Efficiency assessment of an oil dispersant (Adt type 3) by Swirling flask test & Baffled flask test under laboratory condition

M.V.K.S Wickramanayake, R. A. Maithreepala and H.B. Asanthi

Department of Limnology and Water Technology, University of Ruhuna, Matara, Sri Lanka

Abstract- This study was focused to assess efficiency and toxicity of an oil dispersant (Adt type 3) under the laboratory conditions. Swirling Flask Test (SFT) and Baffled Flask Test (BFT) were used for effectiveness assessments using Murban crude oil (density 0.837gcm^{-3}) as the reference oil. The locally made baffled flask was used with 100µl of oil, 4µl of dispersant and120ml of filtered seawater at 1:25 dispersant to oil ratio. The SFT was followed using1:10 dispersant to oil ratio and the dispersant efficiency was expressed as a ratio between amount of oil initially added to test flask and amount of oil extracted to dichloromethane (DCM). Oil was extracted by using DCM and then the absorbance was measured at wavelengths of 300nm, 370nm and 400nm to determine the extracted oil amount. A marine fish, *Neopamacentrus azysron*(yellow tail damsel fish)was used as the target organism for the toxicity test which is common at shore areas as well live up to 20m depth. Seven glass tanks (44x29x31cm^3) were used as six replicates and one control tank and each tank was filled with 30L of filtered sea water. Median LC50 was calculated using Trimmed SPEARMAN-KARBER method, version 1.0.The efficiency of Adt concentrated type 3 oil dispersant was determined as 55.29±1.47%, and 16.08±1.19% under BFT and SFT respectively. The swirling flask test showed relatively low efficiency value than BFT and the value was lower than the USEPA recommended efficiency level of 45%. Percentage dispersant occurred due to natural dispensability of oil was obtained as 1.105 (mean of three control experiments). There was no significant difference of absorbance values among the replicates of each set up average values were taken for response factor. Water temperature, salinity, NH_3, and pH did not show significant difference among the replicates of toxicity test. Dissolved Oxygen in the tanks reduced dramatically during first 3 hours and then it was constant. Acute toxicity level of Adt concentrate type 3 oil dispersant for *Neopamacentrus azysron*was 746.16±3.56 ppm and it is moderately toxic under the laboratory conditions. Aggressive behavior and increase of breathing rate were observed in the toxicity test at the lethal concentrations. The present assessment explains that the Adt type 3 is low efficient and low toxic oil dispersant.

Index Terms- BFT, efficiency, LC50, Oil dispersant, SFT, toxicity

I. INTRODUCTION

Remediation method for oil spill is to be decided based on the characteristic features of the oil and sensitivity of nearby environment considering climatic variables [1]. has been reported that natural organic sorbents can adsorb 3 and 15 times their weight in oil, natural inorganic sorbents adsorb 4 to 20 times their weight in oil and synthetic sorbents can absorb up to 70 times their weight in oil. Even though, bio remediation techniques of bio augmentation or bio stimulation recommended as economically feasible methods use of chemical dispersant had been introduced to treat Torry canyon spill off Cornwall, England in 1967 and later chemical oil dispersants have been reformulated due to its successfulness of applications[2]. However, a review of ITPOFs database of past oil spills has been reported that, among the 258 of recorded marine incidents between 1995 and 2005 only 18% had been used chemical dispersants 4at the sea[3].

Dispersants are made of surfactants (surface active agents) dissolved in one or more solvents, composed with both lipophilic and hydrophilic groups in their chemical nature. The lipophilic end of the molecules is attached to the oil phase and the hydrophilic end extended into the water phase [4]. Surfactant creates interface stabilization and it prevents collision with adjacent droplets and reduce adherence to hydrophilic solid particles. Ultimate action of dispersant is making tiny oil particles and breaks the oil slick to small droplets. The behavior of a surfactant is strongly affected by the balance between the hydrophilic and lipophilic groups (HBL) in the molecule[5]. Dispersants are made by mixing different types of surfactants at different ratios as such modern dispersant formulations containing one or more nonionic surfactants (15 to 75 percent of the formulation), anionic surfactants (5 to 25 percent of formulation) and one or more solvents [5].

