RESEARCH ARTICLE   **Open Access**

Biochemical toxicity of Corexit 9500 dispersant on the gills, liver and kidney of juvenile *Clarias gariepinus*

A. P. Ugbomeh[*], K. N. O. Bob-manuel, A. Green and O. Taylorharry

Abstract

Corexit 9500 is a dispersant commercially available in Nigeria that is used to change the inherent chemical and physical properties of oil, thereby changing the oil's transport and fate with potential effects on the environment. The aim of this study was to assess the biochemical (enzymes and electrolyte) toxicity of Corexit 9500 dispersant on the gills, liver and kidney of juveniles of *Clarias gariepinus* after exposure for 21 days. One hundred sixty fish were used without gender consideration. Range-finding tests were conducted over a 96-h period after acclimatisation of the test organisms in the laboratory. The test organisms (10/treatment) were exposed to Corexit 9500 in the following concentrations—0.00, 0.0125, 0.025 and 0.05 ml/l in triplicate. Twenty-one days later, fish was dissected. 0.5 g from each of the following organs—gills, liver and kidney tissues—was removed, homogenised and tested for enzymes [superoxide dismutase (SOD), catalase (CAT), alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP)], urea, creatinine and electrolytes (sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-)) following standard methods. In the gills, SOD and ALT to AST ratio were significantly lower than in control while the creatinine was significantly higher in the toxicant. In the kidney, creatinine was significantly higher in fish exposed to the toxicant. In the liver, ALP increased in the toxicant while urea was decreased. The mean electrolyte concentrations (Na^+, K^+, Cl^- and HCO_3^-) increased significantly in the concentration of the toxicant ($P < 0.05$). The alterations observed in the activities of these electrolytes and enzymes indicated that Corexit 9500 interfered with transamination and metabolic functions of the fish.

Keywords: Enzymes, Corexit 9500, Creatinine, Electrolytes, *Clarias gariepinus*

Background

Clarias gariepinus contributes greatly to the commercial catch of the artisanal fishers in freshwater communities of the Niger Delta and Nigeria at large and is also a preferred species for aquaculturist (Potts et al. 2008; FAO 2010-2016). In Nigeria, the Niger-Delta region has been affected by oil spills, with the resultant use of dispersants thus triggering acute responses in fish and other aquatic lives. Fishes are veritable tools for assessing the effect of pollution in aquatic environments because of their mobile nature (Van der Oost et al. 2003) and some physiological responses from the kidney, gills, liver and heart serve as biomarkers that indicate changes in biological response (ranging from molecular, cellular and physiological to behavioural changes), caused by exposure or toxic effect of environmental chemicals (Sribanjam et al. 2018). Biochemical markers are quantifiable responses of the exposure of an organism to a pollutant, and the use of different factors is more beneficial than the use of a single biomarker (Kanchan et al. 2011). The analysis of these biochemical alterations is often used to determine the effect of pollutants on the various organs in the field or in experimental studies (Fontainhas-Fernandes et al. 2008).

Corexit 9500 is one of the several dispersants that are commercially available in Nigeria and are used to change the inherent chemical and physical properties of oil, thereby changing the oil's transport, fate and potential effects on the environment (NRC [National Research Council], 2005). The use of dispersants in Nigeria is restricted to the offshore environment, and Corexit 9500

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which is found in our markets is not among the approved dispersants in Nigeria (DPR [Department of Petroleum Resources] 2018, 2002). Its availability however implies that there is a demand for it. Approximately 250 barrels of dispersant (Gold Crew, Corexit 9500 and Corexit 9527) was applied in the 1998 rupture of a 24-in. pipeline from the IDOHO platform of Mobil in the Qua Ibo Terminal (Olagbende et al. 1999). Corexit 9500A has been observed to increase the mobility of polycyclic aromatic hydrocarbons (a component of crude oil) making them easily available to a larger number of organisms (Zuijdgeest and Huettel 2012) thereby increasing their potential toxicity. There is a paucity of information on the enzymatic and electrolytic responses of Corexit 9500 on the gills, liver and kidney of the juveniles of *Clarias gariepinus*. This study is focused on providing start-up information about the biochemical effects of Corexit 9500 on the gills, liver and kidney and the physicochemical interactions of the medium over a 3-week period.

