Research Journal of Environmental Sciences

ISSN 1819-3412

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Environmental Sciences 4 (2): 115-126, 2010
ISSN 1819-3412

Joint Action Response and Haematological Profile of *Macrobrachium vollenhoevenii* (Herklots, 1857) Exposed to Binary Mixtures of Spent Lubricant Oil and Dispersant

L.O. Chukwu and O.S. Lawal
Aquatic Toxicology and Ecophysiology Laboratory,
Department of Marine Sciences, University of Lagos, Akoka, Yaba, Lagos, Nigeria

Abstract: The lethal effects of Spent Lubricant Oil (SLO) and dispersant acting singly and jointly as binary mixtures in predetermined (9:1) and equitoxic (3:1) ratios on *Macrobrachium vollenhoevenii* were evaluated and haematological parameters were used to assess effects of sub lethal exposure. The concentration-addition model (relative toxic units RTU) and Synergistic model (SR) were used to assess their interaction of the tested mixtures meant to simulate environmental control settings in aquatic ecosystems. On the basis of derived toxicity indices, the dispersant (96 h LC_{50} = 0.06 ml L^{-1}) was found to be about 1.33 times more toxic than the SLO (96 h LC_{50} = 0.08 ml L^{-1}) when acting singly against *M. vollenhoevenii* while the joint action response indicated that the equitoxic ratio (96 h LC_{50} = 0.56 ml L^{-1}) was relatively more toxic. The evaluation of the binary mixtures based on RTU (with reference to SLO) revealed conformity to model of synergism (RTU = 5.66; 1.33) for predetermined and equitoxic ratios while the synergistic evaluation indicated antagonism (SR = 0.44) and synergism (SR = 1.33) for predetermined and equitoxic mixtures, respectively. The haematological parameters of *M. vollenhoevenii* exposed to sub lethal concentrations (1/10th, 1/100th and 1/1000th of 96 h LC_{50} values) of the tested mixtures indicated that the total leucocyte count and differential polymorpho-neutrophils count increased significantly ($p>0.05$) with exposure period and concentrations while the differential lymphocyte count had an inverse relationship. The differential eosinophils count appeared to have no significant changes ($p>0.05$). The environmental significance of the results in establishing safe limits for dispersant use in aquatic ecosystems are discussed.

Key words: Joint action response, haematological profile, binary mixtures, prawn

INTRODUCTION

The possible toxic effects of dispersed oil in the water column are a primary consideration when assessing the use of dispersants to deal with an oil spill. The hydrocarbon types of dispersants used in the past have had adverse effects on the environment due to their toxicity. Modern dispersants used today are significantly less toxic than the oils they disperse. These dispersants are used when the impact of the floating oil has been determined to be greater than the impact of mixing of oil into the water column.

Corresponding Author: L.O. Chukwu, Aquatic Toxicology and Ecophysiology Laboratory, Department of Marine Sciences, University of Lagos, Akoka, Yaba, Lagos, Nigeria

Although, dispersants have been found to be very useful in some oil spill clean-up operations, the applications of some dispersants to spilled oil have also been found to cause more damage to the living organisms inhabiting such ecosystems than the spilled oil acting singly (Otitoloju, 2003).

The potential impact of dispersed oil on aquatic ecosystem should be thoroughly considered prior to use. Earlier studies have indicated that acute tests gave little or no relevant information on sub-lethal effects of dispersants or oil/dispersant mixtures (Baklien *et al*., 1986; 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; 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).

Many researchers have shown the surface molecules like dispersant in spite of being differentially toxic at times greatly synergise or enhance the toxicity of spilled oil on the aquatic organism (Adams *et al*., 1999; Chukwu, 2006). It is postulated that dispersants are known to synergize the toxic effects of oil during clean up by solubilising delicate membrane like those of the gills leading to enhanced entry or penetration of toxicants.

Furthermore, the effects of pollutants on physiological processes are relatively well-known and include respiratory, cardiovascular, osmoregulatory, neurological and endocrine disturbance (Wendelaar-Bonga and Lock, 1992; Randall *et al*., 1996; Bamber and Depledge, 1997; Chukwu, 2006; Velisek *et al*, 2006; Abdel-Moneim *et al*., 2008). Physiological assays attempts to measure responses within the normal physiological scope of the organism and correlate these with pollutant exposure or effect. A variety of haematology, respiratory, cardiovascular, osmoregulatory or neurological assays have been developed (Larsson *et al*., 1985; Baatrup *et al*., 1990; Bradbury *et al*., 1991; Depledge and Andersen, 1990; Aagaard *et al*., 1991). Physiological assays are especially useful for monitoring fluctuating exposures, acting as early warning systems for acute events, because the toxic response is usually instantaneous and or sensitive to low exposure concentrations.

