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Synergistic Evaluation of Oil-Dispersant and Oil-Detergent Mixtures Using African Catfish, *Clarias gariepinus* Fry

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Abstract: The single action toxicities of Spent Lubrication Oil (SLO), dispersant (SAF Power), detergent (Sodium Dodecyl Sulphate, SDS) and the joint action toxicities with the synergistic interactions of their mixtures (SLO-dispersant, 9:1 and SLO-detergent, 9:1) were evaluated against *Clarias gariepinus* fry in laboratory bioassays. The synergistic interaction of the tested mixtures was evaluated using the concentration-addition (relative toxic units, RTU), synergistic ratio, SR and isobolograms (pictorial isobole) models. The dispersant (96 h LC_{50} = 0.008 ml L^{-1}) and the detergent (96 h LC_{50} = 1.00 ml L^{-1}) was about 150 times and 1.2 times more toxic than SLO (96 h LC_{50} = 1.20 ml L^{-1}), respectively when acting singly against *C. gariepinus.* Based on the joint action toxicity, the SLO-dispersant mixture (96 h LC_{50} = 0.008 ml L^{-1}) was 1318 times more toxic than SLO-detergent mixture (96 h LC_{50} = 10.54 ml L^{-1}). Synergistic evaluation of the test mixtures based on RTU and SR models (with reference to SLO) revealed that the SLO dispersant mixture and SLO-detergent mixture conformed to the model of synergism (RTU = 135.10, SR = 150.00) and model of antagonism (RTU = 0.11, SR = 0.11), respectively. The resultant pictorial isobole using isobologram model was in agreement with RTU and SR models. The environmental implication of the synergistic interactions of the tested mixtures was discussed.

Key words: Joint toxicity, synergism, spent oil, fish

INTRODUCTION

Most human activities (industrial and domestic) result in the introduction of wastes directly or indirectly into the aquatic environment. In many developing countries including Nigeria, the activities of informal mechanical workshops usually result in uncontrolled discharge of spent lubrication oil into gutter and open drains and this eventually end up in the aquatic bodies. Globally, Bartz (1998) estimated that about 12 million tons of lubrication oil is likely to be discharged into the environment annually. Spilled oil on the surface of water bodies limits gaseous exchange, entangles and kills surface organisms and coats the gills of fish. It also depresses phytoplankton photosynthesis, respiration and growth, kills or causes developmental abnormalities in zooplankton and young stages of many aquatic organisms and causes tainting of fish and shellfish (Otitoloju, 2005).

The indiscriminate discharge of spent lubrication oil into the environment and its negative effects on the environment demands the development of various control strategies. Mechanical workshops now use dispersant, emulsifier, degreasers or detergents in washing off spent lubrication oil, thus

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ensuring easy dispersal. Basically, when two or more chemicals are exposed simultaneously against a living system, the interactions between the constituents may result in an enhancement (synergism), non-interaction (additive action) or reduction (antagonism) of the toxic effects of the chemicals (Cleuvers, 2003; Otitoloju, 2003, 2005). It is important to note that most of the dispersant that have been initially introduced into the market were found either to be very toxic on their own against organisms inhabiting the receiving ecosystem or to in some cases, to enhance the toxicity of the spilled oil to the exposed organism when deployed or utilized to control oil spills (Singer *et al*., 1998; Adams *et al*., 1999). Dispersants are imported into Nigeria from Europe, North America, Asia and other temperate countries. Aquatic species are not equally susceptible to toxic substances and much information on the toxicities of imported dispersant was based on species not native to Nigeria and in environmental conditions different from those of Nigeria. The Department of Petroleum Resources (DPR), the apex body in Nigeria regulating activities of the petroleum industries has since 1980, demanded 96 h LC_{50} toxicity tests of drilling mud systems, base oil, oil-based muds, chemical dispersant and degreaser on local species under Nigerian conditions to determine the safety of their use before these chemicals are approved to be used in the petroleum industry in Nigeria (Odiete, 2003). The DPR also demands the evaluation of synergistic interaction existing between oil-dispersant (9:1) mixtures in comparison with synergistic interaction of oil-sodium dodecyl sulphate (SDS) (9:1) mixtures before approval is given for the use of dispersant in Nigerian environment. Elsewhere (Edwards *et al*., 2003), SDS has been used as a reference chemical for evaluating the sensitivity of organisms to toxicants.

