**Joint Action Toxicity and Biochemical effects of Binary Mixtures of Forcados Light Crude Oil and Three Dispersants against *Clarias gariepinus***

Sogbanmu, T. O. and Otitoloju, A. A.

Ecotoxicology Unit, Department of Zoology, Faculty of Science, University of Lagos, Lagos, Nigeria

**ABSTRACT:** Laboratory-scale experiments were conducted to evaluate the joint action toxicity and biochemical effects of sublethal concentrations of Forcados light crude oil (FLCO) and three dispersants against *Clarias gariepinus* over a period of 28 days. The derived 96-hr LC₅₀ values revealed that the dispersant, DS/TT/066 (0.03mL/L) was the most toxic, followed by dispersant, OC/TT/OSI (0.19mL/L), FLCO (5.06mL/L) and crystal clear oil dispersant (CCOD = 12.06mL/L) the least toxic when acting singly. Joint action toxicity evaluations of FLCO and dispersants showed that the interaction between FLCO : DS/TT/066 and FLCO : OC/TT/OSI was synergistic (synergistic ratio (SR) > 1) with SR values of 10.5 and 3 respectively. However, for the mixture of FLCO : CCOD, the interaction was antagonistic (SR < 1) with SR value of 0.97. The result of the biochemical effects study revealed that malondialdehyde (MDA) levels decreased significantly (P<0.05) in the exposed fishes, reduced glutathione (GSH) and glutathione-s-transferase (GST) activities increased significantly (P<0.05) in fishes exposed to FLCO : CCOD mixture alone while there was no significant difference (p>0.05) in superoxide dismutase (SOD) and catalase (CAT) activities in all the exposed fishes compared to control animals. The observed increase in GSH and GST levels in conjunction with a decrease in MDA concentration in the liver of test animals exposed to binary mixtures of FLCO and CCOD reveals the ability of the animals to overcome the effects of lipid peroxidation in this group. Further studies on the mechanism of toxicity of these dispersants in field and laboratory assays are recommended.

**Key words:** *Clarias gariepinus*, Biochemical parameters, Forcados light crude oil, Dispersants, Joint action toxicity

**INTRODUCTION**

The potential impact of dispersed oil on aquatic ecosystems should be thoroughly considered prior to use of a dispersant. Dispersants had generated considerable interest because of their use as chemical clean up agents for crude oil spill in aquatic ecosystems in Nigeria and globally. Earlier studies have indicated that acute tests gave little or no relevant information on sublethal effects of dispersants or oil/dispersant mixtures (Baklien et al., 1986 and Oyewo, 1986). Dispersant toxicity can be affected by physiological and biochemical processes not normally tested for in acute tests on single life-stage (Singer et al., 1990; Volkman et al., 1994 and Odiete, 2003). It is generally considered that the toxicity of mixtures of oil and dispersant is of greater concern than the dispersant alone (Samuel et al., 2008). Aquatic organisms detoxify organic xenobiotics by phase I metabolism, however, during the detoxification process, highly reactive oxygen species (ROS) such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH⁻) are produced as by-products. Excessive ROS production in response to xenobiotic detoxification can overwhelm natural defense mechanisms leading to cumulative damage of biomolecules namely nucleic acid, proteins and lipids (Kelly et al., 1998). A radical attack on lipids leads to the formation of lipid peroxides, which can decompose to yield alkanes, ketones and aldehydes. The aldehydes most extensively studied are 4-hydroxy-2-nonenal, 4-hydroxy-2-hexenal and malondialdehyde (MDA) (Zielinski and Portner, 2000). The variety of lipid peroxidation (LPO) by-products can also exert adverse biological effects in exposed organisms (Catala, 2009). The quantification of the diverse products of peroxidation especially the level of MDA is now being exploited as biomarkers of oxidative stress.

*Corresponding author E-mail: topesho@gmail.com*
Sogbanmu, T. O. and Otitoloju, A. A.

