EVALUATION OF THE ACUTE TOXICITY OF COREXIT 9527/FORCADOS CRUDE OIL MIXTURE ON *TILAPIA GUINEENSIS* AND *SAROTHEDRON MELANOTHERON*

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Abstract

Oil spills constitute enormous environmental problems and present significant threat to marine and shoreline ecosystems. This problem is compounded by the fact that the toxicity of dispersed oil has been shown to be greater than that of oil alone. This study evaluated the acute toxicity of Corexit 9527/Forcados (C/F) blend crude oil mixture to Sarothedron melanotheron (brackish water specie) and Tilapia guineensis (fresh water specie). Also the effects of the oil/dispersant mixture on the physicochemical parametres of fresh and brackish waters were assessed. Results showed that the oil/dispersant mixture remarkably influenced the physicochemical attributes of the dilution waters. The 96 h LC_{50} values estimated by the probit method were 62.15 and 45.63 litre^{-1} for the fresh and brackish water species *respectively. The oil/dispersant mixture was more toxic to the brackish water specie as shown by the lower LC_{50} value and the greater variation in the physicochemical variables of brackish water.*

Introduction

Oil spills are well-documented environmental and economic catastrophe which presents significant threat to marine and shoreline ecosystems. Most oil spill on waters rapidly spread into a slick, the most damaging effect being observed when the oil strand on shorelines or enters restricted shallow waters such as estuaries; resulting in long term negative consequences (Pritchard and Costa, 1991). Oil spills are managed by the use of oil spill dispersants which prevent coalescence of oil droplets (Nes and Norland, 1988) and the formation of an oil and water emulsion, removes the fire hazard of an oil spill, inhibits the contamination of shorelines, and facilitates the evaporation, biodegradation, and solubilization of the oil (Slade, 1982). Although advocates of dispersant application believe that chemical dispersion serves to accelerate the natural process of oil dispersion in a form that is safer, this treatment might present hazards to the organisms within the water column.

Dispersants contain surfactants as the active ingredient and serve to reduce the interfacial tension between oil and water, thus forming small oil droplets which move into the water column to facilitate quicker and natural dispersion (Nes and Norland, 1988). Chemically dispersed oil degrades faster than undispersed oil as the surface area of oil exposed to the water is increased (Nes and Norland, 1988). Studies on the effects of use of first generation dispersants to clean large oil spills have shown that though oil alone was not very toxic, however use of dispersants caused extensive mortalities of animals and algae in proportion to the dose used (Smith, 1968). Also, the toxic concentration of dispersants is much lower than the concentration required to disperse the stranded oil (Southwards and Southwards, 1978). Nevertheless newer dispersants are thought to be safer. Various studies have shown that the toxicity of dispersed oil was greater than that of oil alone (Thorhaugh and Marcus 1985; Thorhaugh et al., 1986; USNRC, 1989; Koyama and Kakuno, 2004).

Some physicochemical variables of water which have been shown to influence the toxicity of a dispersant formulation include temperature (Ordzie and Garafola, 1981; Wells, 1984), salinity, (Blondina et al, 1999; Rogerson and Berger, 1981, Doe and Wells, 1978; Wells and Harris, 1980; Lonning and Falk-peterson, 1978), alkalinity (Eisher, 1980; Canevari and Foy, 1982), bicarbonate (Ernst and Arditti, 1983), total dissolved solids (TDS) (Linden et al., 1981) and body oxygen demand (BOD) (Velz, 1984).

Forcados is a Nigerian crude oil, and Corexit 9527 is a common oil dispersant widely used to clean up oil spills in both brackish and fresh waters. Aquatic organisms indigenous to these waters are exposed to the toxic effects of this dispersant in combination with the spilled oil.

This study was undertaken to ascertain the acute toxicity of corexit 9527/forcados mixture (C/F) to *Tilapia guineensis* (fresh water species) and *Sarothedron melanotheron* (brackish water species).