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[6]. However, the major factor is considered as the composition of oil followed by sea energy and amount of dispersant added [7]. Rapid dispersion of dispersant-treated oil begins at a wind speed of approximately 7 knots with wave height of 0.3m. The formation of water-in-oil emulsion by wave action increases the viscosity of oil and thereby reduces the dispersant effectiveness [6]. Water chemistry is also important for oil dispersant efficiency and most

oil dispersants designed for working in salt water with 30 to 35psu (practical salinity unit). [7] Describes the increasing of 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. [6] Report explains four types of effectiveness tests including laboratory tests, mesoscale tests, and open water experiments. [8] 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 effectiveness. In a formal laboratory tests, some parameters need to be control, such as salinity, mixing type and energy input, method of applying dispersant, dispersant to oil ratio, temperature and oil related characters[9].Dilution due to advection, turbulent diffusion and energy regime are some factors of laboratory tests make difficulties to extrapolate[8]. [10] Said that the all chemical dispersant products need to pass the efficiency tests before it testing for toxicities.

Therefore, the present study focused to evaluate the effectiveness of oil dispersant chemical following two laboratory test methods and to evaluate acute toxicity of the dispersant on marine fish (*Neopamacentrus azysron*) with reference to [11].

II. MATERIALS AND METHODS

2.1. Effectiveness test for oil dispersant

Swirling Flask Test (SFT), approved by [12] and Baffled Flask Test (BFT), suggested by [13] 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.

2.1.1. 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 was taken from Marine Environmental Protection Authority Sri Lanka and Murban crude oil (density:0.837g/cm^3) was taken from the laboratory of Ceylon Petroleum Corporation, Sapugaskanda Refinery, Sri Lanka. 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.

2.1.1.1. 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 1. The absorbance of extracted sample was measured at three different wavelengths (340nm, 370nm, and 400nm) by using the spectrophoto meter (HACH DR4000U spectrophotometer HACH Company, Colorado) and response factor (RFx) was calculated for checking linear stability of the instrument as follows:

$RF_x = C/A_x$ (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 is considered as acceptable [12].

2.1.1.2. 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 200µl of stock mixture with 120±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 obit, Lab companion SK300) tightly and agitated by 20±1 min at 150 ±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:
$C_{mean} = (C_{340} + C_{370} + C_{400}) /3$ - (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:

$EFF\ (\%) = (C_{mean}/C_{TOT}) \times 100 - (4)$
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 of blank and control.

Final efficiency of oil dispersant was calculated by using equation 5:
$EFF_D = EFF_d - EFF_c$–(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 [12].

2.1.2. Baffled flask test

Baffled flask test was followed [13] [14] using a locally made baffled flask (Figure 1) by attaching four Perspex baffles inside the 250ml Erlenmeyer flask originally produced by SCHOTT, Duran Germany.

Figure 1: Locally produced Baffled flask

2.1.2.1. 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 [14]) have similar densities. Hence that those oil were used for preparing the standard solutions for calibrating spectrophotometer using the amounts reported by [15]. The standard series was prepared using 2ml of Murban crude oil and18ml of DCM and six points in the calibration curve were taken at 20, 50, 100, 150, 200, 300µl 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 100µl of oil was carefully placed at the center of the test flask by using micro pipette and after that 4µl 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±2^{0}C until analysis. The absorbance values of the sample were determined using spectrophotometer (HACH DR4000) at the wave lengths of 340nm, 370nm and 400nm.