Sodium Dodecyl Sulphate (SDS) is a widely employed detergent, with applications in household products, industrial mixtures and cleansing products in cosmetics. SDS is considered one of the world's most commonly used surfactants, it is found in liquids soaps and shampoos, bubble baths, bath and shower gels and toothpastes (Sirisattha *et al*., 2004).

This study focus on acute toxicity evaluation of dispersant (SAF Power), SDS and spent lubrication oil when acting singly against *Clarias gariepinus* fry and on synergistic interactions existing in oil-SAF Power (9:1) and oil-SDS (9:1) mixtures against *C. gariepinus* fry. This will enable the determination of how toxic or non-toxic SAF Power is compared to SDS and whether either SAF power or SDS will or will not potentiate the toxicity of the spent lubrication oil to *C. gariepinus* fry under Nigerian conditions. SAF power is a dispersant under consideration for approval in controlling oil spill in Nigeria. The effects of the accidental and deliberate discharge of spent lubrication oil, detergent and dispersant into surface water channels are good reasons for the initiation of stringent measures to control these practices.

According to Odiete (2003), test organism to be used for toxicity test must be ecologically and economically important, occupy trophic position leading to humans or other important species, have adequate background biology, be widely distributed, be genetically stable, have its early stages (larvae, frys, juveniles) available throughout the year and be sensitive. The African catfish, *Clarias gariepinus* is an ecologically important and commercially valued fish in Nigeria. These mudfish are frequently and widely cultured in ponds and they also occur freely in Nigerian natural fresh waters. *C. gariepinus* fry has been chosen because it is more sensitive being early life stage when compared to the adult (Odiete, 2003).

MATERIALS AND METHODS

Test Animal

The African catfish, *Clarias gariepinus* fry (Burchell, 1822) (2 weeks old and total length of 18-22 mm) were utilized for the bioassays in this study. The animals were purchased (August, 2006) from Agboola fish farm, Ipaja-Ayobo, Lagos, Nigeria and transported in aerated polythene bags to the laboratory.

The catfish, *C. gariepinus* were held in 3 rectangular glass tanks (75×37×37 cm) (300 in a tank) for a minimum of 6 days to allow them acclimatize to laboratory conditions (Temp. 30±2°C; Salinity 0‰; RH 79±2; pH 7.2±0.2). The tanks were filled to quarter capacities with dechlorinated tap water. During the period of acclimatization, the animals were fed on Coppens feed (0.5-0.8 mm) (50 g per 300 animals) twice daily. The dechlorinated tap water in the holding tanks was changed every other day to prevent accumulation of waste metabolites and decaying food materials.

Test Chemicals

Spent Lubrication Oil

Two litres of Spent Lubrication Oil (SLO) was collected from a mechanical workshop at University of Lagos, Akoka, Lagos, Nigeria.

Detergent

Sodium dodecyl (lauryl) sulphate (SDS) (CAS No. 151- 21-3, purity >90%) was purchased from MON Scientific Nigeria Ltd., Sabo-Yaba, Lagos, Nigeria. A stock solution of 10 g L^{-1} (10 g of SDS in a litre of distilled water) was used.

Dispersant

SAF power (a viscous liquid, green in colour) dispersant was collected from Department of Petroleum Resources (DPR), Lagos, Nigeria.

Single Action Bioassays

A static bioassay procedure (no renewal of test media) was adopted for all the toxicity tests. Depending on the test concentrations, a given volume of dechlorinated tap water was measured into bioassay glass tank (22×15×18 cm) and a predetermined volume of spent lubrication oil (SLO), SDS (detergent) and dispersant was added into the water to make it up to 1000 mL (total volume of test media), to achieve the desired test concentration.