Materials and Methods

Animals

Tilapia guineensis was obtained from Ellah lake, while *Sarothedron melanotheron* was obtained from Ogbakiri creek, both in Rivers State, south-south, Nigeria. They were identified by Mr. Sylvester Ohwo of Thermosteel Analytical Laboratory Warri, Delta State, Nigeria. Fish were maintained in 70-liter aquaria (28 ± 2ºC) and fed with fish mash. The fish were allowed a 1 week acclimatization period before commencement of the tests.

Collection of dilution water

Dilution water (the water from the natural habitat of the test organism) was obtained from the natural habitat of the fish (test animals/organisms). Fresh water was obtained from Ellah lake, while brackish (salt) water was collected from Ogbakiri creek both in Rivers State, south-south, Nigeria.

Analysis of the dilution water

Some critical physicochemical parametres of the water were analyzed as follows:

Temperature

This was determined using a mercury thermometre calibrated in 0.2 units from 0 - 100ºC. The thermometre was dipped into the dilution water and the temperature read off after 5 min.

pH

The pH of the dilution water was measured using a Scott Gerate pH metre which was standardized and checked against buffer solutions of pH 4 and 10 under ambient temperature. The electrode was immersed in the dilution water and the pH read immediately.

Conductivity

This was read off by inserting the electrode of the conductivity metre in the water.

Total Dissolved Solids (TDS)

Dilution water (1L) was poured into a beaker of known weight and allowed to completely evaporate to dryness by heating in a mantle. The beaker was re-weighed and the TDS calculated using the relation:
TDS (mgL^{-1}) = D-C/Vs; where D=weight of the dilution water + the beaker on drying, C = weight of the empty beaker, Vs = volume of the dilution water (APHA, 1989).

Dissolved Oxygen

The Scott Gerate dissolved oxygen metre was calibrated using saturated sodium sulphite solution. The electrode was then immersed in the dilution water and the value read off. The reading was converted to dissolved oxygen concentration (mgL^{-1}) with reference to standard tablets.

Biological Oxygen Demand (BOD)

The dissolved oxygen of the dilution water was determined as stated above. A BOD bottle filled with the dilution water was stoppered without trapping air bubbles and placed in an incubator (20 ºC) for 5 days. Distilled water was used as control. After 5 days, the dissolved oxygen of the dilution water and distilled water were determined. The BOD was calculated using the relation (APHA, 1989):

BOD (mgL^{-1}) = (DOB-DOA) – (DOSB-DOSA)/D; where DOB = dissolved oxygen of the dilution water before incubation, DOA = dissolved oxygen of the dilution water after incubation, DOSB = dissolved oxygen of the distilled water before incubation, DOSA = dissolved oxygen of the distilled water after incubation, D=dilution factor (0.5).

Salinity

The salinity of dilution water was determined as described by APHA (1989). Dilution water (10 ml) was poured into a 1000 ml conical flask and made up to the 1000 ml mark with distilled water. The conical flask was stoppered and mixed thoroughly. The solution (10 ml) was poured into an Erlenmeyer flask and the pH adjusted to between 6.0 and 8.5 using sodium bicarbonate solution. Potassium chromate indicator (1ml) was added to the mixture, which was subsequently diluted to 20 ml with distilled water. The mixture was then diluted with standard silver nitrate to a permanent red tinge of silver chromate. The chloride content was determined using the relation: chloride (mg L^{-1}) = $V_A \times N_A \times 35500$; where V_A = volume of silver nitrate (ml), N_A = normality of silver nitrate, 35500 = constant.

Alkalinity

Alkalinity was analyzed as described by APHA (1989). Dilution water (100 ml) in an Erlenmeyer flask to which was added standard phenolphthalein indicator (5 drops) was titrated with sulphuric acid, while stirring to end point (red to colourless). The titre volume was recorded and hydroxide was calculated using the relation:
Hydroxide (mgL^{-1}) = V_A x N_A x 17 x 1000 / V_D; where V_A = volume of sulphuric acid (ml), N_A = normality of sulphuric acid, V_D = volume of dilution water, 17 = molar mass of hydroxide, 1000 = constant.