Blood is a pathophysiological reflector of the body because it is highly susceptible to internal and external environmental fluctuations (Seth and Saxena, 2003). Physiomorphological changes in blood indicate the changes in the quality of the environment and therefore blood parameters are important in diagnosing the functional status of the animal exposed to toxicants. Haematological parameters act as a physiological indicator to changing external environment (Abdel-Moneim *et al*., 2008) as a result of their relationship with energetic (metabolic levels), respiration (haemoglobin levels) and defense mechanisms (leucocytes levels). There is a growing body of literature on the effects of pollutants on blood and other extracellular fluids; and this has largely been obtained during experimental exposures to identified toxicants (Velisek *et al*., 2006).

Macrobrachium sp. being an arthropod has an open circulatory system in which the blood comes in contact with the tissue fluid (lymph) to give the haemolymph. Crustacean haemocytes play important roles in a host's immune response; however, there is no uniform classification scheme for crustacean haemocytes. The circulating haemocyte number is a stress indicator and haemocyte counts may be a valuable tool in monitoring the health status of crustacean species. Studies on the haemocytes of crustaceans contribute to the accumulation of the basic knowledge on haemocytes, especially with regard to the physiological condition of the animal.

Therefore, an evaluation of the toxicological and physiological effects of spent lubricant oil and dispersant in various mixing ratios before setting environmentally safe optimal dispersal limits for managing spills in fragile aquatic ecosystems will be very useful. The aim

of the present study was to investigate the lethal effects of Spent Lubricant Oil (SLO) and dispersant (OSD 94-60) acting singly and jointly as binary mixtures in predetermined (9:1) and equitoxic (3:1) ratios on *M. vollenhoevenii* and use haematological parameters to assess effects of sub lethal exposure.

MATERIALS AND METHODS

Test Animals

The test organism, *M. vollenhoeveni* (Arthropoda, Decapoda, Crustacea) was selected for this study because it is one of the organisms approved for toxicity testing involving dispersants in Nigeria by the Department of Petroleum Resources (DPR). They were obtained from Lagos lagoon with a scoop net and conveyed in plastic bucket holding water from that habitat. The collection was made between 6-7 am in the morning to reduce stress on the organisms. The test animals collected were of similar sizes ranging from 4.0 to 6.0 cm weight ranging from 225.0-243.0 mg. Efforts were made to obtain the animal from the same location over the experimental period (January-June, 2007) to reduce variability in biotypes.

Acclimatization of Animals

The prawns, *M. vollenhoevenii* were kept in plastic holding tanks (75×30×32 cm) which were three-quarter filled with water from lagoon and left for a minimum of 7 days to allow them to adapt to experimental conditions (temperature 29.0±2°C, Relative Humidity, 78.9±3.0, Salinity, 16%). During this period of acclimatization, the test animals were fed with dried and grounded crayfish once daily. The lagoon water in the plastic holding tanks were aerated with a 220V air pump and was changed once every 3 days to prevent accumulation of toxic waste metabolite and decaying of food materials. Dead organisms were removed with a hand net. Organisms were monitored until the population stabilized before being used for experiment.

Test Chemicals

The test chemicals used were the spent lubricant oil and dispersant (OSD 94-60).

Spent Lubricant Oil (SLO)

The spent lubricant oil was collected from different mechanic workshops around University of Lagos, Akoka Yaba, Lagos, Nigeria and stored in a 25 L keg. The collection of the SLO in the different places was to simulate a natural situation, since the spent lubricant oil that impact aquatic organisms come from different sources.

Dispersant

The dispersant, OSD 94-60 is water based biodegradable hydrocarbon approved for use in oil spill control by the Nigerian Regulatory Agency, Department of Petroleum Resources (DPR).

Preparation of Test Media and Application of Toxicants

Lagoon water was used for the preparation of the test media. The bioassay containers used were glass tanks (22×15×18 cm) which are observed to be advantageous when compared to plastic containers as they minimize absorption of toxicants and prevent risk of corrosion and chemical reactions. Some plastics are known to react with some crude oil components.

A given volume of Lagos lagoon water was measured using a measuring cylinder into clean dry bioassay containers and a predetermined volume of Spent lubricant oil or dispersant or the mixture (9:1 and 3:1; SLO: dispersant) was added into the Lagos water to make it up to 1000 mL (total volume of test media) to achieve the desired test concentrations. The measurements of the SLO and dispersants were done using syringes of different volumes. The joint action tests were carried out based on the ratio 9:1 which represents the prescribed ratio for oil spill control in aquatic systems by the regulatory body in Nigeria while the ratio 3:1 serves as the equitoxic ratio of SLO and dispersant.

Bioassays

Single Action Toxicity of Spent Lubricant Oil against *M. vollenhoevenii*

Ten *M. vollenhoeveni* specimens of similar sizes were introduced randomly into each of the 6 bioassays containers in three replicates per treatment giving a total of 30 prawns including the untreated control. The prawns in the bioassay container were exposed to varying concentration of SLO as follows: 1.0, 2.0, 3.0, 4.0 and 5.0 mL and untreated control. Mortality assessment was carried out every 24 h for 4 days.