Methods
Procurement of Corexit 9500
Corexit 9500 (lot no. BPOJ1337A) was purchased from a chemical shop in Port Harcourt in 4-l plastic containers and used in the preparation of the test solution. In order to determine the appropriate concentration, preliminary range-finding test was carried out on a group of five fishes which were exposed to six spaced sample dilutions of the stock solution in logarithmic series of 0.1, 1, 3, 5, 7, 9 and 15 ml/l for 24 h. The test fishes and control were first observed after an exposure period of 8 h (USEPA 2010) and 4-h intervals. The lowest concentration with signs of behavioural anomaly (erratic swimming, hyperventilation) was noticed at 5 ml/l plastic basin after about 8 h.

Procurement and acclimatisation of fish
Live juveniles (300) of *Clarias gariepinus* were obtained from a private fish farm in Port Harcourt City and acclimatised for 14 days in the laboratory (Lelei and Sikoki 2013). The mean length of fish was 8.40 ± 2.40 cm and mean weight 3.28 ± 1.28 g SE. The preparation of test stock solution for chronic testing was according to Taylorharry et al. (2019). A 25-l plastic basin was used for dilution of test solution. However, the test concentrations of 0.00 (control), 0.0125, 0.025 and 0.05 ml/l of Corexit 9500 in dilution water were prepared by separately mixing 0.2, 0.4 and 0.8 ml/l of Corexit 9500 in 16 l of water. A 2-ml syringe was used for measuring the dispersant into the 1 l of water (OECD 1992) while the fish was randomly introduced into the test basins. The test concentration and water in the plastic basins were renewed after every 2 days. All treatment concentrations had 3 replicates of ten fish, and there were no gender considerations. The pH, dissolved oxygen

(DO) and temperature of the test media were measured in situ to determine its correctness for fish survival based on recommended quality parameters (Lelei and Sikoki 2013; APHA 1992) using a standard Hanna multi-parameter probe (model no: HI98130).

Dissection and tissue homogenisation
At the end of 3 weeks, the fishes from each concentration were immobilised by cervical dislocation before dissection. The gills, liver and kidneys were separated from the anterior and posterior part of the gut, placed in separate Petri dishes and preserved in appropriate solution for biochemical analysis. 0.5 g each of the gills, liver and kidney tissues was homogenised separately on ice, in 4 ml of phosphate buffer (0.1 M) at pH 7.4 using laboratory pestle and mortar. It was immediately spun at 3000 r/min in a centrifuge for 10 min, and the supernatants were collected for enzymes (SOD, CAT, ALT, AST, ALP), urea, creatinine and electrolyte (Na^+, K^+, Cl^-, HCO_3^-) assay.

Biochemical analysis
Enzymes
Superoxide dismutase (SOD) and catalase (CAT) levels were measured quantitatively according to the sandwich enzyme-linked immunosorbent assay (ELISA) method (Uotila et al. 1981, Lequin 2005) using Elabscience® Kits. ALT and AST were estimated according to Reitman and Frankel (1957) method using Randox® Kits, while ALP was estimated according to the phenolphthalein method (Romel et al. 1968) using Teco® Kit. Urea was estimated according to the Urease-Berthelot method (Fawcett and Scott 1960) using Randox® Kit while creatinine was estimated according to the Jaffe-Slot method (Aitken et al. 2003) using DiagnosticumZrt® Kit. Calculations were made with formulae based on the methodology of the various trade names as follows:

