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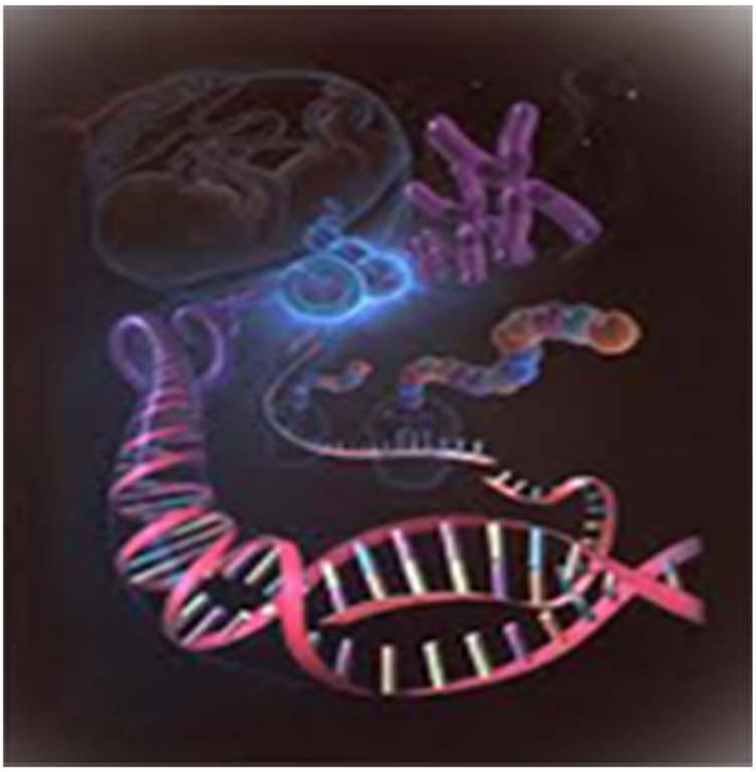

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Research Paper

ACUTE TOXICITY OF THE MIXTURES OF GREASE AND ENGINE WASH OIL ON *FISH, PANGASIUS SUTCHI* UNDER LABORATORY CONDITION

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Oil contamination causes morphological, cytological and behavioral abnormalities in fish. This study was undertaken to evaluate the short-term exposure effect of the mixture of grease and engine wash oil on *Pangasius sutchi.* Fishes exposed to the mixtures of grease and engine wash oil showed various behavioral patterns depending on the amount of doses and exposure time. The test fishes showed different behavioral signs like restlessness, continuous discomfort in swimming, jumping, jerky movement, frequent surfacing, opening and closing operculum, mouth and loss of equilibrium at the final stage. The changes in the mortality of *Pangasius sutchi* was observed at various exposure periods, i.e., 24, 48, 72 and 96 hrs respectively. The higher exposure period revealed higher percentage of mortality and it was further intensified at higher concentrations. At the concentrations of 3.0 ml/L, 3.5 ml/L, 4.0 ml/L and 4.5 ml/L, the mortality rate found for this fish for 24 h and 48 h were 0%, 0%, 10%, 20% and 0%, 20%, 30%, 40% respectively, while at the same concentrations the mortality rate of 72 and 96 h were 10%, 30%, 50%, 60% and 20%, 50%, 70%, 90% respectively. In this study a statistical software EPA probit analysis was used to find out the effective dose. The lethal concentrations (LC_{50} values) of 24 h and 48 h were 5.10 ml/L and 4.65 ml/L respectively, while the LC_{50} values of 72 and 96 h were 4.10 ml/L and 3.54 ml/L respectively. The values of LC_1, LC_5, LC_{10}, LC_{15}, LC_{50}, LC_{85}, LC_{90}, LC_{95} and LC_{99} varied inversely with the increase of exposure time. This contribution provides valuable information about the lethal concentration of oil and shows that as the exposure time increases the toxicity also increases.

Keywords: Grease and engine wash oil, Behavioural pattern, Mortality rate, LC50, Probit analysis

INTRODUCTION

Petroleum or crude oil is a naturally occurring liquid found in forming the earth consisting of a complex mixture of hydrocarbons of various lengths. The approximate length range is C_5H_{12} to $C_{18}H_{38}$. It is usually black or dark brown but varies greatly in appearance, depending on its composition. Petroleum is used mostly, by

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volume, for producing fuel oil and gasoline, both important "primary energy" sources. 84% by volume of the hydrocarbons present in petroleum is converted into energy-rich fuels, including gasoline, diesel, jet, heating and other fuel oils and liquefied petroleum gas (Tong *et al*., 1999).