Oxidative damage is counteracted by antioxidant defense systems and repair mechanisms. The antioxidant defense systems comprise of a number of enzymes which act as scavengers of the highly reactive intermediates produced in cells during hydrocarbon metabolism to maintain cell homeostasis. Notable antioxidant enzymes include (a) superoxide dismutase (SOD), which converts superoxides (O$_2^-$) generated in peroxisomes and mitochondria to hydrogen peroxide; (b) catalase (CAT) which removes the hydrogen peroxide by converting it to water and oxygen; (c) glutathione-s-transferase (GST); (d) glutathione peroxidase and (e) glutathione reductase all of which are involved in the removal of hydrogen peroxide from the system in conjunction with reduced glutathione (GSH). The antioxidant defense system is being increasingly studied because of its potential utility to provide biochemical biomarkers that could be used in environmental monitoring systems (Oruc et al., 2004). According to Wu et al. (2005), the use of biomarkers in environmental monitoring confers significant advantages over traditional chemical measurements because measured biological effects can be meaningfully linked to environmental consequences so that environmental concerns can be directly addressed. Although the activity of antioxidant enzymes may be increased or inhibited under chemical stress, there is, however, no general rule for the different enzymes (Cheung et al., 2001). The antioxidant enzymes tend to respond differently to various chemical compounds, therefore, the activity of an individual antioxidant enzyme cannot serve as a general marker of oxidative damage. Hence, multiple antioxidant values are often measured together to indicate the total oxyradical scavenging capacity and this has been observed to provide greater indicating value (Regoli et al., 2002).

This study was undertaken to assess the joint action toxicity and biochemical effects of forcados light crude oil and three dispersants against *Clarias gariepinus* in order to exploit their use as biological markers of environmental stress related to crude oil and dispersants.

**MATERIALS & METHODS**

Fingerlings (weight range: 6 – 10 g; length range: 4.8 – 6.0 cm) and Juveniles (weight range: 17 – 25 g and length range: 14.5 – 17.1 cm) of *C. gariepinus* (Chordata, Osteichthyes, Siluriformes, Clariidae) also known as the African catfish used in the bioassays were purchased from Apostolic Faith Church fish farm, Anthony Village, Lagos. The fishes were caught early in the morning and transported to the laboratory in oxygenated polythene bags containing bore-hole water. The fishes were kept in a plastic tank (28 x 51 x 29 cm) which was three-quarter filled with dechlorinated water obtained by aerating tap water in a plastic tank for at least 24 hours. The purpose was to allow the rapid evaporation of chlorine gas in the water. During acclimatization, they were fed with Coppens fish feed twice daily (morning and evening) and the water was changed every other day to prevent the accumulation of waste metabolites and food particles. They were maintained in the holding tank for a minimum of 3 days to enable acclimatization to laboratory conditions (temperature: 28 ± 2°C; relative humidity: 78 ± 4%) before commencement of the experiment. The experiments involving the utilization of whole and live fishes were approved and complied with University of Lagos research ethics/regulations.

Forcados Light Crude Oil (FLCO): used for this experiment was obtained from Shell (SPDC) production platform in Forcados, Burutu Local Government Area of Delta State, Nigeria. The physico-chemical properties of the crude oil include: sulphur content = 0.2%, API Gravity = 60/60F, rapid vapour pressure = 2.5psi and pour point = 25. It was stored in a sealed plastic vessel in the laboratory at room temperature and used within a period of 30 days.

Dispersant I (DS/TT/066): Brown-coloured liquid containing surfactant mixed with a hydrocarbon solvent. Manufactured by Oil Pollution Environmental Control Limited, West Yorkshire, Great Britain.

Dispersant II (Crystal Clear Oil Dispersant (CCOD)): White milky liquid, non-ionic emulsifier, viscosity at 25°C = 2000 to 4000cP (Brookefield DV-II + Pro viscometer, spindle LV-4, Speed 10 RPM), pH at 1% solution = 6.6 to 8.4. Manufactured by Cork ‘N’ Seal Limited, Lagos, Nigeria.

Dispersant III (OC/TT/OSI): Blue-coloured liquid, moderate alkaline (pH = 8.8), density = 0.9877gc$m^{-3}$. Manufactured by Synergy Trend International Limited, Dopema, Lagos, Nigeria.

Physico-chemical characteristics were measured at the beginning of the experiment and at the end (that is, before change of the test media). The parameters measured were: dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity (EC), salinity (using Hanna instruments) and pH (using Mettler Toledo pH Meter).

