recorded higher mortality than the brackish water in all 5 tanks throughout the bioassay durations (Figs. 1-4). The trend of mortality in all bioassay setups was tank 1< tank 2< tank 3< tank 4< tank 5.

Probit toxicity analysis

Toxicity of PW on oreochromis niloticus

Rationale used in determining toxicity was based on whether the slope is significantly different from zero.

Estimation of PW toxicity in percentage

The slope of graph plotted for 96 hr acute toxicity of PW on *O. niloticus* was significant greater than 1. This implies a significant toxicity of PW on the fresh water teleost. This was supported by the outcome of the ANOVA result of the PW toxicity and the calculations of toxic unit (TU), which was relatively high. All analysis indicated high toxicity of the PW on *O. niloticus.*

From the Probit line equation given as: $Y = 1.296X + 3.625$; to solve for X, when $Y = 5$ (the probit for 50% mortality):

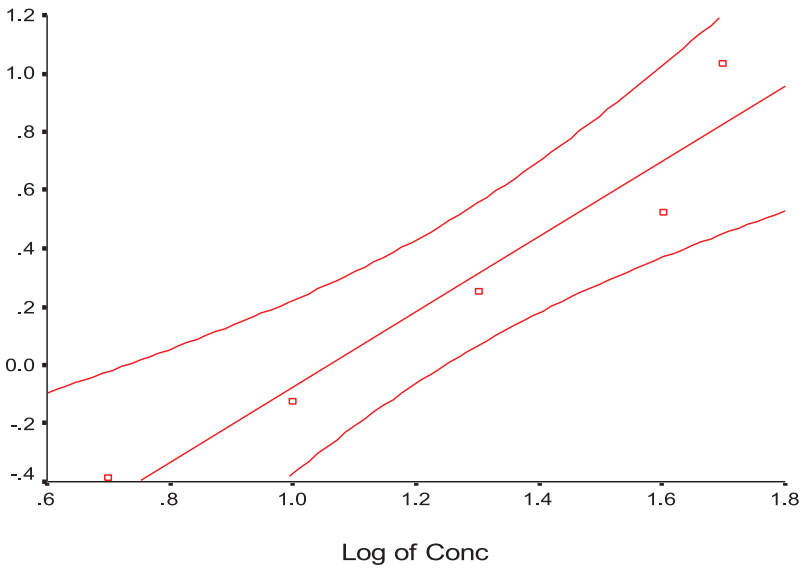

Fig. 5. Linear Regression graph for 96 h acute toxicity testing of PW (%) on *O. niloticus*.

$X = \underline{5 - 3.625} = 1.0610$, and; LC_{50} = antilog 1.0610 = 11.51% (v/v)
1.296

We calculated the toxicity units (TUs) at concentrations of PW in percentage used in the tanks thus;

$$TUintank1 = \frac{5\%}{LC_{50}} = \frac{5}{11.51} = 0.43$$

$$TUintank2 = \frac{10\%}{LC_{50}} = \frac{10}{11.51} = 0.87$$

$$TUintank3 = \frac{20\%}{LC_{50}} = \frac{20}{11.51} = 1.74$$

$$TUintank4 = \frac{40\%}{LC_{50}} = \frac{40}{11.51} = 3.48$$

$$TUintank5 = \frac{50\%}{LC_{50}} = \frac{50}{11.51} = 4.34$$

The concentrations (in percentage) of PW administered to *Oreochromis niloticus* which were below the significant TU value of 1 were 5 and 10% PW in tanks 1 and 2 respectively (Fig. 5). Tanks 3, 4, and 5 which contained 20, 40, and 50% PW respectively had TUs which were greater than 1; thus indicating significant toxicity impacts on the freshwater fish.

Estimation of pw toxicity in parts per million (ppm)

The slope of graph plotted for 96 hr acute toxicity of PW on *O. niloticus* was significant greater than 1. This further supports the indication of significant toxicity of PW on *O. niloticus*. Furthermore, significant toxicity was indicated by the ANOVA result of the PW toxicity and the calculations of TUs, which were high in most of the test tanks.