Oil constitutes several hydrocarbon of which includes gasoline, kerosine, diesel and gas fractions. Spilled oil and grease floats on the surface of water bodies limit gaseous exchange, entangles and kill surface organism and coats the gill of fish (Wells *et al*., 1995, Spies *et al*., 1996). It also retards phytoplankton photosynthesis, respiration and growth, kills or causes development abnormalities in zooplankton and the young stages of many aquatic organisms and cause tainting of fish, shell fish (Afolbai *et al*., 1985; Otitoloju and Don-Pedro, 2002). A study (Anderson and Weber, 1975) reported that when petroleum products are high in concentration, the higher part of the petroleum causes toxic effect on flora and fauna. Fish also suffer from various effects of oil contamination including morphological, cytological and behavioral abnormalities, in addition to abnormal differentiation and mitotic abnormalities in fish eggs. Due to their mobility, fish are able to avoid high concentrations of oil-contaminated water, and most mortality following an oil spill is found in the immediate vicinity of the spill. A large portion of this mortality is due to the effects of dispersants rather than the oil itself (Koyama and Kakuno, 2004). Fish eggs and larvae are more sensitive to PAHs than adults, as they tend to float in surface waters proximal to contaminating oil (Irwin *et al*., 1997).

Oil residuals coming from workshops, automobiles, refinery, oil terminals, depots, merchant ships and tankers discharge into the rivers which contaminate the river water and sediment and find its way to the coastal environment ultimately. In addition the coastal region in Bangladesh especially estuaries, lagoons, mangrove swamps are often exposed to petroleum hydrocarbon arising from major and minor oil spillages. The indiscriminate discharge of oil and grease into our coastal aquatic ecosystem may bring deleterious effect (Hossain *et al*., 2005).

Fish is one of the main sources of dietary protein for the people of Bangladesh. The present stock of fish is not adequate to meet the demand of the country. Although a number of different fish species are being cultured but maximum supply of the fresh water fish comes from non cultured practice, i.e., from age old conventional sources. These fishes are from rivers, canals, streams, haors, lakes, ponds etc. of the country. *Pangasius sutchi* popularly known as Thai catfish is a beautiful fish to water as they are very graceful and elegant swimmers. It *(P. sutchi)* has been imported to Bangladesh from Thailand in 1990 (Sarker, 2000). This fish is basically a riverine species and the culture practice of this fish has been spread out very quickly throughout the country due to its high demands in the market. Its white creamy and delicate flesh with fewer intramuscular bones has a special appeal to consumers. This fish can tolerate a salinity level of 20‰ and very much suitable for growing in brackish water ponds. It is fast growing, easy to culture and is profitable for commercial purposes. Because of good taste it is liked by people of all ages and groups. These species is important as main dietary animal protein source especially for the common people (Rahman, 1989). Reports of the works done elsewhere on the effect of grease and engine wash oil on behavior, and mortality of different fish species is available

(Clark, 1982; Nelson-Smith, 1990 and Chukwu, 2006) but any published report in this regard on indigenous and exotic fish species of Bangladesh is not yet available. Therefore, the need to establish toxic action of the mixtures of grease and engine wash oil against *Pangasius sutchi* as the basis of standard aimed at protecting aquatic lives prompted the present study. In view of above mentioned facts, the aims of this study were i) to observe the toxic action of grease and engine wash oil on behavioral pattern of test fish, *Pangasius sutchi,* ii) to determine the mortality rate of test fish, *P. sutchi* at different concentrations of grease and engine wash oil and iii) to detect the lethal concentration (LC_{50}) of grease and engine wash oil for the test fish, *P. sutchi.*

MATERIALS AND METHODS

The present research work was conducted in the Environmental Pollution Laboratory of the Institute of Marine Sciences and Fisheries, University of Chittagong, Bangladesh during the year 2012. Prior to set the experiment, live and healthy fingerlings of *Pangasius sutchi* used for this study were collected from a farm at Hathazari upazilla under the district of Chittagong of Bangladesh. Fishes were transported to the laboratory in oxygenated polythene bags as soon as it was harvested from the culture pond. In the laboratory, the fishes were kept in glass holding tanks (30"×18"×16") half filled pond water and acclimatized for a period of 12 h. The water was continuously aerated with 220 v air pumps. The mechanical aeration was provided for maintaining the optimal dissolved oxygen level. Each aquarium was filled with 60L of pond water. Ten (10) individuals (mean weight 11.00±0.62 gm, mean length: 4.27±0.22 cm) were released in each aquarium providing no feed and were acclimatized for I2hrs maintaining an ideal condition of the water parameters. Before using them in laboratory, bioassay tests were used in accordance with guidelines developed by Weber, 1993.