Four active catfishes of similar sizes in replicates were introduced randomly into the test media in bioassay tanks. A total of eight fingerlings were exposed per concentration including untreated control (dechlorinated tap water). Mortality assessment was carried out once every 24h for 4 days. Test animals were exposed for 96 hours to graded concentrations of FLCO and dispersants as follows:
A series of bioassays similar to those described for single action tests were carried out but in this instance, animals of similar sizes were exposed to binary mixtures of FLCO and dispersants at ratio 9:1 (v/v) which is the normal application rate for dispersants as specified by the Department of Petroleum Resources in Nigeria. At predetermined concentrations, the proportion of each constituent compound dictated by the ratio was computed and measured. Mortality assessment was undertaken once every 24 h for 4 days. The fingerlings were exposed for 96 hours to varying concentrations of the mixtures as follows:

**FLCO** – 2.5, 5, 10, 15, 20mL/L and untreated control
**DS/TT/O66** – 0.03, 0.06, 0.08, 0.10, 0.20mL/L and untreated control
**CCOD** – 5, 10, 15, 25mL/L and untreated control
**OC/TT/O6I** – 0.12, 0.14, 0.18, 0.20, 0.30mL/L and untreated control

A series of bioassays similar to those described for single action tests were carried out but in this instance, animals of similar sizes were exposed to binary mixtures of FLCO and dispersants at ratio 9:1 (v/v) which is the normal application rate for dispersants as specified by the Department of Petroleum Resources in Nigeria. At predetermined concentrations, the proportion of each constituent compound dictated by the ratio was computed and measured. Mortality assessment was undertaken once every 24 h for 4 days. The fingerlings were exposed for 96 hours to varying concentrations of the mixtures as follows:

**FLCO** – 2.5, 5, 10, 15, 20mL/L and untreated control
**FLCO** – CCOD – 2.5, 5, 10, 15, 20, 25mL/L and untreated control
**FLCO** – OC/TT/O6I – 0.6, 0.9, 1.2, 1.5, 1.8, 2.1mL/L and untreated control

In this series of experiment, the test animals were exposed to sublethal concentrations ((1/10th of 96hrLC50 and 96hrLC50) derived from results of single and joint action toxicity studies) of the test compounds and untreated control in replicates. A semi-static bioassay test protocol was adopted, in which the test media were changed once every 4 days to fresh media of the same concentration and untreated control. At predetermined post commencement periods (14 and 28 days post treatment), fish samples were removed and sacrificed to obtain liver tissues required for biochemical assays. The liver was removed and washed free of blood in ice cold isolation medium (0.25M sucrose, 5 mM tris HCL), lightly blotted and weighed. It was then cut into fragments and homogenized (9% w/v) in 100% methanol and centrifuged at 10,000 × g for 15 min at 4°C as described by Hermes-Lima et al. (1995). The supernatant was collected for substrate and enzyme assays.

The levels of homogenized tissue MDA, as an index of lipid peroxidation were determined by thiobarbituric acid reaction (TBARS Assay) using the method of Yagi (1998). In this method, malondialdehyde is measured spectrophotometrically at absorbance levels of 535 nm to assay for the extent of lipid peroxidation in a sample. The GSH content of liver tissue as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay (1968). The absorbance was read at 412nm. The activity of GST was determined according to the method of Habig and Jakoby (1974). GST activity was measured by monitoring at absorbance level of 340 nm, the formation of a conjugate between 1 Mm GSH and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB). The results were expressed in GST unit/mg protein or U/mg, where one unit is defined as the amount of enzyme that conjugates 1 µmol of CDNB per minute and per milligram of proteins at 25°C and pH7.4.

SOD enzyme activity was determined according to the method by Sun and Zigman (1978). The SOD enzyme assay determined the difference between superoxide anion decomposition and production i.e. its ability to inhibit the autoxidation of epinephrine. Enzyme activity was monitored at absorbance level of 450 nm. Concentrations are expressed as SOD Unit/mg protein or U/mg, where one unit is defined as the amount of enzyme needed to inhibit 50% epinephrine reduction per minute and per milligram of protein at 25°C and pH7.8. Serum catalase activity was determined according to the method of Beers and Sizer as described by Usoh et al. (2005) by measuring the decrease in absorbance at 240nm due to the decomposition of Hydrogen peroxide (H2O2) in a UV recording spectrophotometer. The results were expressed in CAT units/mg protein or U/mg, where one unit is the amount of enzyme that hydrolyzes 1 µmol of H2O2 per minute and per milligram of protein at 30°C and pH8.0.