From the Probit line of equation given as: $Y = 1.289X + (-1.517)$; we solved for X, when $Y = 5$ (the probit for 50% mortality):

$$X = \frac{5 + 1.517}{1.289} = 5.056$$

LC_{50} = antilog 5.056 = 113,762.7 ppm

To solve for the toxic units (TUs) at the various concentrations of PW used in the 5 tanks, we calculated thus;

$$TUoftank1 = \frac{49500.0}{113,762.7} = 0.44$$
$$TUoftank2 = \frac{99000.0}{113,762.7} = 0.87$$
$$TUoftank3 = \frac{198000.0}{113,762.7} = 1.74$$
$$TUoftank4 = \frac{396000.0}{113,762.7} = 3.48$$
$$TUoftank5 = \frac{495,000.0}{113,762.7} = 4.35$$

The concentrations (in ppm) of PW administered to *Oreochromis niloticus* which were below the significant TU value of 1 were 49,500.0 and 99,000.0 ppm in tanks 1 and 2 respectively (Fig. 6). Tanks 3, 4, and 5 which contained 198,000.0, 396,000.0, and 495,000.0 respectively had TUs which were greater than 1; thus indicating significant toxicity impacts on

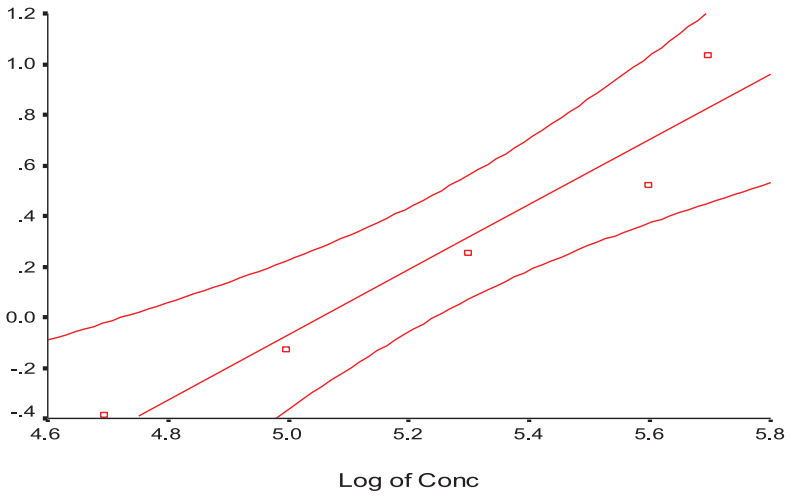

Fig. 6. Linear Regression Graph for 96 h acute toxicity testing of PW (ppm) against *O. niloticus*.

the freshwater fish. This indicates that the toxicity estimation in percentage accurately conformed to estimations in parts per million.

Toxicity of pw on P. africanus

Determination of PW toxicity was based on whether the slope of toxicity graph is significantly different from zero.

Estimation of pw toxicity in percentage

The slope (1.338) of PW's toxicity on *P. africanus* indicated a significant toxicity of the PW on tested *P. africanus*. This result was corroborated by the outcome of the ANOVA analysis of the PW toxicity and the calculations of corresponding toxic units (TUs), which were quite high.

From the Probit line equation given as: $Y = 1.338X + 3.294$; we solve for X, when $Y = 5$ (the probit for 50% mortality):

$$X = \frac{5 - 3.294}{1.338} = 1.275$$

$$LC50 = antilog\ 1.275 = 18.84\%(v/v)$$

We calculated the toxicity units (TUs) at concentrations of PW (in percentage) adopted in the tanks thus;