1. $SOD \left(\frac{pg}{ml}\right) = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times Concentration\ of\ standard$
2. $CAT \left(\frac{ng}{ml}\right) = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times Concentration\ of\ standard$
3. $ALP \left(\frac{IU}{l}\right) = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times Concentration\ of\ standard$
4. $Urea \left(\frac{mmol}{l}\right) = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times Concentration\ of\ standard$
5. $Creatinine \left(\frac{\mu mol}{l}\right) = \frac{Absorbance\ 1\ of\ test - Absorbance\ 2\ of\ test}{Absorbance\ 1\ of\ standard - Absorbance\ 2\ of\ standard} \times Concentration\ of\ standard$

Electrolytes (sodium, potassium, chloride and bicarbonate ions)
Were determined by automation using the ion-selective electrode (ISE) method (Bailey 1976). The samples were

aspirated through the probe of the ISE machine and the results read and copied from the display monitor of the machine.

Statistical method

Data obtained from this study were collated and subjected to one-way analysis of variance (ANOVA), and Tukey was used for mean separation using JMP-SAS version 12. Results were considered significant at $P < 0.05$.

Results

Physicochemical parameters

The physicochemical parameters (Table 1) of all the test media showed that temperature was not different at $P < 0.05$ (26.17–26.53 °C). The DO of control was significantly higher than that of the treatments, with the lowest DO in the 0.05 ml/l treatment. pH was acidic for all treatments including the control but was more acidic with increasing concentration of Corexit 9500 (5.84–5.51). The pH of control was different from treatment 0.025 and 0.05 ml/l but not from 0.0125 ml at $P < 0.05$.

Enzyme activity in the gills, liver and kidneys

The mean level of the enzymes in the gills of *C. gariepinus* treated with Corexit 9500 is shown in Table 2. AST was significantly highest in treatment 0.0125 ml/l (42.00 ± 1.92 µl/l) than other treatments. Control (12.66 ± 0.23 µl/l) had the lowest mean concentration. The AST concentration in all the treatments was higher than the control ($P < 0.05$). The mean level of alanine aminotransferase (ALT) in the gills showed that treatment 0.0125 ml/l (41.33 ± 2.19 µl/l) had the highest mean value while the control (16 µl/l) had the least. The ALT to AST ratio decreased in the treatments (0.88–0.94) compared to the control. Alkaline phosphatase (ALP) was lowest in 0.025 ml/l (12.66 ± 1.09 µl/l) followed by the control

Table 1 Mean ± SD of physicochemical parameters in exposure tanks

Treatments (ml/l)	Temp (°C)		DO (ml/l)		pH	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Control	26.50 ± 0.14[a]	26.3–26.6	6.15 ± 0.33[a]	6.1–6.2	5.84 ± 0.12[a]	5.80–5.85
0.0125	26.53 ± 0.35[a]	26.4–26.6	5.63 ± 0.22[b]	5.5–5.7	5.56 ± 0.08[bc]	5.55–5.60
0.025	26.17 ± 0.16[a]	26.1–26.3	5.76 ± 0.41[b]	5.6–5.9	5.64 ± 0.24[b]	5.62–5.68
0.05	26.31 ± 1.19[a]	26.3–26.4	5.35 ± 0.22[c]	5.2–5.5	5.51 ± 0.10[c]	5.44–5.64

Groups with the different superscript letters in the same column are significantly different from each other ($P < 0.05$)

(16.33 ± 4.01 µl/l) while the highest concentration was recorded in 0.0125 ml/l (208.66 ± 23.10 µl/l). The mean levels of ALP in the control (16.33 ± 4.01 µl/l) and 0.025 ml/l (12.66 ± 1.09 µl/l) treatment were not different but differed from 0.0125 ml/l and 0.05 ml/l (30.50 ± 2.01 µl/l) ($P < 0.05$).Creatinine was highest in the fishes exposed to Corexit 9500 than the control. Although there were differences in the mean values of creatinine across the treatments, they were not statistically significant ($P < 0.05$).