Toxicological data involving quantal response (mortality) for both single and joint action studies were analyzed by probit analysis including equation for probit lines (Finney, 1971). This was executed using Statistical Package for the Social Sciences 16.0 for Windows (SPSS 16.0). The indices of toxicity measurement derived from these analyses were LC50 (lethal concentration that causes 50% response (mortality) of exposed organisms), T.F. and their 95% confidence limits employed as follows:

For the joint action toxicity of heavy metal mixtures, the two models employed for the classifications are the concentration-addition model by Anderson and Weber (1975) with slight modification [relative toxic units (RTU) estimations; Otitoloju (2001)] and synergistic ratios (SR) model after Hewlett and Plackett (1969).

**Model 1:** The concentration-addition model is as follows:

(i) *Additive* if the observed LC50 value of the mixture is equal to the predicted LC50 value i.e. RTU = 1,
(ii) *Synergistic* if the observed value of the mixture is less than the predicted LC50 value i.e. RTU > 1,
(iii) *Antagonistic* if the observed LC50 value of the mixture is greater than the predicted LC50 value i.e. RTU < 1

The relationship of derived LC50 values to predicted LC50 (RTU) is estimated as:
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RTU = \frac{\text{Predicted LC}_{50} \text{ value}}{\text{Observed LC}_{50} \text{ Value}}

Model 2: The synergistic ratio (SR) model is as follows:
\[ \text{SR} = \frac{\text{LC}_{50} \text{ of chemical acting alone}}{\text{LC}_{50} \text{ of chemical + additive (mixture)}} \]

Where: SR = 1 joint action is described as Additive
SR = 1 joint action is described as Antagonistic
SR = 1 joint action is described as Synergistic

The results of the physico-chemical parameters of the test media showed that the dissolved oxygen level ranged from 2.9mg/l (after 4 days of exposure) to 5.5mg/l (after each change to a clean media). The pH and salinity of the test media had values ranging from 8.4 and 0.2ppt to 6.6 and 0.07ppt respectively. The conductivity and total dissolved solids in the test media increased from 139µs/cm and 65.7ppm to 737µs/cm and 374ppm respectively over the period of the observation.

On the basis of the derived 96hrLC_{50} values (Table 1), the dispersant, DS/TT/066 (0.03mL/L) was the most toxic followed by OC/TT/OSI (0.19mL/L), FLCO (5.06mL/L) and CCOD (12.06mL/L). The computed toxicity factor revealed that DS/TT/066 and OC/TT/OSI were approximately 169x and 27x more toxic than FLCO respectively. This is consistent with the report of Otitoloju and Popoola (2009) and Chukwu and Lawal (2010) who reported that some dispersants used in Nigeria were more toxic than forcdas light crude oil and spent lubricant oil respectively. On the other hand, CCOD was approximately 0.4x less toxic than FLCO. This corroborates the report of Otitoloju (2004) which stated that Forcados light crude oil was more toxic than the dispersant, Biosolve when acting alone against Macrobrachium vollenhovenii. The differential toxicity observed among FLCO and the dispersants can be attributed to the fact that the physico-chemical characteristics of the test compounds are different. These characteristics dictate the penetrability of the dispersants into living organisms, site of action of metabolism and hence the toxic actions they exert on exposed organisms.

The analysis of dose-response data for the mixtures of FLCO and dispersants (DS/TT/066, CCOD and OC/TT/OSI) (Table 2) showed that the 96hrLC_{50} values of the mixtures were 0.48, 5.20, and 1.68 respectively. The interactions between the mixtures of FLCO : DS/TT/066 and FLCO : OC/TT/OSI conformed with the model of synergism (SR>1 & RTU>1) with SR values of 10.5 and 3 & RTU values of 9.5 and 2.7 respectively. Consequently, the mixtures were more toxic than FLCO when acting singly against the test animal. This agrees with the report of Otitoloju (2001) & Otitoloju and Popoola (2009). The surface active agents contained in dispersants make membranes more permeable and increase the penetration of toxic compounds into animals. In this way, mixtures of oils and dispersants are often more toxic than either applied individually.

