

Protocol for analysis of toxicity and efficiency of oil dispersant

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Introduction

The spilled oil can be accumulated in sensitive ecosystems such as estuarine wetlands due to physical properties of water and transportation mechanisms like wind and ocean currents (Fuller et al., 2004). The accumulation of oil cause to kill crabs, lobsters, shell fish and the most dramatic symbol is an oiled sea bird (NRC, 1989). However, the effects on aquatic life can be depended on the life time of oil type. Therefore, oil spill is a main ecological and economical problem to all countries and it is important to develop methods to remove oil in sea water.

Application of chemical dispersants is one of the options to accelerate the dispersion of oil from the sea surface into the water column effectively and it helps to accelerate the dilution and biodegradation of oil to reduce the economic and environmental impact from oil spill (Chapman et al., 2007).

Dispersants consist of individual components of surfactants which are specific chemical compounds including oleophilic (oil liking) and hydrophilic (water liking) groups (Karam, 2011). These surfactants act as a chemical bridge between oil and water phases and help to mix with each other more easily and can be reduced the viscosity of surfactant (Lewis et al., 2006). The addition of dispersant rapidly converts a much larger oil slick into small oil droplets and these small oil droplets enhances the biological degradation by increasing surface area available to microorganisms (Trondheim, 2001). Several countries have been used different types of oil dispersants according to their rules and regulations. However, there are no records for usage of oil dispersants in oil spills in Sri Lanka. Therefore, it is important to assess the toxicity of these chemicals to the organisms living in coastal ecosystems before applying the dispersants to oil spills.

Toxicity is defined as negative effects (lethal or sub lethal) on organism caused by exposing to a chemical substance (Lewis et al., 2006). In toxicological studies, Dose-Response relationship use to express the relationship between chemical exposure and toxicity. The acute toxicity of a chemical could be assessed as LC_{50} (Lethal concentration that kills 50% of the test population) and behavioral changes of the organisms can be observed.

Effectiveness of dispersant is still remains as a major issue with chemical oil dispersants and many factors are influenced for dispersant effectiveness, including oil composition, sea energy, state of oil weathering, the type of dispersant used and the amount applied temperature, and salinity of water (IPIECA-IOGP, 2014). However, the major factor is considered as the composition of oil followed by sea energy and amount of dispersant added. Mackay and Wells (1983) describe that the high salinity increases the effectiveness of dispersant by preventing migration of surfactant molecules.

The effectiveness tests have diverse test procedures and evaluating criteria focusing both scientifically and economically limitation factors. IPIECA-IOGP, (2014) report explains four types of effectiveness tests including laboratory tests, mesoscale tests, and open water

experiments. Fingas, (1997) said that these bench scale laboratory tests are widely used to evaluate the performance of dispersant and the physical chemical mechanism of oil dispersants and explain the results as percentage of effectiveness.

Methodology

The protocol for oil dispersant analysis should be covered two major objectives;

1. Analysis of toxicity of the chemical dispersant to coastal organisms
2. Analysis of efficiency of the dispersant

1. Analysis of toxicity

Each dispersant must be tested for 'Toxicity' with the object of determining 72hrs/96 hrs LC₅₀ and to establish incipient lethal limits.

The toxicity of dispersant can be assessed using different organisms under laboratory conditions. The methodology in this protocol explained using two reef fish species. *Abudefduf vaigiensis* (fish 1) (Fig 1) and *Neopamacentrus azysron* (fish 2) (Fig 2) were selected as the test organisms because, they can be easily found around the Sri Lankan coastal marine ecosystems and can be easily handled in a marine aquarium.



Figure 1: The test species (*Abudefduf vaigiensis*, Order: Perciformes, Family: Pomacentridae)



Figure 2: *Neopamacentrus azysron*

1.1 Acclimatization of fish before the Toxicity Test

The test fish should be acclimatized by following trial tests to determine the suitability of the fish for aquarium conditions during two weeks. 16 fish were introduced into two tanks (44×29×31 cm³), and they were fed three times per day up to satiation with commercial Prima diet. Half of the total volume of water was removed and refilled with filtered sea water once in five days in each acclimatization tank. Commercially available Aqua zonic black night top filter (8W, 800 L/h) was fixed and aeration was provided to each tank (Fig 3).