Ten active animals were introduced into the test medium containing either (oil) SLO or detergent or dispersant as the case may be. Each treatment was replicated thrice, given a total of 30 fishes per treatment, including untreated media (control). The concentrations of test chemical tested were as follows:

SLO: 0.1, 0.5, 1.0, 2.0, 5.0, 10.0 and 0 ml L^{-1} (control)
Detergent: 0.4, 0.8, 1.0, 1.4, 2.0 and 0 ml L^{-1} (control)
Dispersant: 0.006, 0.008, 0.010, 0.012, 0.016 and 0 ml L^{-1} (control)

Joint Action Bioassays

A similar experiment to the one described above was also carried out, but in this case, test media containing mixtures involving binary constituents (oil: detergent and oil: dispersant) in pre-determined ratios of 9:1 (prescribed by DPR, Nigeria) were prepared (as described previously). The test animals were exposed to the following concentrations of the test mixtures:

Oil: detergent (9:1): 4.0, 8.0, 10.0, 14.0, 20.0 and 0 ml L^{-1} (control)
Oil: dispersant (9:1): 0.006, 0.008, 0.010, 0.012, 0.016 and 0 ml L^{-1} (control)

Assessment of Quantal Response (Mortality)

Mortality assessment was carried out every 24 h over a 96 h experimental period. Fish was assumed to be dead when there was no body or operculum movement, even when prodded with a glass rod.

Physico-Chemical Parameters of the Test Media

Physico-chemical parameters such as dissolved oxygen, pH and temperature of the test media were measured before and during the experimental period for the various bioassays. The pH and temperature was measured using Hanna instrument (HI 991301). The Dissolved Oxygen (DO) was determined using Jenway DO meter (Model, 9071).

Statistical Analysis

Concentration-Response Data Analysis

Toxicological data involving quantal response (mortality) for both single and joint-action bioassays were analysed by probit analysis after Finney (1971) using SPSS 10.0 for windows. The indices of toxicity measurement derived from the analysis were:

- LC_{50} = The concentration that kills 50% of the exposed population
- TF = Toxicity factor for relative potency measurements

Synergistic Evaluation of the Test Chemical Mixtures

For the joint action toxicity of oil-detergent and oil-dispersant mixtures, the 3 models employed for the synergistic classifications are as follows:

Model A

Concentration-addition model (Anderson and Weber, 1975). This model assumes that when similarly acting toxicants are mixed in any proportion, they will add together to give the observed response. In evaluating the joint-action, a predicted response value(s) (e.g., LC_{50}) is derived by summing up the LC_{50} values of the separate toxicants according to the proportion of their contribution in the mixture. The predicted LC_{50} value is then compared to the observed LC_{50} value of the mixture to classify the type of interaction among the components of the mixture as follows:

- Additive if the observed LC_{50} value of the mixture is equal to the predicted LC_{50} value
- Synergistic if the observed LC_{50} value of the mixture is less than the predicted LC_{50} value
- Antagonistic if the observed LC_{50} value of the mixture is greater than the predicted LC_{50} value

The relationship (RTU, relative toxic units) of derived LC_{50} values to predicted LC_{50} is estimated as:

$$\text{RTU} = \frac{\text{Predicted } LC_{50} \text{ value}}{\text{Experimentally derived } LC_{50}}$$

where, RTU = 1 describes additive action; RTU < 1 describes antagonism and RTU > 1 describes synergism.

Model B

Synergistic Ratio (SR) model (Hewlett and Plackett, 1959). SR is defined as:

$$\frac{LC_{50} \text{ of a chemical acting alone}}{LC_{50} \text{ of chemical + additive (mixture)}}$$

where, SR = 1 describes additive action; SR < 1 describes antagonism and SR > 1 describes synergism

Model C

Isobolograms (Ariens *et al*., 1976). The joint-actions between the test chemicals in binary mixtures are presented in form of isobolograms in Fig. 1. Each isobole (I-IV) represents the amount of