### Table 1. Single action toxicity of FLCO and dispersants against C. gariepinus fingerlings based on 96 hours mortality data

<table>
<thead>
<tr>
<th>Treatment (mL/L)</th>
<th>LC_{50} (95% C.L.)</th>
<th>Slope ± S. E.</th>
<th>Probit Line Equation</th>
<th>D.F.</th>
<th>T.F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLCO 5.06 (30.23 – 0.60)</td>
<td>1.12 ± 1.09</td>
<td>Y = 4.21 + 1.12X</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DS/TT/066 0.03 (0.05 – 0.01)</td>
<td>3.99 ± 1.60</td>
<td>Y = -1.04 + 3.99X</td>
<td>1</td>
<td>168.7</td>
<td></td>
</tr>
<tr>
<td>CCOD 12.06 (19.39 – 7.77)</td>
<td>3.40 ± 1.22</td>
<td>Y = 1.32 + 3.40X</td>
<td>2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>OC/TT/OSI 0.19 (0.20 – 0.19)</td>
<td>48.80 ± 17.43</td>
<td>Y = 40.07 + 48.80X</td>
<td>2</td>
<td>26.6</td>
<td></td>
</tr>
</tbody>
</table>


### Table 2. Analysis (based on concentration-addition and synergistic ratio models) of 96 h LC_{50} values of FLCO alone and binary mixtures of FLCO and Dispersants (ratio 9:1) against C. gariepinus fingerlings

<table>
<thead>
<tr>
<th>Treatment (mL/L)</th>
<th>Observed 96hrLC_{50} (95% C.L.)</th>
<th>Predicted 96hrLC_{50} (95% C.L.)</th>
<th>Probit Line Equation</th>
<th>RTU</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLCO : DS/TT/O66 0.48 (0.60 – 0.40)</td>
<td>4.56 (27.22 – 0.54)</td>
<td>Y = 7.06 + 6.39X</td>
<td>9.5</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>FLCO : CCOD 5.20 (9.63 – 3.03)</td>
<td>5.76 (29.15 – 1.32)</td>
<td>Y = 2.68 + 3.25X</td>
<td>1.1</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>FLCO : OC/TT/OSI 1.68 (9.75 – 1.04)</td>
<td>4.57 (27.23 – 0.56)</td>
<td>Y = 3.81 + 5.28X</td>
<td>2.7</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>FLCO alone 5.06 (30.23 – 0.60)</td>
<td>-</td>
<td>Y = 4.21 + 1.12X</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

KEY: SR: Synergistic Ratio = 96 h LC_{50} of FLCO acting singly / 96 h LC_{50} of the binary mixture, RTU: Relative Toxic Units = Predicted 96hrLC_{50} of binary mixture / Observed 96hrLC_{50} of binary mixture
separately. The implication of this is that in terms of toxicity, the dispersants mentioned above should be carefully considered with other options for oil spill control since they enhance the toxicity of crude oil thus causing more damage in the environment than crude oil itself. On the other hand, the interaction between FLCO and CCOD was found to be antagonistic (SR<1) with SR value of 0.97. Consequently, the mixture was less toxic than FLCO when acting singly against the test organism. This corroborates the report of Chukwu and Lawal (2010) who reported an antagonistic interaction (SR<1) between binary mixture of spent lubricant oil and dispersant, OSD 9460 (ratio 9:1) tested against *M. vollenhoveii*. This implies that this dispersant can be recommended for use as it reduces the toxicity of the crude oil when applied for oil spill control.