$$TU\ in\ tank\ 1 = \frac{5\%}{LC50} = \frac{5\%}{18.84\%} = 0.27$$

$$TU\ in\ tank\ 2 = \frac{10\%}{LC50} = \frac{10\%}{18.84\%} = 0.53$$

$$TU\ in\ tank\ 3 = \frac{20\%}{LC50} = \frac{20\%}{18.84\%} = 1.06$$

$$TU\ in\ tank\ 4 = \frac{40\%}{LC50} = \frac{40\%}{18.84\%} = 2.12$$

$$TU\ in\ tank\ 5 = \frac{50\%}{LC50} = \frac{50\%}{18.84\%} = 2.65$$

The concentrations (in percentage) of PW adopted for *P. africanus* which were below the significant TU value of 1 were 5 and 10% PW in tanks 1 and 2 respectively (Fig. 7). Tanks 3, 4, and 5 which contained 20, 40, and 50% PW respectively had TUs greater than 1, which indicate significant toxicity impacts on the brackish water shrimp.

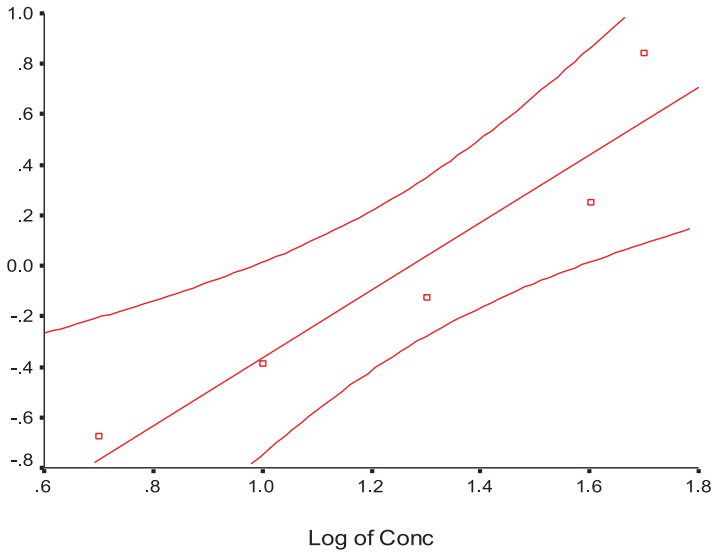

Fig. 7. Linear regression graph for 96 h acute toxicity testing of PW (percentage) on *P. africanus*.

Estimation of PW toxicity in ppm

The slope (1.330) of PW's toxicity indicated a significant toxicity of the PW on tested *P. africanus*. This was corroborated by the ANOVA analysis in which $F = 23.794$, was considered significant when value is 0.0165. The calculations of corresponding toxic units (TUs) were also significant.

From the Probit line equation given as: $Y = 1.338X + (-2.012)$; we solved for X, when $Y = 5$ (the probit for 50% mortality) thus:

$$X = \frac{5+2.012}{1.338} = 5.251, and;$$
$$LC_{50} = antilog 5.251 = 178237.9 ppm$$
$$TU of tank1 = \frac{49500.0}{178,237.9} = 0.28$$
$$TU of tank2 = \frac{99000.0}{178,237.9} = 0.56$$
$$TU of tank3 = \frac{198,000.0}{178,237.9} = 1.11$$
$$TU of tank4 = \frac{396,000.0}{178,237.9} = 2.22$$
$$TU of tank5 = \frac{495,000.0}{178,237.9} = 2.78$$

The concentrations of PW administered in the study which were below the LC_{50} were in the tanks 1and 2, which are 49,500 and 99,000 ppm respectively (Fig. 8). Tanks 3, 4, and 5 with concentrations of 198,000, 396,000, and 495,000 ppm respectively were significantly higher than the determined LC_{50}.