2.1.2.2. Calculation of efficiency

Calculation of efficiency was done according to[14]. 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 6:

$Area = [(Abs340+ Abs370)\times30 + (Abs370 + Abs400\_) \times30] / 2$ (6)

Dispersant performance or effectiveness (Eff%) was given as 7:
Eff % = Total oil dispersed ×100/Density of oil × Voil (7)
Where:
Density of oil expressed as g/L
Voil= Volume (L) of oil added to the test flask (100 µL = 10^{-4}L)
Total oil volume dispersed, g = Mass of oil × [Vtw/ Vew] (8)
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 × VDCM (9)
VDCM = final volume of the DCM extract of water sample
Where;
Concentration of oil l^{-1}= [Area determined by equation 1/slope of calibration curve]

2.2. Statistical analysis

Descriptive data analysis for calculate mean, variance, standard error and standard error was done by Microsoft excel 2013 version. One way ANOVA was used to compare the significance of variations of absorbance values in separate experiments. All statistical analysis was done using SPSS 16.0.

2.3. Toxicity test

Seven glass tanks (44x29x31cm^3) were used and arranged as one control tank (C1) and 6 replicates (R1, R2, R3, R4, R5, and R6). The length of the largest fish (*Neopamacentrus azysron*) was not being more than twice that of smallest in the same test[16] and the range was used from 5.6cm to 6.1cm (fig 2). All experimental tanks were filled with 30Lof filtered sea water and eight acclimatized fish were added into each tank and aeration rate were adjusted to same rate for each tank before 24 hours of applying chemical.

Figure 2: The selected fish species (*Neopamacentrus azysron*)

2.3.1. Determination of effective concentration range

Range finding test was done using broad concentrations and 24 hours exposure time according to [17]. One experimental setup was used with continuous aeration, according to the commendation by [11]. Adt denote the(4-amino,1,2-dithiolane-4-carboxylic acid) concentration series of 0, 50, 100, 150, 200, 250, 300, 350, 400, 450,500,550, 600, 650, 700, 750, 800 ppm were prepared using 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12.0, 13.5, 15.0, 16.5, 18.0, 19.5, 21.0, 22.5, and 24.0g of the dispersant respectively. Mortality and all other functional and morphological changes were recorded during 24 hours period.

2.3.2. Acute toxicity test

Acute toxicity test was conducted according to EPA/600/4-90/02 protocol [12]. Experimental concentration series, 650ppm to 800ppm was selected by considering the preliminary range finding test. 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. LC50 values were calculated using Trimmed SPEARMAN-KARBER method for estimating median lethal concentration in toxicity bioassays. Physico-chemical parameters were measured (Table 2) before and after adding dispersant to the tanks. Fish feeding was ceased 24 hours prior to starting toxicological test and ended after 72 hours.

Table 2: Analytical techniques for measuring physico-chemical parameters

Parameter	**Instrument**
Temperature	pH10, pH meter
pH	pH10, pH meter
Ammonia (NH_3)	Zoolek salt water NH3 test kit
Salinity	YSI 85 meter (Japan)
Dissolve Oxygen (DO)	YSI 85 meter (Japan)

III. RESULTS AND DISCUSSION

3.1. Effectiveness test by swirling flask test

The response factors for the absorbance values in instrument calibration procedure are given in table 4. Response factor (RF) describes the portion of oil concentration which represented by one unit of absorbance converting the absorbance values of experimental sample to concentration of oil [12].

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

There was no significant difference among the absorbance values ($P>0.05$) in eight experimental setups. Dispersant efficiencies of oil plus dispersant mixture and oil alone are given in table 3.

Three calibration concentration curves were obtained for three days and the calculated response factors were used to calculate dispersant efficiency as given in table 4. Since those [15] 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.