The results revealed that the MDA levels in the liver of exposed fishes decreased significantly (P<0.05) compared to control animals after 28 days of exposure (Fig. 1). The level of peroxidation was lowest in animals exposed to FLCO : DS/TT/066 followed by FLCO alone, FLCO : OC/TT/Osi and FLCO : CCOD (least). This is consistent with the findings of Salihu and Bawa-Allah (2012) who reported a reduction in MDA levels in fishes exposed to sublethal concentrations of lead salts (Pb(NO₃)₂). Conversely, this result contradicts the findings of Otitoju and Olagoke (2011), Achuba and Osakwe (2003) and Avci et al. (2005) who reported an increase in MDA levels in tissues of fishes exposed to petroleum hydrocarbons. The significant reduction in the concentration of MDA in the treated groups could be attributed to the action of the antioxidant enzymes in preventing cellular injury by ROS to the animals. The results showed that GSH activity was significantly enhanced (P<0.05) in the liver of fishes exposed to FLCO : CCOD mixture alone compared to control animals over the period of 28 days (Fig. 2). This result contradicts the report of Salihu and Bawa-Allah (2012) who reported a reduction in GSH values of fishes exposed to Lead salts. This suggests an adaptive and protective role of the biomolecule (GSH) against oxidative stress caused by the presence of the constituent compounds. The results showed that the GST activity was significantly enhanced (p<0.05) in the liver of fish exposed to FLCO : CCOD alone compared to control animals (Fig. 3). Similar observation of a statistically significant enhancement in GST in animals exposed to oxidative stress of 2,4- dichlorophenol has been reported by Zhang et al. (2005). GST is a cytosolic or microsomal enzyme that catalyses the conjugation of reduced glutathione (GSH) with oxidative products, such as 4-hydroxyalkenals (membrane peroxides) and/or base propenals, resulting from DNA oxidative degradation (Leaver and George, 1998). Therefore, it also plays an important role in protecting tissues from oxidative stress (Fournier et al., 1992 and Jifa et al., 2006). The results showed that there was no significant difference (p>0.05) in SOD activity between exposed and control animals over the period of 28 days (Fig. 4). SOD is known to provide cytoprotection against free radical induced damage by converting superoxide radicals (O₂⁻) generated in peroxisomes and mitochondria to hydrogen peroxides. The hydrogen peroxide is then removed from the system by the enzyme CAT, which converts it to water and molecular oxygen (O₂). Generally, the inducible enzymes initially increase on exposure to pollutants, then synthesis declines with deteriorating animal condition caused by chronic pollutant-mediated stress (Suteau et al., 1988 and Winston & Di Giulio, 1991). The results showed that there was no significant difference (p>0.05) in CAT activity between exposed and control animals (Fig. 5).

![Fig. 1. MDA levels in liver of *C. gariepinus* exposed to FLCO alone and FLCO : dispersant mixtures (ratio 9:1) over a period of 28 days. Values represent mean ± standard error (n=8 per treatment). Dissimilar letters (a, b, c) are significantly different (P<0.05) from each other.](image-url)
Fig. 2. GSH activity in the liver of *C. gariepinus* exposed to FLCO alone and FLCO : dispersant mixtures (ratio 9:1) over a period of 28 days. Values represent mean ± standard error (n=8 per treatment). Dissimilar letters (a, b, c) are significantly different (P<0.05) from each other.

Fig. 3. GST activity in the liver of *C. gariepinus* exposed to FLCO alone and FLCO : dispersant mixtures (ratio 9:1) over a period of 28 days. Values represent mean ± standard error (n=8 per treatment). Dissimilar letters (a, b, c) are significantly different (P<0.05) from each other.

Fig. 4. SOD activity in the liver of *C. gariepinus* exposed to FLCO alone and FLCO : dispersant mixtures (ratio 9:1) over a period of 28 days. Values represent mean ± standard error (n=8 per treatment). Dissimilar letters (a, b, c) are significantly different (P<0.05) from each other.
CONCLUSION

The use of biochemical responses as biomarkers during environmental monitoring programmes is derived from the basis that a toxic effect manifests itself at the subcellular level before it becomes apparent at higher levels of biological organization. Recent investigations of changes in antioxidant defenses showed that they can be used as biomarkers of oxidative stress by various pollutants in aquatic organisms (Manduzio et al. 2003; Gorinstein et al. 2003; Brown et al. 2004). The results from this study demonstrate the imbalance in hepatic antioxidant homeostasis of fish exposed to binary mixtures oil and dispersants which can serve as a useful tool for environmental monitoring programmes. However, there is need for further studies on the mechanism of toxicity of these dispersants in field and laboratory studies in order to extensively exploit the biomarkers of oxidative stress as cursors of environmental pollution in aquatic ecosystems.

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