The mean ± SE of urea recorded from the samples showed that those treated with 0.025 ml/l had the highest concentration (14.40 ± 2.24 µl/l) followed by the control (12.45 ± 1.18 µl/l). Results showed no difference between control and other treatments ($P < 0.05$), but there was a difference between 0.025 ml/l and 0.0125 ml/l. The distribution of the enzyme catalase among the treated samples showed that the control had the highest concentration (8.76 ± 1.03 µl/l) followed by 0.05 ml/l (7.27 ± 1.12 IU/l) then 0.0125 ml/l (5.68 ± 0.94 IU/l) while 0.025 (4.86 ± 0.83 IU/l) was the least. The control differed significantly from 0.025 ml/l ($P < 0.05$) but was not significantly different from 0.0125 ml/l and 0.05 ml/l ($P > 00.05$) as shown in Table 2. All treatment values were lower than the control, and there was also a variation in the mean concentrations of SOD across the treatments. All fishes exposed to Corexit 9500 had SOD levels lower than the control.

In the kidney, creatinine was higher in the exposed fishes than in the control. Levels increased with increasing concentration of the dispersant (Table 3). AST, ALT, urea and catalase levels were not different in the control and treatments ($P > 0.05$). The ALT to AST ratio increased in higher exposure concentrations. SOD and ALP levels in exposed fishes were different from the control ($P < 0.05$) at concentrations except 0.025 ml/l.

In the liver (Table 4), AST and ALT concentration levels were not different between control and treatments except at 0.05 ml/l ($P < 0.05$). ALP concentrations were different from the treatments ($P < 0.05$) except in 0.025 ml/l. The control differed significantly from the treatments ($P < 0.05$) for urea, whereas creatinine, catalase and SOD treatments did not differ significantly from the control at $P > 0.05$.

Electrolyte levels

Gills

The Na⁺, K⁺, Cl⁻ and HCO₃⁻ ion concentrations in the gills of *C. gariepinus* exposed to Corexit 9500 were all different from the control at $P < 0.05$ (Table 5). Higher concentrations of Na⁺, K⁺ and Cl⁻ with increasing treatment concentrations and the reverse for HCO₃⁻ was observed.

Table 2 Mean (± SE) concentrations of gill enzymes of *C. gariepinus* treated with Corexit 9500 dispersant

Treatments (ml/l)	SOD (mmol/l)	CAT (mmol/l)	ALT (Iμ/l)	AST (IU/l)	ALP (IU/l)	Urea (IU/l)	Creatinine (IIU/l)	ALT to AST (ratio)
Control	2.79 ± 0.23[a]*	8.76 ± 1.03[a]	16.00[d]	12.66 ± 0.23[d]	16.33 ± 4.01[c]	12.43 ± 1.18[ab]	190.00[a]	1.26
0.0125	0.35 ± 0.02[b]	5.68 ± 0.94[ab]	41.33 ± 2.19[a]	42.00 ± 1.92[a]	208.66 ± 23.10[a]	11.44 ± 0.97[b]	123.33 ± 2.97[a]	0.98
0.025	0.92 ± 0.01[ab]	4.86 ± 0.83[b]	27.00[b]	30.66 ± 0.92[a]	12.66 ± 1.09[c]	14.40 ± 2.24[a]	139.66 ± 9.18[c]	0.88
0.05	0.86 ± 0.01[ab]	7.27 ± 1.12[ab]	21.50 ± 3.02[c]	23.00 ± 2.10[c]	30.50 ± 2.01[b]	12.32 ± 4.01[ab]	132.11 ± 2.01[c]	0.93

*Groups with the different superscript letters in the same column are significantly different from each other ($P < 0.05$)

Kidneys

The Na^+ and K^+ ion concentrations in the kidney of fish exposed to Corexit 9500 were not different from those of the controls ($P > 0.05$). Also, the Cl^- and HCO_3^- concentrations of the controls were not different from the lower exposure concentrations of 0.0125 ml/l and 0.025 ml/l ($P > 0.05$) but were different from levels of fish exposed to 0.05 ml/l of Corexit 9500 ($P < 0.05$) (Table 6).