P1.4: Complete Tank Setup

Fig 3: Completed set up of a tank

1.2 Preliminary Test

Preliminary test for toxicological study is important to find the effective concentration series (Hall, & Golding, 1998). Two experimental setups were used respectively in the presence and absence of aeration. Twelve glass tanks (44×29×31 cm³) were used in two series of tanks including six tanks to each series labeled as (C, R1, R2, R3, R4 and R5) (Fig 4). The used oil dispersant was Adt (4-amino-1, 2-dithiolane-4-carboxylic acid) type 3. The concentration series of 0, 20, 40, 60, 80, 100 ppm of oil dispersant were prepared using 0, 0.60, 1.20, 1.80, 2.40 and 3.0g of the dispersant respectively for fish 1. Total length and weight of all fish were measured to select the fish within the length range of 4.2cm to 5.4cm and the weight range was 3.9g to 4.8g. 650,680,710,740,770 and 800 ppm concentration series was prepared by adding 19.5, 20.4, 21.3, 22.2, 23.1 and 24g of oil dispersant to each tank respectively for fish 2.

The length of the largest fish should not be more than twice that of the smallest fish in the same test. All tanks were filled with 30L of filtered sea water and 8 fish were added into each tank and covered by a net to minimize the outside disturbances.

Percentage mortality and other morphological and functional changes such as erratic swimming, loss of relax; lethargy, discoloration, excessive mucous production etc. were observed and recorded in two experimental setups.

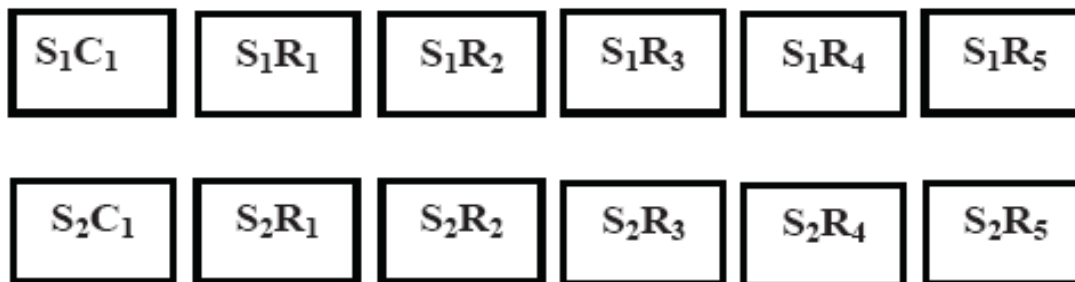


Figure 2.2: Experimental set up for the toxicity test

Fig 4: Diagrammatic explanation for the experimental set up

1.3 Acute toxicity Test

Acute toxicity test was conducted according to EPA/600/4-90/027F protocol (USEPA, 1993a). The selected concentration ranges for the experimental set up of without aeration was 0, 28, 32, 36, 40 and 44 ppm and for the set up with aeration was 0, 65, 70, 75, 80 and 85 ppm. Each experimental setup includes four series for the decided concentration ranges.

Physico-chemical parameters were measured before and after adding the dispersant to the tanks according to the methods given in Table 1. Fish feeding was ceased 24 hrs prior to the test and then the percentage mortality and other behavioral changes were observed and recorded during 24 hrs continuously. LC₅₀ values were calculated using EPA probit analysis program, Version 1.5 (Finney, 1978). Water temperature was measured once in two hours and other parameters were determined before adding the dispersant, after 24 hrs and at the end of the experiment after 72 hrs.

Table 1. Analytical Techniques for Physico-Chemical Parameters

<i>Parameter</i>	<i>Instrument</i>
Temperature	mercury bulb Thermometer
pH	salt water master test kit
Ammonia (NH ₃)	Zoolek salt water NH ₃ test kit
Salinity	YSI 85 meter (Japan)
Dissolve Oxygen (DO)	YSI 85 meter (Japan)

The following Toxicity limits (LC₅₀) will be adopted for the evaluation of toxicity

1. Non toxic : > 10,000
2. Slightly toxic: 1000 – 10000
3. Moderate Toxic: 100 – 1000
4. Toxic < 100

5. Dispersant alone should be moderately to slightly toxic
6. Dispersant should not significantly increase the toxicity of the oil, the involvement of the dispersant on the oil will be allowed at 15% – 20%.

1.4 Effectiveness test for oil dispersant

Swirling Flask Test (SFT), approved by (EPA, 2010) and Baffled Flask Test (BFT), suggested by Sorial et al. (2010) were selected to determine the efficiency. SFT is a currently approved method to determine oil dispersant efficiency in USA and BFT was proposed as newly developed method by EPA and many studies showed that these two methods are reliable and simple small scale laboratory tests can be used for determining oil dispersant efficiency.