Bicarbonate

Dilution water (50 ml) to which was added phenolphthalein indicator (3 drops) was titrated with 0.1N sulphuric acid to end point pH 8.7 (colourless to light red). The titre volume was noted. Titration for bicarbonate was done by adding 3 drops of bromothymol blue indicator to an end point of 3.7 (blue to yellow). The titre volume was used to calculate the bicarbonate concentration using the relation:
Bicarbonate (mgL^{-1}) = V_T x E x N x 1000 / V_S; where V_T = titre volume (ml), E = equivalent weight of the bicarbonate, N = normality of sulphuric acid, V_S = volume of dilution water (ml), 1000 = constant (APHA, 1989).

Total hydrocarbon content (THC)

Xylene (50 ml) was added to 1000 ml of dilution water placed in a separating funnel, followed by vigorous shaking for 2 min. The lower xylene layer was collected, centrifuged and the absorbance read at 400nm using a spectrophotometer (DR 2000). The volume of the aqueous layer was noted. The THC was calculated using the relation:
THC (mgL^{-1}) = V_E x Abs_E / V_{aq}; where V_E = volume of xylene, Vaq = volume of aqueous solvent, Abs_E = absorbance of xylene (APHA, 1989).

General Bioassay Techniques

Preparation of the test substance

The test substance (Corexit 9527/Forcados blend mixture) used was a 1: 4 mixture of Corexit 9527 (dispersant) and Forcados blend (crude oil) (C/F). A 1000 mgL^{-1} stock solution of the mixture in dilution water was prepared and subsequently diluted as needed.

Effect of C/F on the physicochemical parameters of the dilution water inhabited by fish

Bioassay tanks constructed with a transparent glass measuring 40cm x 25cm x 25cm, covered with a 0.5 mm nylon mesh (to prevent the animals from jumping out) were used for the study. The tanks were well aerated with air pumps to ensure adequate circulation of air.

Twenty fingerlings of each species of fish were transferred into the respective bioassay tanks containing one of 5,10,35,70,100 or 450 mgL^{-1} of C/Fin brackish or fresh water at room temperature (28 ± 2ºC). Control groups were held in tanks containing the appropriate dilution water without C/F.

The dilution water was replaced every 24 h by siphoning out the stale/used dilution water from the middle of the bioassay tank, followed by immediate replacement with fresh dilution water from the inlet tube (Mojisola, 2000).

Physicochemical parameters of the dilution water such as temperature, dissolved oxygen, pH, total dissolved solid, alkalinity, bicarbonate and salinity were determined before and after (on stabilization of the test system) renewal at 24 h intervals for 4 days.

Acute toxicity studies

This was done to determine the concentration of the test substance that killed 50% of the exposed organisms (LC_{50}). In the preliminary studies carried out to determine the concentration range to be used in the toxicity test, animals (n=20) in the respective bioassay tanks were exposed to each of a wide range of concentration of C/F (100, 400, 650, 850 and 1000mgL^{-1}) over 24, 48 and 96 h. Subsequently, a series of concentrations between the range that killed all/most of the animals or a few/no animal were selected and used for the study. After treatment, the animals (n=20) were observed regularly and mortality recorded at 3h, 6h, 12h, 24h, 48h, 72h and 96h. Mortality was established at the point when no peculiar movement was detected (Sprague, 1973). Dead animals were removed immediately on detection (APHA, 1989).

Statistical Analysis

Data obtained was analyzed using One-Way analysis of variance (ANOVA) and further subjected to LSD post hoc test for multiple comparisons. The results were presented as Mean ± SEM. Differences between means of treatment and control groups were accepted significant at $P<0.05$.

Results

Baseline physicochemical characteristics of brackish and fresh waters

Results showed differences in the physicochemical characteristics of fresh and salt waters (Table 8.1). The baseline physicochemical properties of the tested waters were within the recommended range (WHO, 1984; APHA, 1989; Environment Canada, 1987).