Liver

Na^+ and HCO_3^- levels in the liver of *C. gariepinus* in the control and 0.025 ml/l exposure level were not different ($P > 0.05$), but were different with the higher exposure concentrations ($P < 0.05$). Levels of Na^+, K^+ and Cl^- increased with increasing exposure concentrations, but HCO_3^- decreased with increasing exposure concentrations of the dispersant (Table 7).

Discussions

Physicochemical parameters

The decrease in dissolved oxygen (DO) with increasing concentrations of Corexit 9500 could be attributed to the ability of the dispersant to reduce soluble oxygen in the medium. The reduced dissolved oxygen with higher concentration of Corexit 9500 might have caused stress in the fish resulting in suffocation and fish death during the acute testing. This perception corroborates prior reports of Baden (1982) that water bodies contaminated with toxicants and other aquatic pollutants usually have a high biological oxygen demand and a reduction in DO. Boyd and Lichthoppler (1979) also reported that low DO of water could have stress-related adverse outcomes on the growth and behaviour of fish. The increased acidity in the treatments may also have impacted the fish.

Enzymes

In this study, the mean concentration of some of the enzymes increased as the concentration of the toxicant increased while some decreased in the organs examined indicating elevation or decrease observed as dose-dependent. This is in accordance with the report of Pesce et al. (2008), who noticed that the disparity in metabolic enzyme activities in fish was directly proportional to the concentration of the toxicant. In fish, the gills are critical organs of respiration and respiratory distress has been observed to be one of the early symptoms of water poisoning in fish (Ortiz et al. 2003). The enzyme activities in the gills of *C. gariepinus* exposed to Corexit 9500 increased significantly when compared to the control (especially AST, ALT and ALP). This may be due to an effect on the micellar layer on fish gill surface responsible for the absorption of anthropogenic substances from the polluted environment through epithelial cells with the subsequent elevation of enzyme activities (Monferran et al. 2008). Enzymes are considered as sensitive biochemical toxicity indicators in the organs of fish. The enzymes AST and ALT are transaminases basically found in most organs of fish, catalyse transamination between an amino acid and α-keto acid and are important in protein synthesis (Fanta et al. 2003).

There was a reduction in creatinine levels in the gills and an increase in the kidney of fish exposed to Corexit 9500 when compared to the control. This shows variations in the effect of the toxicant on fish organs. In the liver, there was no significant difference between control and treatments. Creatinine kinase (CK) is found in several tissues, and it catalyzes the degradation of waste product creatinine. Could increase or decrease in creatinine levels in the gills and kidney respectively be as a

Table 3 Mean (± SE) values of kidney enzymes in *C. gariepinus* treated with Corexit 9500 dispersant

Treatments (ml/l)	SOD (mmol/l)	CAT (mmol/l)	ALT (IU/l)	AST (IU/l	ALP (IU/l)	Urea (IU/l)	Creatinine (IU/l)	ALT to AST (ratio)
Control	4.70 ± 0.98[a]*	10.79 ± 1.99[a]	4.00 ± 1.00[a]	4.50 ± 0.64[a]	7.00[b]	14.72 ± 1.10[a]	30.00[a]	0.89
0.0125	1.50 ± 0.07[c]	14.11 ± 2.13[a]	5.00[a]	6.00[a]	30.66 ± 1.24[a]	11.33 ± 2.63[a]	76.66 ± 2.91[b]	0.83
0.025	4.44 ± 0.98[a]	21.19 ± 2.14[a]	5.33 ± 1.01[a]	4.66 ± 0.17[a]	6.66 ± 1.23[b]	24.09 ± 3.62[a]	100.00[c]	1.14
0.05	2.91 ± 0.71[b]	14.33 ± 4.10[a]	4.00 ± 0.91[a]	3.50 ± 0.24[a]	31.50 ± 2.01[a]	12.55 ± 2.30[a]	200.00[d]	1.14