Table 4: Response factors for calibrating instrument and calculating dispersed oil amount

Oil concentration g/l	Response factor for 340nm	Response factor for 300nm	Response factor for 400 nm
Day 1			
0.0045	0.042	0.109	0.225
0.09	0.399	0.725	1.475
0.45	0.584	1.300	2.054
1	0.916	1.579	2.409
1.81	1.237	1.535	2.078
3.68	2.478	2.606	3.458
Day 2			
0.0045	0.037	0.083	0.136
0.09	0.393	0.810	1.323
0.45	0.579	1.187	1.948
1	0.825	1.494	2.392
1.81	1.290	1.532	2.061
3.68	2.619	2.587	3.524
Day 3			
0.0045	0.037	0.086	0.145
0.09	0.391	0.825	1.343
0.45	0.576	1.2	1.948
1	0.823	1.510	2.392
1.81	1.284	1.540	2.059
3.68	2.595	2.609	3.569

Dispersant effectiveness due to oil dispersant (Table 5) was calculated by subtracting the oil alone dispersant effectiveness from dispersant effectiveness in oil plus dispersant (data given in Table 3).Overall oil dispersant effectiveness was calculated as a mean of eight corrected oil dispersant efficiencies. Percentage dispersant effectiveness due to dispersant and its 95% confidence limit are given in table 5. Percentage oil dispersant effectiveness of Adt concentrate type 3 oil dispersant for Murban crude oil under EPA swirling flask test was 16.08. [13] [18] discussed the repeatability and reproducibility of dispersant effectiveness tests among different laboratories should have high values and the present study showed the relevance (Table 5). This is not a rare value for SFT effectiveness test because [14] and [19] showed that the SFT reported a relatively low percentage dispersant value. However, the recommendation of US EPA national oil and hazardous substances pollution contingency plan (40 CFR Ch.1 (7-1-14 edition) was the average dispersant effectiveness should be at least 45% (50%±5%). Hence that Adt concentrate type 3 oil dispersant for Murban crude oil did not achieve the recommended effectiveness level.

Table 5: Corrected oil dispersant effectiveness and dispersant effectiveness at 95% confidence limit.

Experiment no	Dispersant % effectiveness due to oil dispersant	Dispersant % effectiveness at 95% lower confidence level
1	17.86	15.90
2	19.44	16.91
3	18.51	17.13
4	17.99	16.07
5	18.32	17.42
6	17.05	16.53
7	16.66	14.36
8	16.31	14.31

3.2. Effectiveness test by Baffled flask test

The locally produced Baffled flask was used by obtaining same mixing regime which has been described in [14]. [20] Reported that the geometry is the major factor for obtaining similar conditions same to the sea in the baffled flask. Perspex and epoxy resin glue did not degenerate either by oil, seawater or dispersant and therefore it confirmed that there was no interference for the absorbance readings in the experiment.

There was no significant difference among absorbance values of three baffled flask tests. It was observed that the filtered sea water in experimental flask was become brownish color when it was in an orbital shaker. This brownish water gradually reduced its color and oil slick was re appearing on the water surface as a significant phenomenon in the control flask.

Mean slope of the calibration curve for Oil + DCM concentration series was 0.534. The mean slope of the calibration curve for dispersant, Oil and DCM was 0.432. Percentage dispersant occurred due to natural dispensability of oil was obtained as 1.105 (mean of three control experiments). Mean percentage dispersant of "oil+dispersant" mixture was obtained as 58.32.Summary of mean dispersant values, corrected values by subtracting the natural dispersant and 95% confidence limit of corrected percentage dispersant are given in table 6.

Table 6: Summary of average % oil dispersant effectiveness and 95% lower confidence limits

Experiment	Average % oil dispersant effectiveness	95% lower confidence limit	Average % oil dispersanteff: in controller	95% lower confidence limit for contr ol	Final dispersant effectiveness 95% LCL
1	57.32	54.31	1.06	0.90	53.76
2	58.31	56.10	0.94	0.78	55.43
3	59.79	56.10	0.94	0.78	56.69

Percentage oil dispersant efficiency of Adt percentage effectiveness values obtained under two experimental setups are shown in fig 3. Percentage dispersant effectiveness of 55.29 was a relatively high value than that of SFT value of 16.08.Under same laboratory condition and same chemicals, BFT and SFT show the different percentage effectiveness values. This is a common difference as described in [13] [14].