Effect of C/F on the physicochemical parameters of fresh and salt waters

C/F increased the temperature, alkalinity, pH, TDS, bicarbonate, salinity, but decreased the dissolved oxygen of fresh water inhabited by fish at 24, 48, 72 and 96 h respectively compared to control (Table 8.2).

For salt water, on day 1, C/F increased the temperature, salinity, bicarbonate level, TDS, but decreased dissolved oxygen compared to control. Prior to renewal, the pH and alkalinity were decreased, but increased after renewal compared to control (Table 8.3).

On day 2, C/F increased the temperature, pH, dissolved oxygen, TDS, bicarbonate, and salinity before and after renewal. However it increased alkalinity before renewal, but elicited varying changes after renewal compared to control (Table 8.3).

On day 3, before renewal, there were increases in dissolved oxygen, pH, alkalinity, bicarbonate and salinity, with increased TDS; while after renewal all the parameters were increased compared to control (Table 8.3).

On day 4, dissolved oxygen, pH, alkalinity, bicarbonate and salinity decreased prior to renewal, but were increased after renewal compared to control (Table 8.3).

Also it was observed that in both C/F contaminated and control fresh and salt waters, the values of pH and dissolved oxygen were higher in stale than in freshly renewed water; while temperature, TDS, alkalinity, bicarbonate and salinity were higher in freshly renewed than in stale water.

Acute toxicity (LC_{50}) of C/F against *Tilapia guineensis* and *Sarothedron melanotheron*

The results showed that as the exposure time increased, the LC_{50} values decreased, implying greater toxicity with increased exposure time.

C/F blend was more toxic to the brackish water species as shown by the lower LC_{50} values. At low exposure time, the difference in LC_{50} values between fresh and salt water species was pronounced with LC_{50} values for brackish water approaching half that of fresh water. However, with increase in exposure time, the gap closed. The LC_{50} values of C/F against the organisms over time are as shown in Tables 8.4, 8.5 and 8.6.

Table 8.1: ***Comparison of baseline physicochemical characteristics of brackish and fresh waters***

Parameter	Fresh water	Brackish water
Temperature (ºC)	27.60±0.40	26.55±0.10
pH	7.33±0.04	7.25±0.05
Conductivity (us/cm)	410.80±0.40	1344.85±0.30
Total dissolved solids (TDS) (mg L^{-1})	245.00±0.40	806.30±0.50
Dissolved Oxygen (mg L^{-1})	7.01±0.03	6.70±0.01
Biological Oxygen Demand (BOD) (mg L^{-1})	3.41±0.04	4.40±0.13
Salinity (mg L^{-1})	140.50±0.50	1488.96±0.14
Alkalinity ($MgCaCO_3$/L)	79.90±0.40	300.25±0.99
Bicarbonate (mg L^{-1})	6.16±0.07	12.34±0.05
Total petroleum hydrocarbon content (THC) (mg L^{-1})	0.11±0.03	0.20±0.05

Table 8.2: ***Effect of C/F on critical physicochemical parameters of fresh water before renewal and after renewal at 24, 48, 72 and 96 h***

Treatment	Concentration (mg/L)	Temperature (ºC)	Dissolved O_2 (mgL^{-1})	pH	TDS (mgL^{-1})	Alkalinity ($MgCaCO_3$/L)	Bicarbonate (mgL^{-1})	Salinity (mgL^{-1})
Day 1 (before renewal)								
C/F	5	27.55	6.87	7.39	244.96	75.19	6.33	144.97
	10	27.65	6.68	7.40	244.92	75.39	6.41	145.03
	35	27.60	6.83	7.46	245.20	75.84	6.45	145.09
	70	27.60	6.77	7.54	245.41	75.64	6.30	144.92
	100	27.65	6.70	7.40	245.22	75.90	6.32	145.01
	450	27.65	6.70	7.40	245.22	75.90	6.32	145.01
Control	-	27.48	7.01	7.35	244.71	74.76	6.16	144.89
Day 1 (after renewal)								