*Groups with the different superscript letters in the same column are significantly different from each other ($P < 0.05$)

Table 4 Mean (± SE) values of liver enzymes in *C. gariepinus* treated with Corexit 9500 dispersant

Treatments (ml/l)	SOD (mmol/l)	CAT (mmol/l)	ALT (IU/l)	AST (IU/l)	ALP (IU/l)	Urea (IU/l)	Creatinine (IU/l)
Control	1.76 ± 0.03[a*]	22.24 ± 2.18[a]	5.33 ± 0.45[b]	4.33 ± 0.14[a]	10.33 ± 0.91[c]	15.69 ± 0.93[a]	113.33 ± 2.13[a]
0.0125	1.67 ± 0.1[a]	10.96 ± 1.02[a]	8.33 ± 0.23[ab]	5.33 ± 0.91[a]	223.66 ± 1.72[a]	11.38 ± 1.11[c]	150.00 ± 3.14[a]
0.025	0.77 ± 0.0[a]	11.54 ± 1.93[a]	4.50 ± 0.41[b]	5.50 ± 0.72[a]	14.00 ± 1.87[c]	12.24 ± 1.09[bc]	55.00 ± 1.96[a]
0.05	1.67 ± 0.0[a]	11.74 ± 0.97[a]	16.00 ± 1.20[a]	12.33 ± 0.21[b]	76.33 ± 2.24[b]	13.09 ± 0.9[b]	130.00 ± 4.81[a]

*Groups with the different superscript letters in the same column are significantly different from each other (*P* < 0.05)

result of increased or decreased activity of CK brought about by differing effects of the toxicant? This however was not measured, as elevated CK will result in decreased creatinine levels (Sparling 2016) which might indicate muscle damage. Creatinine leaves the muscle and enters the blood where it becomes a waste item to a greater extent from the muscle breakdown. It is excreted by filtration through the glomeruli of the kidney and discharged as urine (Adamu and Kori-Siakpere 2011). Consequently, it is regularly utilised in the analysis of renal function test particularly the glomeruli filtration rate (Murray et al. 2003). Akani and Gabriel (2016) reported a significant decrease in creatinine level of *C. gariepinus* exposed to concentrations of oilfield wastewater.

Urea is a waste product metabolised in the liver and an end product of protein catabolism excreted by the kidney. The level of urea in the fishes exposed to concentrations of Corexit 9500 was significantly different from the control in the gills and liver but not in the kidney. This means Corexit may affect the metabolism of urea in the liver but might not affect its elimination in the kidney. Mahmoud et al. (2012) recorded a significant increase in urea and creatinine when they exposed *C. gariepinus* to mercury chloride. The low value of urea in the liver in this study may be attributed to its inability to metabolise protein as reported by Kori-Siakpere (2000). The main function of the liver is the detoxification of chemical substances that come in contact with organisms by metabolising xenobiotics to non-harmful substances. Consequently, in the event of contact with xenobiotics or toxicants, the chemistry of the liver may be distorted to counteract the adverse outcome of pollutants (Abdullaev et al. 1985; Obomanu et al. 2009).