Discussions

The pH of 8.65 of the produced water indicates that it is alkaline, with a high salinity of 13.4. Most fishes can tolerate pH values of about 5.0 to 9.0, with preference for water between pH of 6.5 and 8.2 [11]. The temperature of 48 °C of the produced water at the time of sampling was slightly high, as fishes and most aquatic organisms are cold-blooded, consequently their metabolism increases with water temperature and decreases as water cools [8]. Furthermore, retention capacity of dissolved oxygen in water reduces with increase in temperature. Studies have shown that warm water also increases toxicity of xenobiotics such as cyanides, phenol, xylene and zinc to aquatic animals [2]. The relatively high temperature of the produced water is strongly linked to the observed low dissolve oxygen content (DO) of 2.3 mg/l, which corroborates the high chemical oxygen demand (COD). Although other factors that influence the required DO include species-specificity, organismal physical state, physicochemical properties of pollutants, route of entry, etc. However, studies have shown that at 5 °C, trouts use about 50–60 mg O_2/ h; at 25 °C [4]. Fish in the current study also require great amount of oxygen because they are cold-blooded animals, hence they require more oxygen at higher temperatures when their metabolic rate increases. Furthermore, numerous scientific studies suggest that 4–5 ppm of DO is the minimum amount required by a large, diverse fish population [3]. The required DO level for aquaculture generally averages about 9.0 ppm [17].

Analyzed elements in the produced water were all below regulatory limits of [6]. Varied levels of trace elements in produced and formation water samples have been reported over the years by industries and regulatory agencies [17]. This variability can be ascribed partly to different geological characteristics of the reservoirs; for instance, gas fields usually provide higher values of heavy metals than oil fields [18]. Furthermore, produced water generated from early production has significantly higher trace element content than that of prolonged fields [19]. It has also been suggested that corrosion

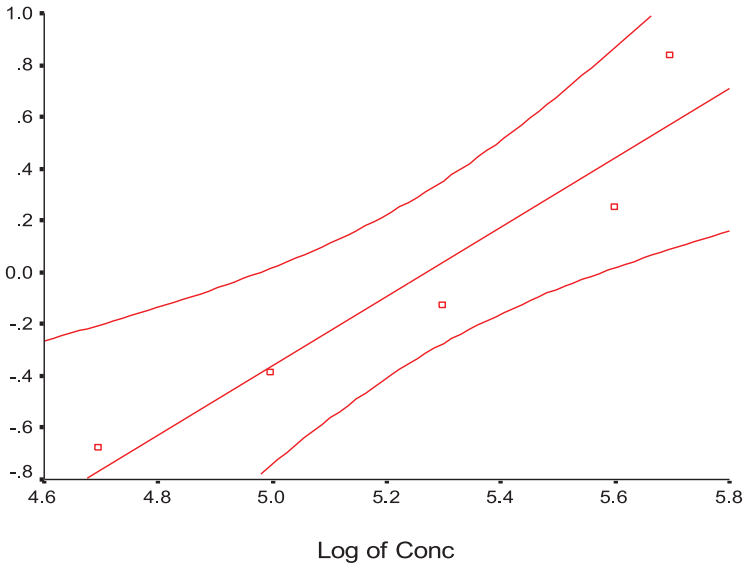

Fig. 8. Linear Regression graph for 96 h acute toxicity testing of PW (ppm) on *P. africanus*.

of galvanized equipment could be a source of zinc and lead in some produced water [17]. The low levels of trace elements obtained in the Mobil QIT produced water may be due to the fact that the water emanates from prolonged oil fields of over 20 years [24].

Current regulations in the United States require that oil and grease in produced water discharged into the aquatic environment does not exceed a daily maximum of 42 mg/l, or a monthly average of 29 mg/L [20]. The Kuwait Convention requires that produced water discharges do not exceed a monthly average of 40 mg/l [15]. In Mediterranean Sea, the offshore protocol of Barcelona Convention has the same provisions. In Australia, the limit for daily average discharge concentration was set at 30 mg/l [8]. It is notable that the oil and grease level (14.1 mg/l) of the water in current study was below all the regulatory limits [19].

The eco-toxicity test was conducted using 50% of the produced water as the highest concentration. This is due to the fact that during the range finding test 55% of the produced water had a 100% mortality rate within 24 h duration of the test. At the end of the 96 h test the results in both fresh and brackish water showed mortality increased with increase in the percentage of produced water in the test tanks. The percentage mortalities of *Oreochromis niloticus* at the end of the 96 h test were 35%, 45%, 60%, 70% and 85% which occurred at concentrations of produced water of 5%, 10%, 20%, 40% and 50% respectively in each test tank. In like manner, *P. africanus* had a percentage mortality of 25%, 35%, 45%, 60% and 80% at the respective concentrations of the produced water. Mortality rates of both test organisms being directly proportional to the concentrations of produced water suggest that produced water inflicted acute toxicity on the test species. Zero mortality recorded in the control experiments of both fresh and brackish water samples at the end of 96 h reliably establishes the fact that mortality in the treatment set ups was associated with the produced water.