C/F	5	28.70	4.76	6.28	247.98	75.81	8.81	160.93
	10	29.05	4.69	6.33	248.32	76.27	8.72	161.04
	35	29.07	4.84	6.36	247.61	76.20	8.77	161.22
	70	28.88	4.88	6.38	247.93	77.13	8.64	160.98
	100	28.80	4.77	6.42	248.08	76.65	8.69	160.84
	450	28.80	4.77	6.42	248.08	76.65	8.69	160.84
Control	-	28.20	4.99	6.23	247.11	75.48	8.61	160.68
Day 2 (before renewal)								
C/F	5	27.55	6.89	7.37	244.97	75.22	6.39	144.90
	10	27.50	6.70	7.42	245.10	75.77	6.42	145.02
	35	27.65	6.81	7.40	245.22	75.41	6.58	145.10
	70	27.60	6.46	7.40	245.33	75.81	6.39	144.99
	100	27.70	6.81	7.42	245.35	75.88	6.31	145.07
	450	27.70	6.81	7.42	245.35	75.88	6.31	145.07
Control	-	27.10	7.03	7.35	244.68	74.77	6.18	144.51
Day 2 (after renewal)								

C/F	5	28.77	4.70	6.38	247.92	75.91	8.70	161.04
	10	29.04	4.63	6.38	248.17	76.48	8.89	161.09
	35	29.09	4.93	6.32	247.85	77.10	8.97	160.78
	70	28.88	4.72	6.44	247.83	77.17	8.79	160.75
	100	28.75	4.70	6.47	248.03	77.11	8.63	160.88
	450	28.75	4.70	6.47	248.03	77.11	8.63	160.88
Control	-	28.15	4.95	6.28	247.09	75.61	8.59	160.62
Day 3 (before renewal)								
C/F	5	27.50	6.88	7.36	244.91	75.10	6.29	144.92
	10	27.58	6.69	7.40	244.95	75.70	6.31	145.08
	35	27.60	6.97	7.80	245.20	75.32	6.49	145.09
	70	27.64	6.88	7.38	245.28	75.80	6.30	144.94
	100	27.55	6.80	7.45	245.15	75.82	6.29	145.05
	450	27.55	6.80	7.45	245.15	75.82	6.29	145.05
Control	-	27.48	7.00	7.33	244.68	74.65	6.15	144.88
Day 3 (after renewal)								

C/F	5	28.65	4.71	6.35	247.97	75.70	8.86	161.08
	10	28.80	4.69	6.49	248.06	76.27	8.94	161.03
	35	28.85	4.88	6.54	247.69	76.20	8.83	161.22
	70	29.10	4.92	6.39	247.96	77.17	8.78	160.96
	100	28.85	4.85	6.46	248.05	77.13	8.69	160.83
	450	28.85	4.85	6.46	248.05	77.13	8.69	160.83
Control	-	28.65	5.01	6.24	247.02	75.44	8.59	160.57
Day 4 (before renewal)								
C/F	5	27.50	6.89	7.37	244.88	75.12	6.34	144.99
	10	27.55	6.67	7.41	244.94	76.20	6.32	145.09
	35	27.62	6.84	7.41	245.18	75.33	6.49	144.98
	70	27.66	6.88	7.45	245.25	76.47	6.31	145.08
	100	27.58	6.81	7.48	245.20	76.82	6.47	145.06
	450	27.58	6.81	7.48	245.20	76.82	6.47	145.06
Control	-	27.50	7.01	7.28	244.61	74.62	6.15	144.90
Day 4 (after renewal)								
C/F	5	28.75	4.76	6.3	247.92	75.8	8.7	161.0

				1		0	1	2
	10	28.95	4.56	6.37	248.06	76.51	8.87	161.08
	35	28.85	4.81	6.33	247.88	77,17	8.98	161.19
	70	28.87	4.77	6.38	247.99	77.06	8.74	160.99
	100	28.42	4.72	6.37	248.03	77.11	8.79	161.08
	450	28.42	4.72	6.37	248.03	77.11	8.79	161.08
Control	-	28.70	4.99	6.24	247.08	75.76	8.57	160.60