Single Action Toxicity of Dispersant against *M. vollenhoevenii*

Ten *M. vollenhoeveni* specimens of similar sizes were introduced randomly into each of the 6 bioassays container. This was done in three replicates per treatment giving a total of 30 prawns including the untreated control. The prawns in the bioassay containers were exposed to varying concentrations of dispersant as follows: 0.2, 0.4, 0.6, 0.8 and 1.0 mL and untreated control. Mortality assessment was carried out every 24 h for 4 days.

Joint Action Toxicity of Mixtures of SLO and Dispersant against *M. vollenhoevenii*

Three replicates of 10 *M. vollenhoevenii* were introduced per tank (6 tanks in all) including control tanks. For the predetermined test the ratio of SLO and dispersant was in the ratio 9:1 and the prawns were then exposed to varying concentration of the mixture as follows: 1.0, 2.0, 3.0, 5.0 and 6.0 mL and untreated control. For the equitoxic ratio 3:1, the prawns were exposed to varying concentrations of the mixture as follows: 0.8, 1.0, 1.2, 1.4 and 1.6 mL and untreated control. Mortality assessment was carried out once every 24 h in 4 days.

Assessment of Quantal Response (Mortality)

The test animal, *M. vollenhoevenii* was considered dead if there was no movement or response to stimulus even when prodded with a blunt glass rod.

Sublethal Studies

Acclimatization of the prawns for 7 days was also necessary before the start of the sub-lethal studies. After the acclimatization period, twenty prawns were exposed per tank to 1/10th 1/100th and 1/1000th of 96 h LC_{50} values for the predetermined ratio, 9:1 and the equitoxic ratio, 3:1. For both the predetermined and equitoxic experiments, there was a control. The exposure of the prawns was done for 2 weeks (14 days) with the toxicants (SLO and dispersant) replaced every 48 h before which the prawns were fed at least 2 h prior to toxicant renewal.

Extraction of Haemolymph

Haemolymph was collected from three prawns using 1 mL syringe which was injected at the base of the carapace and at the tail (telson). The haemolymph was immediately

transferred into an EDTA bottle (treated bottle to prevent the coagulation of the haemolymph). Haemolymph extraction was done every 7 days and the haemolymph samples were taken to the Haematological unit of Lagos University Teaching Hospital (LUTH) at Idi-Araba, Lagos, Nigeria where they were analysed. The tests carried out were Total leucocyte count, Differential polymorpho-neutrophils count, Differential lymphocyte count and Differential eosinophil count.

Laboratory Analysis of Haemolymph

The materials that were used in the sub-lethal test were the commercially prepared Leishman stain and distilled water. A drop of the blood sample was placed on a clean glass slide and a cover spreader was used to make a thin film of the blood. It was allowed to dry on a staining rack. The slide was flooded with Leishman stain for 2 min. Staining was differentiated with 3-4 drops of distilled water and left for 9 min. The slide was the rinsed with running water and left to dry before examining under a microscope using immersion oil.

Statistical Analysis

Concentration Response Data Analysis

Toxicological concentration data involving quantal response (mortality) for both single and joint action studies were analysed by Probit analysis (Finney, 1971). The indices of toxicity measurement derived from these analyses was LC_{50} (median lethal concentration that causes 50% mortality of exposed organisms) and Toxicity factor (T.F) for relative potency measurements.

Analysis of Data and Measurement of Joint Action Toxicity of Binary and Multiple Mixture of Test Compounds

The models used for the analysis of joint action toxicity data are the concentration-addition model (Anderson and Weber, 1975) and the synergistic ratios model. The concentration-addition 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 separate toxicants according to the proportion of their contribution in the mixture. The predicted LC_{50} value(s) 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 of derived LC_{50} values to predicted LC_{50} (RTU) is estimated as:

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

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

Measurement of joint-action toxicity by synergistic ratios was defined after Hewlett and Plackett (1959) 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.

Analysis of Variance (ANOVA), Student Newman Keuls (SNK) Tests and Duncan Multiple Range Tests (DMRT)

One way Analysis of Variance (ANOVA) and comparison of means by Student Neuman Keuls (SNK) (Zar, 1996) test were used to test for statistical differences of the median lethal toxicity tests. One way analysis of variance (ANOVA) and comparison of means by Duncan Multiple Range Test (DMRT) (Zar, 1996) were used to test for statistical differences between extrapolated fractions (1/10th, 1/100th and 1/1000th 96 h LC_{50}) and untreated control in the results (computed means) of the sub lethal tests at 7 day and 14 day exposure periods.

RESULTS

Physico-Chemical Parameters of the Test Media

The mean values obtained for the physico-chemical parameters of the test media (Lagoon water) throughout the period of the experiment were dissolved oxygen (8.40 mg L^{-1}), pH (6.84), Total dissolved solids (3.48 mg L^{-1}), Salinity (16‰) and temperature (29.0°C).

Single Action Toxicity of Spent Lubricant Oil and Dispersant against *M. vollenhoevenii*

The result of dose mortality analysis of spent lubricant oil and dispersant against *M. vollenhoevenii* reveals that the derived toxicity indices (LC_{50}) for spent lubricant oil ranged from 0.19 ml L^{-1} (24 h LC_{50}) to 0.08 ml L^{-1} (96 h LC_{50}), while for dispersant the derived toxicity indices (LC_{50}) ranged from 0.12 mL (24 h LC_{50})/L to 0.06 ml L^{-1} (96 h LC_{50}) (Table 1).