The mixture of the grease and engine wash oil was used as a test compound. The grease and engine wash oil used for this experiment was collected from automobile workshop at Bhadarhat in Chittagong metropolitan city of Bangladesh. Oil and grease was properly mixed by stirring with glass rod before applying in the aquaria.

The bioassays were carried out in glass tanks. These glass tanks were preferred to plastic container as they minimized absorption of toxicant and prevent risk of corrosion and chemical reactions. Some plastics are known to react with some crude oil compounds (Otitoloju and Don-Pedro, 2002*).* A well ventilated and damp proof room was selected to save the experiment from detrimental effects of the surrounding. Five aquaria of equal size (30"×18"×16") were set up in the room before starting the experiment. All the aquaria were washed thoroughly with tap water. Necessary equipments and chemicals were collected and set in the laboratory for measuring the water parameters and room temperature. Pond water was used as the medium for all the bioassay tests conducted. Water was measured using measuring cylinder into clean dry bioassays containers and predetermined volume of mixtures of grease and engine wash oil was added into the water to make it up to 60L to achieve the desired test concentration. A glass rod was used to stir the mixture.

Ten fishes of similar sizes were released in each aquarium providing no food and acclimatized for 12 h maintaining an ideal condition of the water parameters like temperature (30°±0.5°C), pH (6.68±0.20) and dissolved oxygen (5.20 ± 0.25

ml/L). Time to time bottom of the each aquarium was cleaned off by siphoning. Out of five aquaria, one aquaria designated as control group, was not provided with pollutant but all other conditions remained same as that of the other aquaria where pollutant was provided. After the application of pollutant at different concentrations (3.0 ml/L, 3.5 ml/L, 4.0 ml/L, 4.5 ml/L*)* at four aquaria, behavioral patterns and mortality were observed and recorded after 24, 48, 72 and 96 h intervals respectively. The doses were applied randomly to each aquarium and two replicate experiments were practiced for getting the precise results. Room temperature and temperature of water were measured by a centigrade laboratory thermometer having a range from 0°-110°C. Dissolved oxygen content of water was determined by Winkler's azide modifications of Iodometric method (APHA, 1976). pH was determined by using laboratory digital pH meter (Model TOA HM5B, Tokyo Electronics Ltd. Japan).

The test compound was measured by a micropipette having ten divisions in one ml scale. Each of the main divisions of the micropipette has ten sub divisions having the volume 0.01 ml for each division. With the help of above mentioned micropipette, the test liquid compound was applied and mixed with water of the aquarium very carefully. Statistical software (EPA probit analysis program, version 1.5) was used for analyzing the data on mortality rates of test fishes obtained from the experiments (Finney, 1971; Fisher and Yates, 1963).

The fish, *Pangasius sutchi* were assumed to be dead if there was no movement of appendages, opercula and mouth or failed to respond to the touch of forceps/glass rod.

RESULTS AND DISCUSSION

Behavioral Effects

At low concentration of grease and engine wash oil (3.0 ml/L) the test fishes show no significant abnormalities in their behavior during the early period of exposure. At the end of the exposure (96 h) erratic movements resulted and some fishes die (Table 1). At the concentration of 3.5 ml/L and 4.0 ml/L the fish showed erratic movement after a short period of time. With the increase in time, movement of the test fishes was near the surface of water. Besides, gulping of air and jumping behavior were revealed by a number of fish and they died within 96 h at different intervals of time (Table 1). A peculiar behavior observed when the test fish species were

Table 1: Percentage Mortality *of Pangasius sutchi* at Different Concentrations of the Mixtures of Grease and Engine Wash Oil for Different Exposure Periods