Table 8.3: ***Effect of C/F on critical physicochemical parameters of brackish (salt) water before renewal and after renewal at 24, 48, 72 and 96 h***

Treatment	**Concentration (mgL^{-1})**	**Temperature (ºC)**	**Dissolved O_2 (mgL^{-1})**	**pH**	**TDS (mgL^{-1})**	**Alkalinity ($MgCaCO_3/L$)**	**Bicarbonate (mgL^{-1})**	**Salinity (mgL^{-1})**
Day 1 (before renewal)								
C/F	5	26.57	6.69	7.25	806.87	300.28	12.44	1488.07
	10	26.51	6.68	7.28	806.89	300.30	12.42	1488.02
	35	26.55	6.74	7.27	806.82	300.27	12.41	1488.09
	70	26.55	6.72	7.29	806.93	300.27	12.37	1488.11
	100	25.50	6.71	7.27	806.84	300.29	12.39	1488.10
	450	25.50	6.71	7.2	806.8	300.29	12.39	1488.1

				7	4			0
Control	-	26.49	7.01	7.28	806.84	300.30	12.32	1488.02
Day 1 (after renewal)								
C/F blend	5	27.62	5.42	6.51	807.70	301.95	13.12	1583.37
	10	27.65	5.33	6.60	807.93	301.97	13.17	1583.35
	35	27.68	5.51	6.55	807.60	301.99	13.17	1583.31
	70	27.62	5.27	6.47	807.75	302.08	13.19	1583.29
	100	27.62	5.25	6.58	807.68	302.06	13.16	1583.33
	450	27.62	5.25	6.58	807.68	302.06	13.16	1583.33
Control	-	27.55	5.38	6.45	807.04	301.90	13.02	1583.03
Day 2 (before renewal)								
C/F blend	5	26.55	6.72	7.27	806.88	300.23	12.33	1488.62
	10	26.58	6.69	7.30	806.87	300.33	12.41	1488.65
	35	26.55	6.74	7.29	806.84	300.32	12.52	1488.52
	70	26.55	6.71	7.28	806.91	300.34	12.49	1488.58
	100	25.59	6.72	7.27	806.89	300.31	12.46	1488.82
	450	25.59	6.72	7.27	806.89	300.31	12.46	1488.82
Control	-	26.51	6.70	7.2	806.8	300.28	12.31	1488.0

				6	0			3
Day 2 (after renewal)								
C/F blend	5	28.65	5.44	6.49	807.69	301.95	13.16	1583.32
	10	27.63	5.31	6.62	807.95	301.97	13.16	1583.41
	35	27.67	5.49	6.47	807.95	302.07	13.13	1583.47
	70	27.69	5.47	6.50	807.66	301.96	13.18	1583.39
	100	27.69	5.29	6.55	807.62	302.04	13.17	1583.44
	450	27.69	5.29	6.55	807.62	302.04	13.17	1583.44
Control	-	27.55	5.27	6.41	807.55	301.99	13.03	1583.01
Day 3 (before renewal)								
C/F blend	5	26.20	6.69	7.27	806.86	300.26	12.36	1488.00
	10	26.60	6.70	7.27	806.89	300.29	12.42	1488.37
	35	26.40	6.73	7.28	806.83	300.27	12.46	1488.93
	70	26.40	6.70	7.25	806.87	300.26	12.58	1488.10
	100	26.20	6.69	7.29	806.82	300.28	12.53	1488.80
	450	26.20	6.69	7.29	806.82	300.28	12.53	1488.80
Control	-	26.40	6.68	7.2	806.8	300.20	12.28	1487.2