Enzymes and electrolytes are components of liver function test, which are dependable indicators of liver metabolism and wellness of the organism under test (Sreekala and Zutshi 2010; Iweala and Okeke 2005; Uboh et al. 2011). The observed increase in liver enzymes was an indication of underlying liver injury in the fish. Elevation of tissue enzymes AST, ALT and ALP indicates liver damage which may be hepatitis or necrosis of cells (Gabriel and George 2005; Yousafzai and Shakoori 2011). Toxicants have been observed to amplify the cellular membrane permeability, thereby enhancing enzyme leaching or leaking out of the liver to the blood, or they may have decreased the permeability, forcing the enzymes to accumulate in the cells (Gabriel et al. 2009). Increase in AST and ALT suggests active transamination, so as to maintain energy cycle (Adams et al. 1996). ALP in the cellular external membrane plays a significant role in phosphate metabolism, and it prevents the external membrane from being damaged (Durrieu and Tran-Minh 2002). The increase in this enzyme implies overproduction of phosphate bound esters, which helps in the preservation of cell integrity (Gabriel et al. 2009; Gabriel and George 2005).

Superoxide dismutase (SOD) is a collection of metalloenzymes that play a vital antioxidant role, and comprises the main protection against the toxic effects of superoxide radicals in aerobic organisms. SOD catalyses the conversion of superoxide radicals to hydrogen peroxide (H_2O_2) and oxygen, and it is the first enzyme of response in oxi-radicals (Kappus 1985). In the liver, the mean concentration of SOD decreased significantly as the concentration of the toxicant increased (*P* < 0.05). The SOD was reduced in the gills, liver and kidney. This means that there were no oxi-radicals initiated by the Corexit 9500. This finding in this study disagrees with Mandeep and Rajinder (2017), who observed oxidative stress response in the liver, kidney and gills of *Ctenopharyngodon idellus* exposed to chlorpyrifos

Table 5 Mean (± SE) concentration values of electrolytes in the gills of *C. gariepinus* treated with Corexit 9500

Treatments (ml/l)	Na^{+} (mmol/l)	K^{+} (mmol/l)	Cl^{−} (mmol/l)	HCO$_3$[−] (mmol/l)
Control	138.33 ± 5.27[c*]	4.26 ± 0.65[c]	97.33 ± 3.76[c]	24.00 ± 2.03[a]
0.0125	151.33 ± 9.57[b]	5.90 ± 0.43[a]	103.00 ± 5.3[b]	20.66 ± 1.99[b]
0.025	154.00 ± 12.01[ab]	5.70 ± 1.12[ab]	104.33 ± 5.86[ab]	20.00b[c]
0.05	155.00 ± 3.45[a]	5.55 ± 0.97[b]	106.00 ± 3.67[a]	18.50 ± 2.86[c]

*Groups with the different superscript letters in the same column are significantly different from each other (*P* < 0.05)

Table 6 Mean (± SE) values of electrolytes in the kidney of *C. gariepinus* treated with Corexit 9500

Treatments (ml/l)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO^- (mmol/l)
Control	$137.50 \pm 11.0^{a*}$	3.55 ± 0.12^a	96.00^b	24.50 ± 2.31^a
0.0125	137.33 ± 4.91^a	4.16 ± 0.09^a	97.66 ± 7.23^{ab}	24.66 ± 3.33^a
0.025	138.33 ± 7.10^a	4.00^a	96.66 ± 5.21^b	22.66 ± 3.01^{ab}
0.05	136.50 ± 4.23^a	4.25 ± 0.47^a	99.50 ± 2.87^a	20.50 ± 6.02^b

*Groups with the different superscript letters in the same column are significantly different from each other ($P < 0.05$)

(a toxicant). The significant decrease of SOD in gills could be attributed to an inability to produce the enzyme in the presence of Corexit 9500, also indicating some form of stress.

Catalase (CAT) is an enzyme that is situated in the peroxisomes and assists in the removal of hydrogen peroxide, which is metabolised to oxygen and water (Van Der Oost et al. 2003). The concentration of catalase fluctuated across the various concentration of the treatment showing no significant difference between the control and the treatments ($P < 0.05$). The initial elevation in the enzyme level indicates an elevated antioxidant level in order to neutralise the impact of reactive oxygen species (ROS). Furthermore, the decrease in CAT activity might be as a result of influx of superoxide radicals. The current observations are in agreement with the findings of other workers—Ballesteros et al. (2009) (*Jenynsia multidentata* exposed to endosulphan) and Ferreira et al. (2010) (*Rhamdia quelen* exposed to agrochemicals).