Concen. ml/L	No. of Fish Taken		24 h				48 h				72 h				96 h			
	R1	R2	R1 No. of Died	R2 No. of Died	Total No. of Died	Percentage of mortality	R1 No. of Died	R2 No. of Died	Total No. of Died	Percentage of mortality	R1 No. of Died	R2 No. of Died	Total No. of Died	Percentage of mortality	R1 No. of Died	R2 No. of Died	Total No. of Died	Percentage of mortality
Control	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.0	10	10	0	0	0	0	0	0	0	0	1	1	2	10	2	2	4	20
3.5	10	10	0	0	0	0	1	3	4	20	3	3	6	30	6	4	10	50
4.0	10	10	1	1	2	10	3	3	6	30	5	5	10	50	8	6	14	70
4.5	10	10	2	2	4	20	3	5	8	40	7	5	12	60	9	9	18	90

released in the toxic solutions of high concentration (4.5 ml/L) of grease and engine wash oil. Rapid movement started after their exposure to high concentrations. Immediately placing into high concentration they showed high excitement and they tried to come out of water with frequent jumping.

There are few adequate studies on behavioral effects. Respiratory stress and erratic movement of fish were noticed by Belding (1929) in the toxic environment. EPA, US (1976) compiled data on lethal toxicities of various petroleum products to aquatic organisms and reported that adverse behavioural effects can result from petroleum product concentrations as low as 10 to100 μg/L. The fish, *Fundulus similus,* exhibits abnormal behavior at concentrations of 200 ppm. Suppression in the locomotor activity of an amphipod and a coelenterate medusa was observed after exposure to extracts of four oils at 15 ppm or greater (Anderson, 1979). In general, levels of 0.1 to 0.3 total naphthalenes, regardless of the oil, appear to be the lowest concentrations in water which produce abnormal and deleterious responses during long-term exposure to sublethal levels of petroleum hydrocarbons. The behavioral activities may be divided into three stages as observed by Kabir and Begum (1978) and Islam (2001) of which erratic movement was the first stage, paralysis was the second stage and settle down of the organisms at the bottom resulting death was the final stage. Similar type and sequence in behavioral activities were observed in the present study which confirmed the findings of the previous studies. Abnormal movements of the fishes in this study at the first stage were probably due to nerve inhibiting action of test compound. This result approved the findings that were reported by the authors (Oyewo, 1986; Chukwu, 2006; Chukwu and Ugbeva, 2003).

Toxic Effects

Toxic effect of different concentrations of grease and engine wash oil on fish species, *Pangasius sutchi* was studied during the exposure period of 24, 48, 72 and 96 h. Percentage mortality of *Pangasius sutchi* at different concentrations of the mixtures of grease and engine wash oil at different exposure periods has been given in Table 1. Mortality rates recorded at 24 and 48 h exposure were 0%, 0%, 10%, 20% and 0%, 20%, 30%, 40% at the concentrations of 3.0 ml/L, 3.5 ml/L, 4.0 ml/L and 4.5 ml/L respectively. On the other hand mortality rates recorded at 72 and 96 hrs exposures were 10%, 30%, 50%, and 60% and 20%, 50%, 70% and 90% respectively at the same concentrations. The LC_{50} value at 24 hrs exposure was 5.10 ml/L where lower and upper limit were 4.59 ml/L and 28.00 ml/L respectively (Table 2). The value of chi-square for the 24 h exposure was insignificant with 3 degrees of freedom ($\lambda^2 = 0.598$, $p > 0.05$, Table 2).

The LC_{50} value at 48 h exposures was 4.65 ml/L with 95% confidence limit where the lower and upper limits were 4.23 ml/L and 6.34 ml/L respectively (Table 3). The value of chi-square for 48 h exposure data was insignificant at 5% level with 3 degrees of freedom ($\lambda^2 = 1.967$, $p > 0.05$, Table 3).

The LC_{50} value at 72 h exposure was 4.10 ml/L with 95% confidence limit where the lower and upper limits were 3.79 ml/L and 4.76 ml/L respectively (Table 4).