				6	8			0
Day 3 (after renewal)								
C/F blend	5	27.64	5.38	6.48	807.73	301.96	13.19	1588.92
	10	27.65	5.40	6.42	807.97	302.02	13.17	1588.98
	35	27.60	5.34	6.39	807.99	301.99	13.22	1588.89
	70	27.65	5.30	6.47	807.78	301.97	13.15	1589.02
	100	27.70	5.35	6.59	807.66	302.05	13.19	1589.01
	450	27.70	5.35	6.59	807.66	302.05	13.19	1589.01
Control	-	27.60	5.25	6.31	807.51	301.88	13.11	1588.04
Day 4 (before renewal)								
C/F blend	5	26.70	6.70	7.29	806.88	300.34	12.35	1488.14
	10	26.84	6.72	7.29	806.84	300.29	12.44	1488.09
	35	26.39	6.71	7.31	806.80	300.29	12.53	1488.18
	70	26.55	6.69	7.30	806.87	300.30	12.42	1488.09
	100	26.67	6.70	7.28	806.82	300.37	12.37	1488.88
	450	26.67	6.70	7.28	806.82	300.37	12.37	1488.88
Control	-	27.50	6.67	7.27	806.8	300.26	12.23	1487.1

					5			2
Day 4 (after renewal)								
C/F blend	5	27.40	5.37	6.40	807.68	301.94	13.19	1588.88
	10	28.10	5.35	6.42	807.97	301.99	13.25	1588.93
	35	27.75	5.34	6.45	807.97	302.03	13.20	1588.84
	70	27.87	5.33	6.43	807.72	302.92	13.17	1589.02
	100	28.08	5.31	6.45	807.66	301.99	13.22	1588.97
	450	28.08	5.31	6.45	807.66	301.99	13.22	1588.97
Control	-	27.45	5.28	6.39	807.51	301.85	13.10	1588.10

Table 8.6: ***LC_{50} of C/F on Tilapia guineensis and Sarothedron melanotheron***

Time (h)	**LC_{50} of Corexit 9527/Forcados blend mixture**	
	Tilapia guineensis **(Fresh water species) (mgL^{-1})**	***Sarothedron melanotheron*** **(Brackish water species) (mgL^{-1})**
3	619.80 ± 10.02	397.04 ± 10.22
6	443.86 ± 8.73	227.04 ± 9.87
12	282.67 ± 7.66	154.49 ± 7.55
24	197.00 ± 6.12	121.34 ± 6.77
48	132.87 ± 4.33	100.75 ± 4.28
72	76.46 ± 5.04	55.34 ± 4.40
96	62.15 ± 4.50	45.63 ± 4.46

Discussion

Despite the benefits of the use of dispersants to manage and contain oil spills, there is great concern about their ecological impact and toxicity to ecosystems. Toxicity of a dispersant/oil mixture to an ecosystem is a function of the dispersant, the oil being dispersed, the nature of exposure (i.e. concentration and time), the organism involved, and the life stage of the organism (Michelli et al., 1991). The combination of these factors as well as others that may be relevant in specific situations will determine the ultimate impact.

This study evaluated the acute toxicity of Corexit 9527/Forcados mixture in brackish and fresh waters by assessment of the effect of the dispersant/oil mixture on the physicochemical characteristics of the waters and the LC_{50} of the fish species that inhabit the waters.

Analysis of fresh and salt waters showed significant differences in their baseline physicochemical characteristics. In addition, the waters were found to be within the recommended range (WHO, 1984; APHA, 1989; Environment Canada, 1987) and hence suitable for the study, considering that significant changes in the baseline values of these parameters could affect the results of the study.

The observation that in both C/F contaminated and control waters the values of pH and dissolved oxygen were higher in stale than in freshly renewed water; while temperature, TDS, alkalinity, bicarbonate and salinity were higher in freshly renewed than in stale water may be due to the metabolic and other activities of the fish.