Electrolytes

The examination of biochemical reactions of electrolytes in an organism exposed to a toxicant revealed that aquatic pollutants act by destroying the permeability of cell membranes replacing the structural or electrochemical important elements in the cell which cause functional failures (Yelwa and Solomon 2016; Adhikari et al. 2004). The dual function of electrolytes in animals lies in controlling fluid distribution and maintaining a normal muscular irritability (Abernathy et al. 2003). Na^+, Ca^{2+} and K^+ are the main cations of the extracellular fluid while carbonate HCO_3^- and chloride ions (Cl^-) are the chief anions of the intracellular fluid (Celik et al. 2005). In this study, the mean level of Na^+, Cl^- and K^+ increased significantly in the gills of *C. gariepinus* while HCO_3^- decreased

with increasing concentration of Corexit 9500 in the liver. The increase in the ionic concentrations and decrease in HCO_3^- of the treated samples could be as a result of the effects of Corexit 9500 on the electrolyte balance of *C. gariepinus*. In the kidney, there was a decrease in HCO_3^- concentration but Cl^- increased with increase in the concentration of Corexit 9500, while in the gills, Na^+ and K^+ were not different from the control. Circulating levels of Na^+ have been shown as a valuable measurement of stress, mainly due to the active movement of this ion across the gill structure, depending on its concentration in the external medium (Bentley 1971).

Conclusion and recommendations

In conclusion, Corexit 9500 elevated some enzymes (AST, ALT, ALP) in the test organism, inhibited the production of some enzymes (creatinine, CAT, SOD) and produced a significant change in the levels of urea in the liver. Some electrolyte levels (Na^+, K^+, Cl^-) were elevated, while there was inhibition in the levels of HCO_3^- in the test organism.

Corexit 9500 can be concluded to be highly toxic to *C. gariepinus* even at very low concentration (LC_{50} of 0.115 ml/l at 96 h) as its administration altered the level of liver and gill enzymes, and intracellular electrolytes, thereby affecting adversely the anti-oxidative defense system in various organs of the fish.

The physicochemical parameters measured could provide useful information for evaluating the toxicological effects of Corexit 9500 on the fish and help in the diagnosis of the pollution. Study on long-termeffects of dispersants on growth, reproduction and normal functions of the aquatic organism are recommended. Concentrations of dispersant used in oil spills should be appropriately assessed, and their use near fish breeding grounds should be avoided.

Table 7 Mean ± SE values of electrolytes in the liver of *C. gariepinus* treated with Corexit 9500 dispersant

Treatments (ml/l)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO^- (mmol/l)
Control	$136.66 \pm 2.90^{b*}$	3.83 ± 0.24^c	97.33 ± 2.17^c	23.00 ± 1.71^a
0.0125	142.00 ± 3.37^b	5.03 ± 0.82^b	101.66 ± 5.18^b	22.33 ± 3.01^a
0.025	152.50 ± 2.12^a	5.35 ± 0.12^{ab}	$104.00. \pm4.13^{ab}$	19.50 ± 1.71^b
0.05	152.66 ± 3.24^a	5.50 ± 0.67^a	105.00 ± 2.17^a	19.66 ± 1.11^b

*Groups with the different superscript letters in the same column are significantly different from each other ($P < 0.05$)

Abbreviations
ALP: Alkaline phosphatase; ALT: Alanine amino transferase; AST: Aspartate transaminase; Ca^+: Calcium ion; CAT: Catalase; Cl^-: Chloride ion; DO: Dissolved oxygen; GFR: Glomerular filtration rate; HCO_3^-: Bicarbonate ion; K^+: Potassium ion; Na^+: Sodium ion; ROS: Reactive oxygen species; SOD: Superoxide dismutase

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