The high mortality rate may be attributed to the low DO in the test tanks during the experiment. Results of DO recorded before and after assay at 24 h intervals indicate drastically reduced DO at the end of each day's semi-static renewal test. This is an indication of the presence of oxygen utilizing substances in the test media. This underscores the importance of the test method used, the semi-static renewal method, where the test material is replaced every 24 h.

Significant acute toxicity occurred in the 3rd tank, and followed an ascending trend with the tanks in both the freshwater and brackish water experimental set ups. In the freshwater experiment, with 20% PW toxicity unit of 1.74 was detected and at PW of 198,000 ppm, toxicity unit was 1.7. On the other hand, toxicity unit of 1.06 at 20% PW, while at PW concentration of 198,000 pm, toxicity unit of 1.11 was detected. These data suggest that PW was more toxic to the freshwater organism than the brackish water.

Higher mortality recorded in the fresh water, *O. niloticus* than the brackish water, *P. africanus* could be attributed to the increase of salinity following the introduction of the produced water in the fresh water test media, but for the remarkable euryhaline nature of the teleost which affords it its tolerance of salinity up to 20 ppt [21]. In any case a low oxygen level combined with a higher salinity level compared to their cultured (pond) environment may be attributed to the higher mortality in the freshwater than the brackish water *P. africanus*, whose habitat water is as brackish as the produced water. The median lethal time (LT50) for *O. niloticus* was 68.6 h at the effluent concentration of 20% while LT50 of *P. africanus* was also 68.6 h but at effluent concentration of 40%. This further implies that the effluent was more toxic to the fresh water *O. niloticus* than the brackish *P. africanus*.

The physicochemical results of the test media (percentage of produced and habitat water) showed that as the percentage of habitat water increased (increased dilution), the various nutrients (phosphate, sulphate, nitrate, etc.) in the test media decreased. This is an indication of the dilution effect on toxicity. In the marine environment, physicochemical processes control the toxicokinetics and toxicodynamics of constituent compounds in produced water especially at elevated concentrations. Following discharge into the sea, compounds in produced water undergo a variety of changes, collectively known as weathering. The most important weathering processes are dilution, evaporation or volatilization, adsorption, precipitation, biodegradation and photoxidation. Individually and collectively, these processes tend to reduce the concentration of compounds in the receiving environment, thereby decreasing their potential toxicity to marine organisms [21,22].

It is notable that the oil and grease content of the produced water is 14.1 mg/l, which is below the Nigerian oil and gas regulatory limit for near-shore environment. This simply implies that the toxicity may be due to certain other reasons such as low dissolved oxygen levels, high chemical oxygen demand and high salinities for the fresh water organism. Effluent sample was more toxic on the fresh water organisms than the brackish water, possibly due to the abruptly elevated salinities coupled with the low DO levels. The high levels of COD in the produced water may be attributed to the chemicals used for the treatment and separation of crude oil from water. It was observed from the semi-static renewal test, that the dissolved oxygen levels in each test tank, reduced from an average of 4.6 mg/l to an average of 0.4 mg/l approximately. This drastic decrease in DO is attributable to the presence of organic and inorganic substances (corrosion inhibitors, oxygen scavengers, biocides, etc.) in the produced water, which bind to available oxygen, thereby threatening the organisms with the condition of hypoxia.

Conclusion

We have demonstrated through the study that the produced water collected from the Mobil QIT effluent point source was toxic to the aquatic organisms, particularly freshwater *O. niloticus*. We therefore recommend 99,000 ppm of PW as the no observed effect concentration (NOEC).

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Declaration of competing interests

Authors declare no conflict of interest.

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