1.4.1 Swirling flask test

Swirling flask test was done according to the guidelines published in U.S. Environmental Protection Agency. Adt concentrate Type-3 oil dispersant and Murban crude oil (density:0.837g/cm³) were used in the test. A 200ml Erlenmeyer flask, SCHOTT, DURAN Germany and 250 ml separation funnel (SCHOTT) was used as a replacement of modified Erlenmeyer flask with a side spout. Erlenmeyer flask was used to mix the oil in the dispersant with filtered sea water via 45µm mesh. A separation funnel was used to takeout mixed water sample without disturbing to top layers after settling. Dichloromethane (DCM) was used to extract the oil from the sea water.

1.4.2 Calibration of the UV-Visible spectrophotometer

Stock standard solution was prepared each day by mixing 1 part of oil to 9 part of DCM in an amber glass bottle and used for calibrating the spectrophotometer. A known volume of stock solution was added to 30ml filtered seawater in a 250ml separation funnel. Then 5ml of fresh DCM was used three times to extract the oil after vigorous shaking for 15 seconds and the setting time was 2 minutes. Extraction was done three times and the final volume was adjusted to 20ml and the different volumes of oil+DCM mixture and the final concentration of oil in extracted DCM are given in Table 3. The absorbance of extracted sample was measured at three different wavelengths (340nm, 370nm, and 400nm) by using the spectrophotometer (HACH DR4000U spectrophotometer HACH Company, Colorado) and response factor (RF_x) was calculated for checking linear stability of the instrument as follows:

$$RF_x = C/A_x \quad (1)$$

Where:

RF_x = Response factor at wavelength X (X= 340, 370, or 400 nm)

C = Oil concentration, in mg of oil/ml of DCM in standard solution

A_x = Spectrometric absorbance of wave length X

When RF_x for five standards of extracted oil are <20% different from the overall mean value of the five standards was considered as acceptable (EPA, 2010).

Table 3: Average oil dispersant effectiveness for oil plus dispersant and oil alone

Experiment Number	Mean % dispersant effectiveness for oil plus dispersant	Mean % dispersant effectiveness for oil alone
1	19.08	1.22
2	20.94	1.50
3	19.53	1.02
4	18.94	0.95
5	19.57	1.25
6	18.81	1.76
7	18.14	1.48
8	18.11	1.80

Dispersant efficiencies of oil plus dispersant mixture and oil alone are given in table 3 as a reference of a test and there was no significant difference among the absorbance values ($P>0.05$) in eight experimental setups. Three calibration concentration curves were obtained for three days and the calculated response factors were used to calculate dispersant efficiency. Since those (Chandrasekar, 2006) observed the effect of salinity on the dispersant efficiency immediately collected sea water was filtered and used for the experiment. The calculated average values for the replicates in each experimental setup were considered as the mean dispersant effectiveness.

1.4.3 Preparation and analysis of experimental sample

A series of experiment consists with four replicates of dispersed oil in a water mixture, one blank with only sea water and a control with only oil and seawater. Experimental sample was prepared by mixing 200 μ l of stock mixture with 120 \pm 2ml of filtered sea water in a 200ml Erlenmeyer flask. The stock mixture was added very carefully to the center of the flask by using a micropipette. The all 6 flasks were placed on the orbital shaker (2cm orbit, Lab companion SK300) tightly and agitated by 20 \pm 1 min at 150 \pm 10 rpm for preparing oil dispersant stock mixture, with one part of dispersant and ten parts of oil.

Then all samples were quickly and carefully poured to six 250ml separation funnels and allowed 10 minutes for settling. After 10min of settling, 30ml of sea water sample was carefully drained to another 250ml separation funnel and extracted to DCM. First 2ml of sample was drained out and the next 30 ml was taken. The sample was extracted three times by using 5ml portions of DCM and final volume was adjusted to 20ml. settling period for phase separation was 2 min. DCM are extracted to glass vials with Teflon cap with aluminum seal off. Finally Spectrophotometric absorbance was measured at wavelengths of 300nm, 370nm and 400nm and oil quantity in the DCM extraction was determined using equation 2:

$$C_x = (A_x) \times (RF_x) \times (V_{DCM}) \times (V_{tw} / V_{ew}) - (2)$$

Where:

C_x = Total mass of dispersed oil in swirling flask at wave length X

A_x = Spectrophotometric absorbance at wave length X

V_{DCM} = Final volume of DCM extract of water sample (20ml)

V_{tw} = Total water volume in swirling flask (120ml)

V_{ew} = Volume of water extracted for dispersed oil content (30ml)

Three values of oil concentration for each sample were obtained and mean values were calculated using equation 3:

$$C_{\text{mean}} = (C_{340} + C_{370} + C_{400}) / 3 \quad (3)$$

Dispersant performance (dispersed oil amount or EFF) was based on the ratio of the total oil dispersed in the test system to the total oil added to the flask and calculated according to equation 4:

$$\text{EFF} (\%) = (C_{\text{mean}} / C_{\text{TOT}}) \times 100 \quad (4)$$

Where:

C_{mean} = Average value for the mass of dispersed oil in swirling flask

C_{TOT} = Total mass of oil initially added to swirling flask

EFF was calculated for four experimental samples, blank and control.