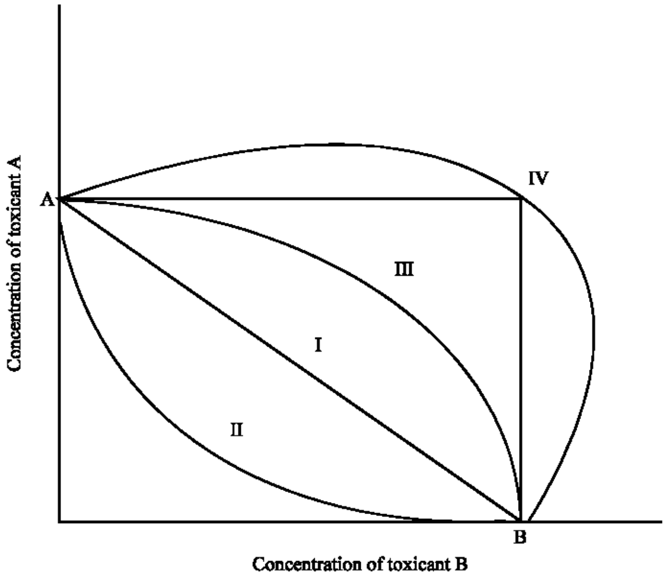

Fig. 1: Isoboles depicting the types of interactions between two chemicals A and B (Ariens *et al.*, 1976). Isobole 1 depicts additive action; Isobole 2 depicts synergism; Isobole 3 depicts subadditive action; Isobole 4 depicts antagonism

the toxicants in the formulations. In the theoretical isobole (Fig. 1) points A and B represent the amounts of toxicant A and B which individually (singly) produced the biological response (LC_{50} or median response levels in this research) which when connected gives the additive line. Isoboles (1-4) where the two constituents of the test mixture are separately active are described in the legend to Fig. 1.

In this research binary mixtures of oil-detergent and oil-dispersant (at 9:1 combination) were separately tested against *C. gariepinus* fry, as were the individual constituents on their own. The data based on the derived 96 h LC_{50} values were fitted into isobolograms and compared to the theoretical model (Fig. 1) in order to extrapolate the type of interaction depicted by the binary mixtures of the test chemicals after Ariens *et al.* (1976).

RESULTS

Physico-Chemical Characteristics of the Test Media during Toxicity Testing

The values obtained for the physico-chemical parameter of test media throughout the 96 h acute toxicity evaluations remained fairly constant. The measured dissolved oxygen, pH and temperature values were 6.2±0.2 mg L^{-1}, 7.0±0.2 and 27±2°C, respectively.

Single Action Toxicity of SLO, Dispersant and Detergent Tested against *C. gariepinus* Fry

The analysis of concentration-mortality data of SLO when tested against *C. gariepinus* revealed that the derived toxicity indices (LC_{50}) ranged from 1.20 (96 h LC_{50}) to 439.41 ml L^{-1} (24 h LC_{50}), while for the dispersant and detergent, the LC_{50} ranged from 0.008 (96 h LC_{50}) to 0.017 ml L^{-1} (24 h LC_{50}) and 1.00 (96 h LC_{50}) to 2.11 ml L^{-1} (24 h LC_{50}) respectively (Table 1). Based on the computed toxicity factor, TF using 96 h LC_{50} ratios, the dispersant, SAF Power was found to be 150 times more toxic against *C. gariepinus* than the SLO (Table 1). However, the detergent, SDS was found to be slightly (1.2 times) more toxic than SLO against *C. gariepinus* (Table 1).

Table 1: Relative toxicity of SLO, detergent and dispersant on *Clarias gariepinus*