Table 1: Relative toxicity of spent lubricant oil and dispersant against *M. vollenhoevenii*

Exposure time (h)	LC_{50}	LC_{95} (95% CL ml L^{-1})	LC_{50}	Slope±SE	TF^2	Probit line equation
Spent lubricant oil						
24	0.19 (0.00-0.32)	5.94 (3.62-150.34)	190.01 (28.07-4.05x109)	1.09±0.45	-	Y = 4.15+1.09x
48	0.18 (0.00-0.52)	2.78 (1.85-4.41)	42.75 (14.35-3649.57)	1.38±0.43	-	Y = 4.39+1.38x
72	0.17 (0.01-0.48)	2.11 (1.29-2.89)	24.72 (10.64-417.56)	1.54±0.43	-	Y = 4.50+1.54x
96	0.08 (0.01-0.29)	1.09 (0.29-1.67)	15.55 (7.30-295.07)	1.43±0.44	1	Y = 4.94+1.43x
Dispersant						
24	0.12 (0.03-0.21)	0.79 (0.62-1.25)	5.37 (2.53-42.33)	1.99±0.48	-	Y = 5.19+1.99x
48	0.89 (0.02-0.16)	0.54 (0.42-0.69)	3.24 (1.83-12.73)	2.11-0.46	-	Y = 5.57+2.11x
72	0.07 (0.014-0.13)	0.44 (0.32-0.57)	2.89 (1.65-11.33)	2.02±0.45	-	Y = 5.71+2.02x
96	0.06 (0.01-0.11)	0.34 (0.22-0.43)	2.05 (1.28-6.11)	2.10±0.45	1.33	Y = 5.99+2.10x

CL: Confidence limit, LC: Lethal concentration, TF: Toxicity factor, TF = (LC_{50} of test compound at 48 h/LC_{50} of test compound at other h (72 and 96 h)), TF^2: Toxicity factor, TF^2 = (96 h LC_{50} of least toxic compound/96 h LC_{50} of test compounds)

On the basis of the computed toxicity factor (96-h LC50) ratios, the spent lubricant oil was found to be about 1.33 times more toxic against *M. vollenhovenii* than the dispersant. The randomized analysis of variance (ANOVA) showed that there was significant difference ($p<0.05$) in response to different treatments (concentrations) at 24, 48, 72 and 96 h of exposure.

Joint Action Toxicity of Binary Mixtures of Spent Lubricant Oil and Dispersant (9:1) and (3:1) against *M. vollenhoevenii*

The analysis of concentration response data for the joint action toxicity of SLO and dispersant in *M. vollenhoevenii* revealed from Table 2 that the median lethal concentrations of the binary mixture (9:1) and (3:1) of SLO and dispersant were 0.18 and 0.56 ml L^{-1}, respectively indicating that the predetermined ratio was about 3 times more toxic than the equitoxic ratio. In each case, it was observed that toxicity increases with increase in exposure time.

Further analysis of the joint action toxicity data based on the concentration-addition model gave Relative Toxicity Unit (RTU) values of 5.66 and 1.33 for predetermined (9:1) and equitoxic (3:1) ratios, respectively indicating conformity with the model of synergism (Table 3). Furthermore, analysis of the joint action results based on the synergistic ratios

Table 2: Relative toxicity of the binary mixtures of SLO and dispersant against *M. vollenhoevenii* in predetermined ratio, 9:1 and equitoxic ratio:3:1

Exposure time (h)	LC_{50}	LC_{95} (95% CL ml L^{-1})	LC_5	Slope±SE	TF^2	Probit line equation
Predetermined ratio						
24	0.78 (0.31-1.19)	4.07 (3.26-5.49)	21.31 (12.25-69.99)	2.29±0.44	-	Y = 3.61+2.29x
48	0.38 (0.,08-0.71)	2.92 (2.19-3.91)	22.75 (11.86-107.52)	1.85±0.39	-	Y = 4.14+1.85x
72	0.22 (0.03-0.47)	1.71 (1.08-2.26)	13.38 (7.82-46.92)	1.84±0.40	-	Y = 4.57+1.84x
96	0.18 (0.02-0.41)	1.3 (0.73-1.77)	9.46 (5.94-27.55)	1.91±0.42	3.11	Y = 4.78+1.91x
Equitoxic ratio						
24	0.68 (0.48-0.81)	1.31 (0.20-1.46)	2.5 (2.02-3.93)	5.85±1.13	-	Y = 4.32+5.85x
48	0.59 (0.36-0.73)	1.22 (0.11-1.36)	2.53 (2.00-4.30)	5.18±1.07	-	Y = 4.55+5.18x
72	0.55 (0.36-0.68)	1.04 (0.92-1.13)	1.96 (1.67-2.72)	5.96±1.10	-	Y = 4.90+5.96x
96	0.56 (0.40-0.67)	0.96 (0.86-1.04)	1.65 (1.46-2.06)	7.04±1.20	1	Y = 5.12+7.04x