Final efficiency of oil dispersant was calculated by using equation 5:

$$\text{EFF}_D = \text{EFF}_d - \text{EFF}_c \quad (5)$$

Where:

EFF_D = % dispersed oil due to dispersant only

EFF_d = % dispersed oil with dispersant added

EFF_c = % dispersed oil with no dispersant added

All calculations were based on; Part-300 national oil and hazardous substances pollution contingency plan (EPA, 2010).

1.4.4. Baffled flask test

Baffled flask test was followed by Sorial et al. (2006), Sorial (2006) and using a locally made baffled flask (Figure 3) by attaching four Perspex baffles inside the 250ml Erlenmeyer flask originally produced by SCHOTT, Duran Germany.



Figure 3: Locally prepared Baffled flask

1.4.5 Preparing the calibration curve and experimental samples for BFT

Murban crude oil (density 0.837gcm^{-3}) and South Louisiana crude oil (SLC) (density 0.839gcm^{-3}), used by Sorial, (2006) have similar densities. Hence that those oil were used for preparing the standard solutions for calibrating spectrophotometer using the amounts reported by Chandrasekar, (2006). The standard series was prepared using 2ml of Murban crude oil and 18ml of DCM and six points in the calibration curve were taken at 20, 50, 100, 150, 200, 300 μl of SLC-DCM stock solution with 30ml of sea water in a separation funnel.

The Test flask was placed on an orbital shaker and 120 ml of filtered sea water was added to the test flasks. Then 100 μl of oil was carefully placed at the center of the test flask by using micro pipette and after that 4 μl of dispersant was placed on the center of oil at 1:25 dispersant to oil ratio. The orbital shaker was set up to 150rpm for 20 min and then the samples were put into 250ml separation funnels and allow 10 minutes for settling. After settling 30ml of sample was

drained out and the extracted sample was stored under $4 \pm 2^{\circ}\text{C}$ until analysis. The absorbance values of the sample were determined using spectrophotometer (HACH DR4000) at the wavelengths of 340nm, 370nm and 400nm.

1.4.6 Calculation of efficiency

Calculation of efficiency was done according to Sorial, (2006). Oil dispersant efficiency was based on ratio of the total oil dispersed by oil dispersant to the total amount of oil added to experimental test flask. The area under the absorbance vs wavelength curve for experimental samples between 340nm and 400nm was calculated using trapezoidal rule according to equation 1:

$$\text{Area} = [(\text{Abs}_{340} + \text{Abs}_{370}) \times 30 + (\text{Abs}_{370} + \text{Abs}_{400}) \times 30] / 2 \quad (1)$$

Dispersant performance or effectiveness (Eff%) was given as 2:

$$\text{Eff \%} = \text{Total oil dispersed} \times 100 / \text{Density of oil} \times \text{Voil} \quad (2)$$

Where:

Density of oil expressed as g/L

Voil= Volume (L) of oil added to the test flask ($100 \mu\text{L} = 10^{-4}\text{L}$)

Total oil volume dispersed, g = Mass of oil \times [Vtw/ Vew] (3)

Where:

Vtw= Total water volume in the testing flask (120mL)

Vew= volume of water extracted for dispersed oil content (30mL)

Mass of oil, g = Concentration of oil \times VDCM (4)

VDCM = final volume of the DCM extract of water sample

Where;

Concentration of oil $l^{-1} = [\text{Area determined by equation 1/slope of calibration curve}]$

Conclusions:

The following conclusions can be taken based on the above explained experimental results.

The toxicity level of oil dispersant can be categorized as to the fish species, *Abudefduf vaigiensis* based on the LC₅₀ value (ppm).

The effectiveness of the oil dispersant was reached at the acceptable effectiveness limit (45%) according to the effectiveness test of SFT or not. The effectiveness test of BFT showed the value as..... for the dispersant.

Pass or fail criteria defined as,
Slightly toxic to non toxic: Pass
Effectiveness limit \geq (45%): Pass

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