Exposure time (h)	LC_{50} (95% CL) (ml L^{-1})	Slope±SE	Probit line equation	TF
SLO				
24	439.41 (233.06- 636.37)	0.50±0.21	Y = 3.69 + 0.50x	
48	141.62 (98.26-225.16)	0.53±0.19	Y = 3.87 + 0.53x	
72	5.13 (3.36-9.53)	1.24±0.46	Y = 4.12 +1.24x	
96	1.20 (0.86-1.67)	1.55±0.20	Y = 4.87 + 1.55x	1.0
Dispersant (SAF Power)				
24	0.017 (0.013-0.042)	2.48±0.79	Y = 9.38 + 2.48x	
48	0.014 (0.011-0.026)	2.32±0.75	Y = 9.31 + 2.32x	
72	0.011 (0.009-0.014)	2.92±0.75	Y = 10.73 + 2.92x	
96	0.008 (0.007-0.009)	3.75±0.79	Y = 12.83 + 3.75x	150.0
Detergent (SDS)				
24	2.11 (1.59-4.12)	2.28±0.57	Y = 4.26 + 2.28x	
48	1.49 (1.22-2.03)	2.63±0.54	Y = 4.55 + 2.63x	
72	1.27 (1.06-1.61)	2.80±0.53	Y = 4.71 + 2.80x	
96	1.00 (0.85-1.18)	3.29±0.54	Y = 5.00 + 3.29x	1.2

CL = Confidence limit, SE = Standard error, TF = Toxicity factor = 96 h LC_{50} value of SLO/96 h LC_{50} value of SLO, dispersant and detergent

Table 2: Synergistic analysis of test compound binary mixtures (based on concentration-addition model) tested against *C. gariepinus*

Test compound mixtures	Experimentally observed 96 h LC_{50} (95% CL) (ml L^{-1})	Predicted 96 h LC_{50} (95% CL) (ml L^{-1})	RTU
SLO: Dispersant (9:1)	0.00796 (0.00669-0.00897)	1.0808 (0.7747-1.5039)	135.78
SLO: Detergent (9:1)	10.54 (8.85-12.67)	1.180 (0.859-1.621)	0.11

CL = 95% Confidence limit, Predicted 96 h LC_{50} = Sum total of the single action 96 h LC_{50} values of constituent toxicants according to proportion of contribution in the test mixture

$$\text{RTU (Relative toxicity unit)} = \frac{\text{Predicted 96 h } LC_{50}}{\text{Experimentally observed 96 h } LC_{50}}$$

RTU = 1 indicates addition action
RTU > 1 indicates synergism
RTU < 1 indicates antagonism

Joint Action Toxicity of SLO-Dispersant and SLO-Detergent Mixtures (9:1) against *C. gariepinus*

The analysis of concentration-mortality data for the mixture of SLO and dispersant at 9:1 revealed that the observed 96 h LC_{50} was 0.008 ml L^{-1} (Table 2). The 96 h LC_{50} observed for the mixture of SLO and detergent (SDS) based on 9:1 was 10.54 ml L^{-1} (Table 2).

Comparison of the 96 h LC_{50} of the SLO-dispersant and SLO-detergent mixture revealed that the SLO-dispersant mixture was 1318 times more toxic than the SLO-detergent mixture. Further comparison of the 96 h LC_{50} value of the mixtures to that of SLO, dispersant and detergent when acting alone showed that the SLO-dispersant (9:1) mixture (96 h LC_{50} = 0.008 ml L^{-1}) was more toxic than any of the SLO (1.20 ml L^{-1}) and detergent (1.00 ml L^{-1}) acting singly. No difference was observed between the 96 h LC_{50} of SLO-dispersant (9:1) mixture and 96 h LC_{50} of dispersant (0.008 ml L^{-1}) acting singly (Table 3). The SLO-detergent mixture (96 h LC_{50} = 10.54 ml L^{-1}) was less toxic than any of the SLO, dispersant and detergent acting alone (Table 3).

Synergistic Evaluation of SLO-Dispersant and SLO-Detergent Mixtures (9:1) against *C. gariepinus*

The synergistic evaluation of the SLO-dispersant and SLO-detergent mixtures (9:1) against *C. gariepinus* based on concentration-addition model showed that the interaction between the SLO and dispersant based on ratio 9:1 mixture was in conformity with the model of synergism (RTU = 135.10)

Table 3: Analysis (based on synergistic ratio model) of the 96 h LC_{50} values of test compounds when acting jointly or singly against *C. gariepinus*