CL: Confidence limit, LC: Lethal concentration, TF: Toxicity factor, TF = (LC_{50} of test compound at 48 h/LC_{50} of test compound at other h (72 and 96 h)), TF^2: Toxicity factor, TF^2 = (96 h LC_{50} of least toxic compound/96 h LC_{50} of test compounds)

Table 3: Analysis (Based on concentration-addition and synergistic ratio models) of the 96 h LC_{50} values of spent lubricant oil and dispersant when acting singly and jointly against *M. vollenhoevenii*

Mixture of spent engine oil and dispersant	Observed 96 h LC_{50} (95% CL ml L^{-1})	Predicted 96 h LC_{50} (95% CL ml L^{-1})	Probit line equation	TF	RTU	SR^a	Sr^b
9:1	0.18 (0.02-0.41)	1.02 (0.28-1.55)	Y = 4.78+1.91x	3.11	5.66	0.44	0.33
3:1	0.56 (0.40-0.67)	0.90 (0.27-1.36)	Y = 5.12+7.04x	1.00	1.61	1.33	0.11
Spent lubricant oil alone	0.08 (0.00-0.29)	-	Y = 4.94+1.43x	1.00	-		
Dispersant alone	0.06 (0.01-0.11)	-	Y = 5.99+2.10x	1.33	-		

RTU: Relative toxic unit, RTU = Predicted LC_{50} value binary mixture/Observed LC_{50} value binary mixture, SR: Synergistic ratio, SR = LC_{50} of spent engine oil or dispersant/LC_{50} (spent engine oil+dispersant), TF: Toxicity factor, T.F = 96 h LC_{50} of least toxic compound/96 h LC_{50} of test compounds, SR^a: Synergistic ratio (SLO), SR^b: Synergistic ratio (dispersant)

Table 4: Mean haematological parameters of *M. vollenhoevenii* exposed to sublethal concentrations of binary mixtures of SLO and dispersant in pre-determined ratio (9:1) for 14 days

Concentration	No. of test	Total leucocyte count		Differential polymorpho-neutrophils count		Differential lymphocyte count	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Control	3	141.00^{A}	141.33^{A}	57.33^{A}	57.00^{A}	24.00^{A}	14.69^{A}
1/10th (0.130 ml L^{-1})	3	200.67^{B}	270.33^{B}	60.00^{A}	65.67^{B}	30.33^{B}	27.00^{B}
1/100th (0.0130 ml L^{-1})	3	291.33^{C}	380.00^{C}	61.67^{A}	71.67^{C}	37.67^{C}	34.33^{C}
1/1000th (0.00130 ml L^{-1})	3	342.00^{D}	459.67^{D}	76.00^{B}	86.33^{D}	41.67^{D}	40.33^{D}

Means with the superscript letter in a column are not significantly different in the DMRT (p = 0.05)

Table 5: Mean haematological parameters of *M. vollenhoevenii* exposed to sublethal concentrations of binary mixtures of SLO and dispersant in equitoxic ratio (3:1) for 14 days

Concentration	No. of test	Total leucocyte count		Differential polymorpho-neutrophils count		Differential lymphocyte count	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Control	3	140.33^{A}	141.67^{A}	57.67^{A}	57.00^{A}	32.33^{A}	23.67^{A}
1/10th (0.0.96 ml L^{-1})	3	254.67^{B}	366.67^{B}	58.33^{A}	60.67^{B}	34.33^{B}	28.67^{B}
1/100th (0.0096 ml L^{-1})	3	261.33^{C}	380.00^{C}	59.67^{B}	61.67^{C}	36.33^{C}	30.67^{C}
1/1000th (0.00096 ml L^{-1})	3	469.33^{D}	689.33^{D}	64.67^{C}	77.33^{D}	40.67^{D}	42.33^{D}

Means with the superscript letter in a column are not significantly different in the DMRT (p = 0.05)

model with reference to SLO shows that mixtures prepared based on ratio 9:1 was found to be less toxic (Sr^{a} = 0.44) (antagonism) than the spent lubricant oil when acting singly while the mixture prepared based on ratio 3:1 was found to conform with the model of synergism (Sr^{a} = 1.33) (Table 3). The interaction in SLO-dispersant (9:1 and 3:1) mixtures with reference to dispersant conformed to the model of antagonism (Sr^{b} = 0.33 and Sr^{b} = 0.11) (Table 3).

Sub Lethal Effects of Binary Mixtures of SLO and Dispersant on Haematological Parameters of *M. vollenhoevenii*

The haematological observations on the Total leucocyte count, Differential polymorpho-neutrophils count, Differential lymphocyte count and the Differential eosinophil count on *M. vollenheovenii* under sub lethal exposure to binary mixtures of SLO-dispersant (9:1 and 3:1 ratios) are shown in Table 4 and 5. The results indicate a significant increase (p>0.05) in the total leucocyte-count and differential polymorpho-neutrophils count of *M. vollenheovenii* exposed for 14 days in the different treatments compared to control. It was evident that the differential lymphocyte count of the *M. vollenheovenii* exposed for 14 days resulted in a significant decrease (p>0.05) in mean values in the different treatments. The randomized analysis of variance (ANOVA) and Duncan test showed that there were significance differences (p>0.05) in haematological parameters exposed to different sub lethal concentration at 7 and 14 days (Table 4, 5).