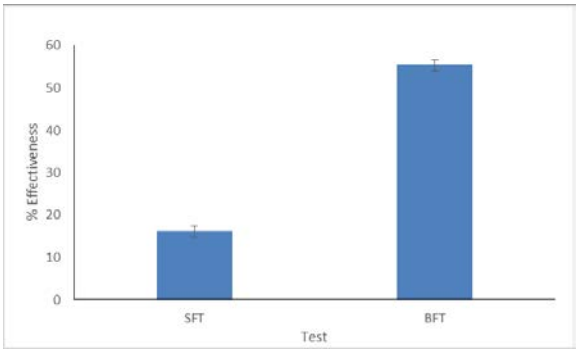

Figure 3: Percentage effectiveness of Adt concentrate type 3 oil dispersant under BFT and SFT

3.3. Water quality in toxicity testing tanks

The statistical data mean values, the maximum and minimum values of water quality parameters in the three replicates are given in the table 7. There was no significant difference ($p>0.05$) in temperature, salinity, pH and ammonia among the three replicates.

Table 7: mean minimum and maximum values of the water quality parameters in six replicates of three series

Parameter	Series 1	Series 2	Series 3
Temperatur e Mean±SD Min-max	29.91±0.2 29-30.2	29.86±0.3 6 29-30.5	29.90±0. 47 29-31
Salinity Mean±SD Min-max	30.07±0.0 4 30-31	30.07±0.4 5 30-30.1	30.07±0. 04 30-30.1
pH Mean±SD Min-Max	9.35±0.20 9-9.8	9.47±0.16 9-9.9	9.47±0.1 6 9.1-9.9
NH3 Mean±SD Min-Max	0 0	0 0	0 0

Mean water temperature in the tanks was around 30^{o}C and it was similar to the ambient temperature. Salinity did not fluctuate significantly (30.0-30.1 ppt) in the experimental tanks. Total $NH_3$was not detected in the tanks and therefore those parameters did not effect on the mortality of fish. Dissolved oxygen content in the experimental tanks reduced from 13ppm to 8 ppm during first five hours of the test and later it became constant. However, there was no evidence to assume DO as a critical factor for fish mortality in the test.

Reduction of DO in the tanks with continuous aeration was observed and it indicated that the dispersant chemical affected to reduction of dissolve oxygen making stressful condition to the test organisms. Although, water pH did not decrease with Adt, Fromm, (1980) discussed fish mortality due to acidic group of Adt (-COOH).

3.4. Mortality of fish

Fish mortality was observed after 3 hours and ended after 19 hours (figure 4) at the all three series of experimental setups. At the end of experiment, a white color layer was observed on the water circulating parts of the aeration pump and also on the tank walls. It can be supposed that the same phenomena can be happen on the fish respiratory organs. [21] reported that some morphological and functional changes of fish gill cause the death of fish. Therefore, the appearance of white foam due to oil dispersant may cause to increase the fish mortality.[22] said that the surface active agents delay the coalescence of gas bubbles and thus making gas-liquid interface layer and reduce the bulk motion of liquid. [22] Described that the increasing stability of air bubbles in water and [23] reported that the stable air bubbles or large number of air bubbles can be affected on the fish gills and cause fish death by gas emboli in blood capillaries.