Test mixtures	LC_{50} values (95% CL) (ml L^{-1})	SR^1	SR^2
SLO: Dispersant (9:1)	0.00796 (0.00669-0.00897)	150.75	1.005
SLO: Detergent (9:1)	10.54 (8.85-12.67)	0.11	0.095
SLO alone	1.20 (0.86-1.67)		
SAF power alone	0.008 (0.007-0.009)		
SDS alone	1.00 (0.85-1.18)		

CL = 95% Confidence limit,

$$SR = \frac{LC_{50}\text{ of a toxicant acting alone}}{LC_{50}\text{ of mixture}}$$

$SR^1/SR^2 = 1$ indicates addition action
$SR^1/SR^2 > 1$ indicates synergism
$SR^1/SR^2 < 1$ indicates antagonism

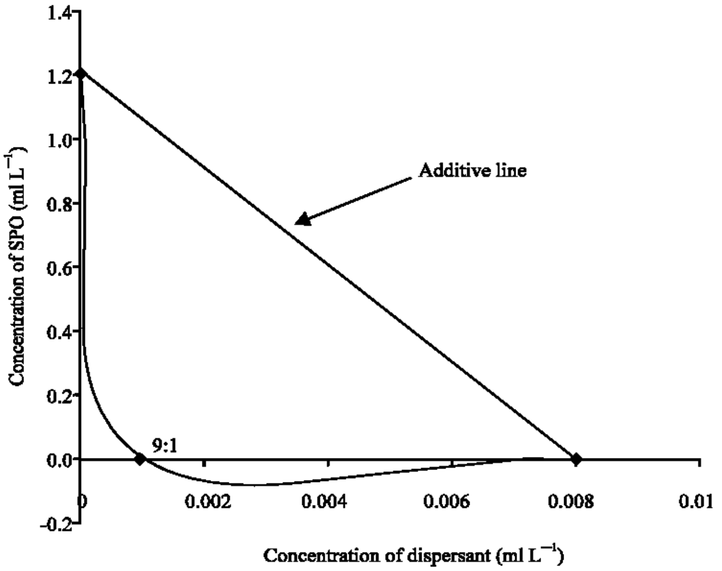

Fig. 2: Isobole representation of the SPO-dispersant (9:1) mixture effect when tested against *C. gariepinus*

while the interaction in the SLO-detergent (9:1) mixture conformed with the model of antagonism (RTU = 0.11) (Table 2).

Further analysis of the concentration-mortality data based on the synergistic ratio (SR) model (Model B) revealed that the SLO-dispersant (9:1) mixture with reference to SLO was in agreement with the model of synergism ($SR^1 = 150.00$) while the mixture with reference to dispersant was found to have similar toxicity level with dispersant when acting alone ($SR^2 = 1.00$, additive action) (Table 3). The interaction in SLO-detergent (9:1) mixture conformed to the model of antagonism with reference to either SLO ($SR^2 = 0.11$) or detergent ($SR^2 = 0.09$) (Table 3).

The subjection of SLO-dispersant (9:1) and SLO-detergent (9:1) mixtures interactions to additional synergistic analysis (Isobologram, Model C) by fitting the derived 96 h LC_{50} values into isobologram and comparing it to the theoretical pictorial isobole (Fig. 1) revealed that the resultant isobole from the SLO-dispersant (9:1) mixture agreed with the model of synergism (Fig. 2) while the resultant isobole from the SLO-detergent (9:1) mixture conformed with the model of antagonism (Fig. 3).