DISCUSSION

In this study, on the basis of derived toxicity indices, the dispersant (96 h LC_{50}= 0.06 ml L^{-1}) was found to be about 1.33 times more toxic than the SLO (96 h LC_{50}= 0.08 ml L^{-1}) when acting singly against *M. vollenhoevenii* while the joint action response indicated that the equitoxic ratio (96 h LC_{50} = 0.56 ml L^{-1}) was relatively more toxic than the pre-determined ratio. This findings are in agreement with those of Otitoloju (2005) and Chukwu (2006), who evaluated the joint action response of prawns against other dispersants approved for use by the Nigerian regulatory agency. However, the dispersant OSD 94-60 used in this study is

more toxic than the biosolve dispersant used by Otitoloju (2005), where he gave the 96 h LC_{50} of biosolve against *M. vollenhoevenii* as 1.90 ml L^{-1}.

From this study, the dispersant OSD 94-60 was found to be 3.2 times more toxic than the SLO against *M. vollenhoevenii* when acting singly. This observation is in agreement with the findings of Oyewo (1986) and Chukwu (2006), who reported that some dispersants used in Nigeria were relatively highly toxic to some brackish water species. The different mixtures of SLO and dispersant in the joint-action toxicity in the ratios 9:1 and 3:1 tested against *M. vollenhoevenii*, showed that there were variations depending on the proportion of addition of the mixture components. The concentration addition model shows that the joint action of SLO and dispersant in the ratios 9:1 and 3:1 were both synergistic with the RTU values of 5.66 and 1.33, respectively. This means that at ratios 9:1 and 3:1 of the SLO and dispersant mixtures, the damages caused on organisms would be synergised when such ratios are applied during spent lubricant oil clean-up exercise.

When comparing the mixtures of SLO and dispersants at the different ratios (9:1 and 3:1) with SLO acting singly using the synergistic ratio model, it was found that the mixture of SLO and dispersant in the ratios of 9:1 and 3:1 was less toxic than when SLO was acting alone on *M. vollenhoevenii*. The findings also indicate that based on the synergistic ratios model with reference to SLO shows that mixtures prepared based on ratio 9:1 was found to be less toxic (Sr^a = 0.44) (antagonism) than the spent lubricant oil when acting singly while the mixture prepared based on ratio 3:1 was found to conform with the model of synergism (Sr^a = 1.33). Therefore, the use of the dispersant with the SLO in the ratio 9:1 would reduce toxic effect on *M. vollenhoevenii* when used in clean-up exercise of SLO spillage in the aquatic environment as the SLO acting singly has shown to be more toxic. However, comparing the mixtures of the SLO and dispersant in the ratio 9:1 and 3:1 to the dispersants acting alone shows that the mixtures in both ratio were less toxic. It is important to note that the SNK test results which show the statistical differences between all pairing concentrations indicated that there were significant differences in the mortality responses in the different concentrations of toxicants both in the single action and joint action toxicity.

Many studies have been conducted using isolated haematological parameters on fish especially (Blaxhall, 1972; Mawdesley-Thomas, 1971; Singer *et al*., 1990) but there is paucity of published data on prawn haematological parameters. It therefore becomes imperative to encourage haematological investigations of tropical prawns, especially the establishment of normal values together with the identifications of those environmental and disease conditions that may affect these values. If these are done, a bank of useful information would be available from which valuable and informative conclusions can be drawn. From results of haematological tests various derived values can be calculated such as the cellular stability, leucocyte count, haemocyte count, neutrophils count among many others.

In this study healthy prawns, *M. vollenhoevenii* were used to ascertain the sub lethal effects on the haemolymph exposed to binary mixtures of SLO and dispersant at various fractions of the 96 h LC_{50} of ratios 9:1 and 3:1. The haematological parameters of interest were the total leucocyte count, differential polymorpho-neutrophils count, differential lymphocyte counts and the differential eosinophil counts. Exposure of *M. vollenhoevenii* to sublethal concentrations of the binary mixture of SLO and dispersant in ratios 9:1 and 3:1 affected the total leucocyte count in that there was significant increase in values as the exposure time increased from day 0 to day 14. Higher total leucocytes values obtained with increase in sublethal concentrations and increase in number of days may be as a result of combating and coping with stress induced toxicants. These haematological responses of the prawn in this study are in agreement with the findings of Donsikova *et al.* (2006) reported that the haematological profile of carp, *Cyprinus carpio* showed significant ($p<0.01$) increase in the

number of erythrocytes, segmented neutrophile granulocytes, developmental forms of myeloid sequence and eosinophils on exposure to sub lethal doses of pryrethoid, Cypermethrin. However, Svobodova *et al.* (2003) reported significantly lower values ($p<0.01$) of erythrocytes, haemoglobin and Packed Cell Volume (PVC) as a result of possible disruption of haemotopoiesis, but found no changes in white blood picture in carp after acute exposure to deltamethrin.