Evaluation of the effect of the dispersant/oil mixture on the physicochemical characteristics of fresh water inhabited by fish showed that C/F increased the temperature, alkalinity, pH, TDS, bicarbonate, salinity, but decreased the dissolved oxygen at 24, 48, 72 and 96 h compared to control. The C/F also elicited varying changes in salt water inhabited by fish. These indicate that C/F affects and changes the physicochemical characteristics of the dilution water in which fish inhabit, this may subsequently affect the physiological and other processes in the fish leading to toxicity. This is underscored by the observation that low dissolved oxygen (Scott and Dutka, 1980), increased alkalinity (Eisher, 1980; Canevari and Foy, 1982), bicarbonate (Ernst and Arditti, 1983), TDS (Linden et al., 1981) and BOD (Velz, 1984) caused an increase in the toxicity of surfactants.

Also, increased salinity has been shown to enhance the toxicity of Corexit 9527/oil mixture (Blondina *et al*, 1999), while higher temperatures have been shown to increase the toxicity of Corexit 9527 (Ordzie and Garafola 1981; Wells, 1984). The changes in these parametres maybe a major contributing factor in the toxicity of C/F blend to *Tilapia guineensis.*

Generally C/F elicited greater fluctuations in the physicochemical parametres of the brackish water characterized by increases in salinity, alkalinity, bicarbonate, TDS and pH; this may also translate to increased toxicity.

Fluctuations/changes in several environmental variables such as salinity can influence the interactions between organisms and pollutants in aquatic organisms and therefore affect the toxicity of xenobiotics (Wang *et al*, 2001).

The 96 h LC_{50} were 62.15 ± 4.50 $mgL^{-1}$and 45.63 ± 4.46 mgL^{-1} for *Tilapia guineensis and Sarothedron melanotheron* respectively, showing greater toxicity to the brackish water organisms. Comparison of the LC_{50} values of C/F in fresh and salt water species over time show significant (p<0.05) greater toxicity against the brackish specie as shown by the lower LC_{50} values. Thus, the higher the salinity of the water, the more toxic the dispersant/oil mixture. This is consistent with previous studies which showed that brackish water species are more sensitive to Corexit 9527/oil mixture than fresh water species (Blondina et al, 1999).

The brackish water had lower dissolved oxygen; this may contribute to greater sensitivity to C/F blend as low dissolved oxygen is known to increase the toxicity of surfactants (Scott and Dutka, 1980).

Also, as alkalinity decreased from brackish to fresh water, there was reduced toxicity as shown by the higher $LC_{50.}$ This is consistent with previous studies (Canevari and Foy, 1982; Eisher, 1980). The LC_{50} also decreased as the bicarbonate level, total dissolved solids and conductance increased from fresh to brackish water. There was a low LC_{50} at the high BOD of brackish water. BOD is the amount of oxygen required for aerobic microorganisms present in a water body to oxidize organic matter to stable inorganic form. High BOD is an indication of high toxicity in a water body (Velz, 1984).

In addition, the nature of exposure (concentration and time) among other factors is a great determinant of the toxicological effect of a dispersant (Michelli et al., 1991). Exposure time is inversely proportional to the LC_{50} (Anderson et al., 1984); as exposure time increases, the LC_{50} decreases and the agent is more toxic.

The greater acute toxicity shown in the brackish and fresh water fishes could be due to the differences in the baseline levels of physicochemical parametres and also the greater fluctuations/changes in these parametres in the brackish water.

It is important to note that the study did not use the values of the dispersant/oil mixture measured in the water to which the animals were exposed (water soluble fraction), but the total oil per unit volume (nominal concentration). This constitutes a limitation to the study since nominal concentration is bound to give a higher toxicity value than the water soluble fraction due to the tendencies of hydrocarbon to adhere to test equipment as well as difficulties of the largely hydrophobic compounds to dissolve in the water (Norton et al., 1978; Anderson et al., 1984).

In conclusion, the C/F blend was more toxic to the brackish than the fresh water species. This greater toxicity may be attributed to the significant differences in the physicochemical characteristics of fresh and brackish waters. In addition, considering the fact that increased salinity, alkalinity, bicarbonate and other changes in the physicochemical variables of water result in increased sensitivity of marine community to toxic effects of xenobiotics, the inherent physicochemical make-up of brackish water may pre-dispose the aquatic organisms to greater toxic effects.

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