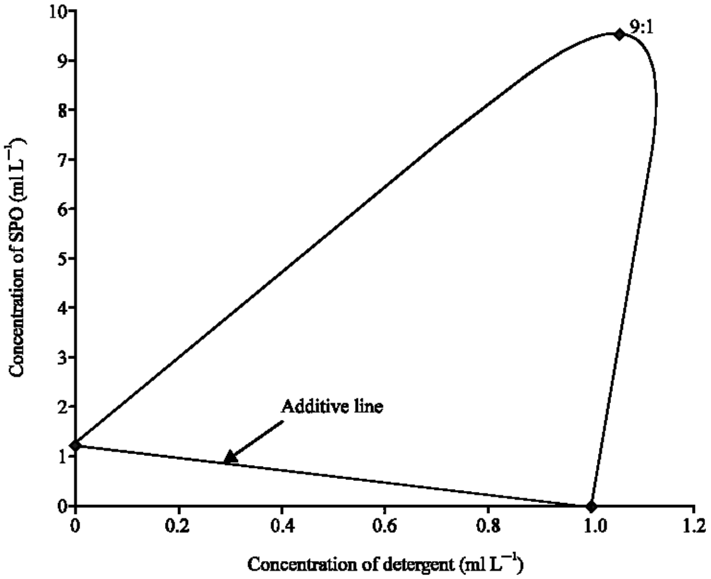

Fig. 3: Isobole representation of the SPO-detergent (9:1) mixture effect when tested against *C. gariepinus*

DISCUSSION

The results obtained in this study indicated that the dispersant, SAF power (96 h LC_{50} value of 0.008 ml L^{-1}) and detergent, SDS (96 h LC_{50} value of 1.00 ml L^{-1}) was about 150 times and 1.2 times (slightly) more toxic than spent lubrication oil (SLO) (96 h LC_{50} = 1.20 ml L^{-1}) respectively when tested singly against *C. gariepinus* (Table 1). This result with reference to the dispersant is in agreement with the findings of Otitoloju (2005), who reported that some dispersant used in Nigeria were relatively highly toxic to aquatic organisms. Although in this study the detergent, SDS was found to be slightly more toxic than the spent lubrication oil, the conversion of the 96 h LC_{50}, 1.00 ml L^{-1} based on the stock solution of 10 g L^{-1} used in this study makes the 96 h LC_{50} to be equal to 0.01 g L^{-1}. Generally, chemicals that have a 96 h LC_{50} of more than 1000 mg L^{-1} (1 g L^{-1}) are considered almost non-toxic (Koyama and Kakuno, 2004). The 96 h LC_{50} (1.00 mg L^{-1} [0.01 mg L^{-1}]) of the detergent, SDS used in this study was far less than 1 g L^{-1}, indicating that the detergent is highly toxic. An extensive review concerning the effects of detergents was done by Cserháti *et al.* (2002), which focused on the biological effects of this class of compound in the environment. In this review SDS was referred to as one of the most toxic compounds even at concentration of 50 ppm (50 mg L^{-1}), for cyanobacterial species. According to Nunes *et al.* (2005), high acute toxicity of SDS can be responsible for harmful effects on aquatic organisms, especially under the direct influence of large human populations, since SDS is widely used in domestic detergents and cosmetic products. Most detergents contain surfactants, enzymes, builders, fabric brighteners, fillers and colouring agents, all of which may contribute to their overall toxicity (Warne and Schifko, 1999).

As envisaged by Otitoloju (2006), the differential toxicity observed between test chemicals such as detergents, dispersant and SLO can be attributed to the fact that the physical characteristics and chemical composition of the test compounds are different. This dictates their site of action and therefore, the toxic action they exert on exposed organisms. It has been well established (Otitoloju, 2005) that spent lubrication oil exerts its action on aquatic animals by forming a barrier on the water surface and thus restricting gaseous exchange through coating of the respiratory surfaces such as spiracles, skin and gills of the exposed organisms. Detergents and dispersant are degreasers and surface active agents, exerting their toxic effects by destabilizing cell membrane structures/barriers and this causing easier influx of toxicants.

The studies on the joint-action toxicity of SLO-dispersant (9:1) and SLO-detergent (9:1) mixtures tested against *C. gariepinus* revealed that the 96 h LC_{50} of SLO-dispersant mixture was 1318 times more toxic than the 96 h LC_{50} of SLO-detergent mixture. The comparison of the test mixtures toxicity against *C. gariepinus* with the 96 h LC_{50} of the SLO acting alone revealed that the SLO-dispersant (9:1) mixture and the SLO-detergent (9:1) mixture was more and less toxic respectively than the SLO acting alone against the *C. gariepinus* fry (Table 3).