According to Johansson *et al.* (1992), the various haematogical parameters are closely attributed to the response of the organisms to its environment (test media in the experimental chambers). The leucocyte indicates any possibility of disease due to the pollutions or environmental conditions. Joshi *et al.* (2002) reported that the increase in white blood cell count can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to sub lethal concentrations of pesticides.

Yamuna *et al.* (2002) stated that the immune defense of invertebrates is extremely efficient and like vertebrates, is mediated by complex cellular and hormonal interactions. They also reported that activation of immune functions worked well in test prawns, *Macrobrachium malcolmsonii* exposed to lowest sublethal concentrations of mercury and copper, hence total hemocytes number, percentile phagocytosis and superoxide anion production were found to be enhanced while higher sublethal concentrations of these metals have inhibitory effects on the hemocytes-mediated immune functions, therefore total hemocytes number, percentile phagocytosis and superoxide anion production were found to be declined.

There was an increase in the differential polymorpho-neutrophils count with increase exposure period from 0 to 14 days. Though there was no regular pattern between sublethal concentrations with differential polymorpho-neutrophils count, the longer the exposure period of the test organism, *M. vollenhoevenii* the higher the level of the differential polymorpho-neutrophils count. The differential polymorpho-neutrophils indicate level of stress on organisms. The differential lymphocyte count was found to decrease with increase in the number of days from day 0 to day 14, though there was no regular pattern between sublethal concentration and the differential lymphocyte count. The ANOVA and Duncan test revealed significant differences ($p>0.05$) in each of the haematological parameters in the different sublethal concentrations. The differential eosinophil count seems to be of no importance as no values were recorded in the test organism. Other authors who have identified blood parameters as potential tools for identification of stress in the environment include, Jusila *et al.* (2001) and Masson *et al.* (2002). According to these researchers, blood parameters such as levels of cortisol, glycemia, haemoglobin, hematocrit, Cl^- content, haemolymph clotting time and total haemocyte counts are very useful indicators of chemical/physical stress in the environment.

In conclusion, the result of this present study suggests the use of dispersants for control of spent lubricant oil spillage may actually reduce biological damage if the varying mixtures of SLO and dispersants at certain ratios are taken into considerations. If this is done the immune functions of the test prawns, *M. vollenhoevenii* would be less susceptible to any disease and stress induced toxicant as indicated by the results obtained in varying sublethal concentrations on the tests prawns. Hence, pollution of natural aquatic environment by spent lubricant oil spillage should be strictly monitored.

REFERENCES

Aagaard, A., B.B. Andersen and M.H. Depledge, 1991. Simultaneous monitoring of physiological and behavioral activity in marine organisms using non-invasive, computer-aided techniques. Mar. Ecol. Prog. Ser., 73: 277-282.

Abdel-Moneim, A.M., N.M. Abou Shabana, S.E.M. Khadre and H.H. Abdel-Kader, 2008. Physiological and histopathological effects in catfish (*Clarias lazera*) exposed to dyestuff and chemical wastewater. Int. J. Zool. Res., 4: 189-202.

Adams, G.G., P.L. Klerks, S.E. Belanger and D. Dantin, 1999. The effects of the oil dispersant Omni-Clean on the toxicity of fuel oil No. 2 in two bioassays with the sheepshead minnow *Cyprinodon variegates*. Chemosphere, 39: 2141-2157.

Anderson, P.D. and L.J. Weber, 1975. The toxicity to aquatic population of mixtures containing certain heavy metals. Proceedings of the International Conference on Heavy Metal in the Environment Institute of Environmental Studies, (HMEIES'75), University of Toronto, pp: 933-953.

Baatrup, E., K.B. Doving and S. Winberg, 1990. Differential effects of mercurial compounds on the electroolfactogram (EOG) of salmon (*Salom salar* L.). Ecotoxicol. Environ. Safety, 20: 269-276.

Baklien, A., R. Lange and L.O. Reiersen, 1986. A comparison between the physiological effects in fish exposed to lethal and sublethal concentrations of a dispersant and dispersed oil. Mar. Environ. Res., 19: 1-11.

Bamber, S.D. and M.H. Depledge, 1997. Responses of shore crabs to physiological challenges following exposure to selected environmental contaminants. Aquat. Toxicol., 40: 79-92.

Blaxhall, P.C., 1972. The haematological assessment of the health of freshwater fish. Review of selected literature. J. Fish Biol., 4: 593-604.

Bradbury, S.P., R.W. Carlson, G.J. Niemi and T.P. Henry, 1991. Use of respiratory-cardiovascular response of rain- bow trout (*Oncorhynchus mykiss*) in identifying acute toxicity syndromes in fish: Part 4 Central nervous system seizure agents. Environ. Toxicol. Chem., 10: 115-131.