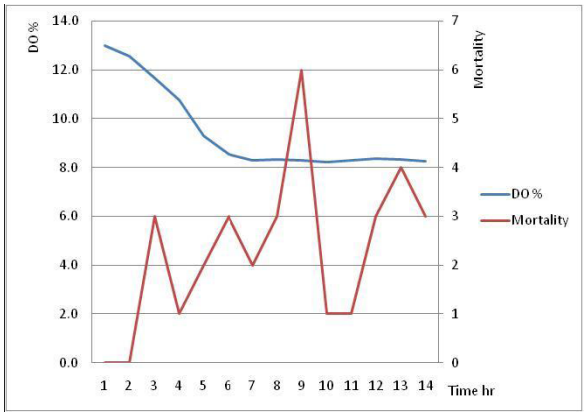

Figure 4: Mean number of mortality and % DO changes in experimental tanks

3.5. Toxicity parameters

Lethal concentration values in three experimental series with lower and upper 95% confidence levels (95% LCL and 95%UCL) are shown in Table 8. The average LC50 value for three experimental test series was 746.16±3.50 and according to US EPA toxicity selection criteria given in Table 9, Adt concentrate type 3 oil dispersant is as practically nontoxic to *Neopomacentrus azysron.* However, according to the selection criteria of [24] Adt concentrate type 3 oil dispersant can be considered as moderately toxic to the fish. [11] Reported mean LC50value for *Abudefduf vaigiensis* as 70.319 ±0.576ppm under the similar laboratory condition and same dispersant type and the value is 10 times lower than that of the present study.

Table 8: LC_{50} values and 95% confidence limit for three experimental series

Test series	LC50 (ppm)	95% LCL	95%UCL
1	742.67	725.62	760.12
2	749.68	731.37	768.68
3	746.14	727.99	764.74

Table 9: EPA five-step scale of toxicity categories used to classify chemicals based on their acute toxicity [12].

LC_{50} (ppm)	Toxicity classification
>100	Practically Non toxic
>10-100	Slightly Toxic
>1-10	Moderately Toxic
0.1-1.0	Highly toxic
<0.1	Very Highly Toxic

3.5. Behavioral observation of fish

After adding oil dispersant, white color plume appeared in the water column and after a few minutes 1 to 2 cm thick white color, foam was observed in the experimental tanks. The thickness of the foam increased with the increasing of concentrations. Many fish responded to the chemical significantly in several ways such as fish were swimming near glass walls of the tanks, breathing rate and fin movement rates were increased at the death time and loss of balance, lethargy behaviors and relaxing movements. At higher concentrations, frequent movement to water surface was observed during the experiment however no breathing movements were observed as described in the test of oil dispersant toxicity test by [11] behavioral changes such as, uncoordinated movement, erratic swimming, convulsion, loss of balance were observed in the present study as discussed by[11][25][26]) in toxicological tests using fish.

IV. CONCLUSION

The effectiveness of Adt concentrate type 3 oil dispersant was failed to reach the acceptable effectiveness limit (45%) according to the effectiveness test of SFT. The observed effectiveness was 16.08% for Murban crude oil at 150 rpm and 1:10 dispersant to oil ratio. Even though there is no criterion for BFT it showed the effectiveness value of 55.29% for the Adt type 3 dispersant in the present study. Toxicity of type 3 dispersant categorized as partially nontoxic to the fish species *Neopomacentrus azysron* and the LC50 value was 746.16ppm.

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AUTHORS

First Author – M.V.K.S Wickramanayake - B.Sc. Fisheries and Marine sciences. University of Ruhuna, Matara, Sri Lanka, Wickramanayake.mvks@gmail.com
Second Author – Dr. R.A. Maithreepala - B.Sc. (Ruhuna), PhD (NTHU, Taiwan), Faculty of Fisheries and Marine Sciences & Technology, University of Ruhuna, Matara, Sri Lanka, Maithree@fish.ruh.ac.lk
Third Author – Dr. H.B. Asanthi - B.Sc. (Honours) (Ruhuna), PhD (Montpellier, France), Faculty of Fisheries and Marine Sciences & Technology, University of Ruhuna, Matara, Sri Lanka, asanthi@fish.ruh.ac.lk

Correspondence Author – M.V.K.S. Wickramanayake, Wickramanayake.mvks@gmail.com, +94712328674, +94766073514