The synergistic evaluation of the joint-action toxicity results based on the concentration-addition model (Model A) showed that the toxicity of the SLO-dispersant (9:1) mixture tested against *C. gariepinus* conform largely to the model of synergism (RTU = 135.10). Further analysis of the mixture by substituting 96 h LC_{50} values of the test mixtures and single compounds in Model C pictorial isobole (Fig. 1) revealed that the resultant isobole also agreed with the model of synergism (Fig. 2). Synergistic analysis of the SLO-dispersant (9:1) joint-action toxicity data based on synergistic ratio, SR model (Model B) revealed that interactions between the constituents of the mixtures with reference to SLO conformed with the model of synergism (SR^1 = 150.00) while with reference to the dispersant, SAF power the interaction was found to be additive (SR^2 = 1). This indicated that the toxicity of the SLO-dispersant (9:1) mixture with reference to SLO was enhanced (potentiated). This is in agreement with Otitoloju (2005) who reported synergistic interaction in crude oil and dispersant exposed to *Macrobrachium vollenhovenii* at 9:1 even when the crude oil, forcados light was about six times more toxic than the dispersant, bisolve when acting alone. Dispersant promote the break-up or dispersion of an oil slick into small droplets that distribute into the water column. High solubility of oil and particulate phase droplets is expected to lead to higher uptake from SLO-dispersant mixture. This may have accounted for the high mortality rate (potentiation) observed in the mixture. The implication of this result is that the mixtures of SLO and dispersant, SAF power at ratio 9:1 would be expected to cause more damage to the exposed aquatic organism if utilized for emulsification of SLO before discharge into open drains (gutter) at this ratio than the damage SLO alone would have caused.

The synergistic interaction of the SLO-detergent (SDS) (9:1) mixture tested against *C. gariepinus* fry based on the concentration-addition model (Model A) was in agreement with the model of antagonism (RTU = 0.11). Additional analysis based on the pictorial isobole (Fig. 1) revealed that the resultant isobole also agreed with the model of antagonism (Fig. 3). Further analysis of the joint-action toxicity of the mixture using synergistic ratio, SR model (Model B) with reference to either SLO or detergent (SDS) revealed that the interaction was in conformity with the model of antagonism (SR^1 = 0.11 and SR^2 = 0.09 respectively) (Table 3). This indicated that both SLO and SDS reduced the toxicity potential of one another when acting jointly in 9:1 mixture against *C. gariepinus*. This mixture would be an environmentally safer mixture ratio if SDS was to be deployed for the cleaning and emulsification of spent lubrication oil being indiscriminately discharged by road side mechanics into the open drains and gutter.

The three models used for the synergistic classification of joint-action toxicity results in this study were in agreement. This means that each of the models can provide on its own a classification that is quite reliable. Furthermore, it is important to note that the SR model of Hewlett and Plackett (1959) has the advantage of classifying the type of joint-action for each of the individual components in a mixture as observed in this study. As reported by Otitoloju (2002) this model would be more useful when joint-action evaluations are carried out with a view of setting environmental safe limits of pollutants.

The results obtained in this study, particularly in view of the synergistic action of spent oil and SAF power 9:1 mixture should motivate environmental regulators in taken stringent measures against the indiscriminate disposal of spent oil, detergent and an unauthorized usage of dispersant. Even though the SDS in this study gave antagonistic action when combined with SLO, there is need to investigate the toxicity of all the detergents being used for cleaning purposes in the country. The detergents also promote the emulsification of oils and once this occurs the oil will no longer be restricted to the surface and may become readily available to exposed organisms.

The findings in this study related to different magnitudes of toxic response and distinct synergistic sensitivities toward the mentioned compounds, underline the need for further caution in order to ensure reduction in the risk of damage caused by multiple pollutants as they occur in ecosystems. Therefore it would be advantageous for future studies to carry out more synergistic evaluations of dispersant, detergent, spent lubrication oil and crude oil mixture using varying dilution ratios to ascertain the environmental safety of dispersant and detergent used for clean-up operations in the country.

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