Chukwu, L.O., 2006. Evaluation of toxic action mechanisms of binary mixtures of spent lubricant oil and detergent against littoral estuarine macro-invertebrates. Pollut. Res., 25: 7-13.

Depledge, M.H. and B.B. Andersen, 1990. A computer-aided physiological monitoring system for continuous, long-term recording of cardiac activity in selected invertebrates. Comp. Biochem. Physiol., 96: 473-477.

Donsikova, R., J. Veliser, T. Wlasow, P. Gomulka, P. Svobodova and L. Novotny, 2006. Effects of cypermethrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). Neuroendocrinol. Lett., 27: 101-105.

Finney, D.J., 1971. Probit Analysis. 3rd Edn., Cambridge University Press, Cambridge, UK.

Hewlett, P.S. and R.L. Plackett, 1959. A unified theory for quantal responses to mixtures of drugs; non-interactive action. Biometrics, 15: 591-610.

Johansson, N., A. Larsson and K. Lawander, 1992. Metabolic effect of PCB (polychlorine biphenyls) on the brown trout (*Salmon trutt*). Comp. Genet. Pharmac, 3: 310-314.

Joshi, P.K., D. Harish and M. Bose, 2002. Effect of lindane and malathione exposure to certain blood parameters in a fresh water teleost fish *Clarias batrachus*. Pollut. Resour., 21: 55-57.

Jusila, J., S. McBride, J. Jago and L.H. Evans, 2001. Haemolymph clotting time as an indicator of stress in western rock lobster (*Panulirus cygnus* George). Aquaculture, 199: 185-193.

Larsson, A., C. Haux and M. Sjöbeck, 1985. Fish physiology and metal pollution results and experiences from laboratory and field studies. Ecotoxicol. Environ. Saf., 9: 250-281.

Masson, N., F. Guerold and O. Dangles, 2002. Use of blood parameters in fish to assess acidic stress and chloride pollution in French running waters. Chemosphere, 47: 467-473.

Mawdesley-Thomas, L.E., 1971. Toxic chemicals-the risk to fish. New Sci., 40: 74-87.

Odiete, W.O., 2003. Standardisation of test animals for toxicity evaluation for environmental regulation in Nigeria. J. Nig. Environ. Soc., 1: 340-350.

Otitoloju, A.A., 2003. Relevance of joint action toxicity evaluations in setting realistic environmental safe limits of heavy metals. J. Environ. Manage., 67: 121-128.

Otitoloju, A.A., 2005. Crude oil plus dispersant: Always a boon or bane? Ecotoxicol. Environ. Safety, 60: 198-2012.

Oyewo, E.O., 1986. The acute toxicity of three dispersant. Environ. Pollut., 41: 23-31.

Randall, D.J., C.J. Brauner, R.V. Thurston and J.F. Neuman, 1996. Water Chemistry at the Gill Surfaces of Fish and the Uptake of Xenobiotics. In: Toxicology of Aquatic Pollution, Taylor, E.W. (Ed.). Cambridge University Press, Cambridge, UK., pp: 1-16.

Samuel, O.B., O.Q. Suleiman and W.O. Odiete, 2008. Synergistic evaluation of oil-dispersant and oil-detergent mixtures using African Catfish, *Clarias gariepinus* fry. J. Fish. Aquatyic Sci., 3: 280-290.

Seth, N. and K.K. Saxena, 2003. Haematological Responses in a Freshwater Fish *Chanus punctatus* due to fenvalerate. Bull. Environ. Contam. Toxicol., 71: 1192-1199.

Singer, M.M., D.L. Smalheer, R.S. Tjeerdema and M. Martin, 1990. Toxicity of an oil dispersant to the early life stages of four California marine species. Environ. Toxicol. Chem., 9: 1387-1395.

Svobodova, Z., V. Luskova, J. Drastichova, M. Svoboda and V. Zlabek, 2003. Effect of deltamethrin onb haematological indices of common carp (*Cyprinus carpio* L.). Acta Vet. Brno, 72: 79-85.

Velisek, J., T. Wlasow, P. Gomulka, Z. Svobodova, R. Dobsikova, L. Novotny and M. Dudzik, 2006. Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). Veterinarni Medicina, 51: 469-476.

Volkman, J.K., G.J. Miller, A.T. Revill and D.W. Connell, 1994. Oil Spills. In: Environmental Implications of Offshore Oil and Gas Development in Australia: The Findings of an Independent Scientific Review. Swan, J. M., J.M. Neff and P.C. Young (Eds.). Australian Petroleum Exploration Association, Sydney, pp: 509-695.

Wendelaar-Bonga, S.E. and R.A.C. Lock, 1992. Toxicants and osmoregulation in fish. Netherlands J. Zool., 42: 93-478.

Yamuna, A., B.P. Saravana and P. Geraldine, 2002. Effects of Hg and Cu on hemocytes-mediated functions in the prawn, *Macrobrachium malcolmsonii*. J. Environ. Biol., 23: 7-13.

Zar, J.H., 1996. Biostatistical Analysis. 3rd Edn., Prentice-Hall, New Jersey, pp: 178.