Table 2: Output from EPA Probit Analysis Program (Version 1.5) for the Exposure of *Pangasius sutchi* to the Mixture of Grease and Engine Wash Oil for 24 H

EPA Probit Analysis Program Used for Calculating LC/EC Values (Version 1.5)

Conc.	No. Exposed	No. Resp.	Observed Proportion Resp.	Proportion Responding Adjusted for Controls	Predicted Proportion Responding
3.0000	20	0	0.0000	0.0000	0.0006
3.5000	20	0	0.0000	0.0000	0.0104
4.0000	20	2	0.1000	0.1000	0.0680
4.5000	20	4	0.2000	0.2000	0.2212

Chi-Square for Heterogeneity (calculated) = 0.598

Chi-Square for Heterogeneity (tabular value at 0.05 level) = 5.991

Mu = 0.707571 Sigma = 0.070755

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-5.000254	4.072796	(-12.982935, 2.982428)
Slope	14.133221	6.490885	(1.411086, 26.855354)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

Point	Exposure Conc.	95% Confidence Limits	
		Lower	Upper
LC/EC 1.00	3.491	0.608	3.889
LC/EC 5.00	3.901	1.808	4.213
LC/EC 10.00	4.139	3.073	4.626
LC/EC 15.00	4.308	3.825	5.663
LC/EC 50.00	5.100	4.587	28.003
LC/EC 85.00	6.038	5.055	150.702
LC/EC 90.00	6.284	5.167	224.640
LC/EC 95.00	6.667	5.336	405.960
LC/EC 99.00	7.450	5.665	1232.433

The value of chi-square for 72 h exposure data was insignificant with 3 degrees of freedom (λ^2 =0.359, p>0.05, Table 4). The value of LC_{50} at 96 hrs exposures was 3.54 ml/L with 95% confidence limit where the lower and upper limits were 3.28 ml/L and 3.77 ml/L respectively. The value of chi-square for 96 h exposure data was insignificant at 5% level with 3 degrees of freedom (=0.175, p>0.05, Table 5).

According to Stephens *et al*. (1997), fishes are sensitive to higher uptake of petroleum-derived hydrocarbons. Several effects like stress, genetic

Table 3. Output from EPA Probit Analysis Program (Version 1.5) for the Exposure of *Pangasius sutchi* to the Mixture of Grease and Engine Wash Oil for 48 H

EPA Probit Analysis Program Used for Calculating LC/EC Values (Version 1.5)

Conc.	No. Exposed	No. Resp.	Observed Proportion Resp.	Proportion Responding Adjusted for Controls	Predicted Proportion Responding
3.0000	20	0	0.0000	0.0000	0.0414
3.5000	20	4	0.2000	0.2000	0.1304
4.0000	20	6	0.3000	0.3000	0.2758
4.5000	20	8	0.4000	0.4000	0.4489

Chi - Square for Heterogeneity (calculated) = 1.967

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 5.991

Mu = 0.667303 Sigma = 0.109605

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-1.088285	1.729397	(-4.477902, 2.301333)
Slope	9.123713	2.900321	(3.439083, 14.808342)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

Point	Exposure Conc.	95% Confidence Limits	
		Lower	Upper
LC/EC 1.00	2.584	1.280	3.073
LC/EC 5.00	3.069	2.003	3.445
LC/EC 10.00	3.364	2.527	3.686
LC/EC 15.00	3.579	2.932	3.888
LC/EC 50.00	4.648	4.227	6.343
LC/EC 85.00	6.038	5.052	12.478
LC/EC 90.00	6.424	5.257	14.682
LC/EC 95.00	7.040	5.572	18.693
LC/EC 99.00	8.361	6.208	29.437

damage, lower growth rate and high mortality have also been reported by the authors (Al-Yakoob *et al*., 1996; Stephens *et al*. (1997); Gravato and Santos, 2002). Similar findings also noticed in the present study. The toxicity (96 hour LC_{50}) of crude oil in water to a variety of adult marine fish and invertebrates tested in static exposures ranges from 1 to 20 ppm (Anderson, 1979). Anderson (1977) has summarized some recent studies used to determine the effects of petroleum hydrocarbons on growth and reproduction.

Table 3: Output from EPA Probit Analysis Program (Version 1.5) for the Exposure of *Pangasius sutchi* to the Mixture of Grease and Engine Wash Oil for 72 H

EPA Probit Analysis Program Used for Calculating LC/EC Values (Version 1.5)

Conc.	No. Exposed	No. Resp.	Observed Proportion Resp.	Proportion Responding Adjusted for Controls	Predicted Proportion Responding
3.0000	20	2	0.1000	0.1000	0.1217
3.5000	20	6	0.3000	0.3000	0.2771
4.0000	20	10	0.5000	0.5000	0.4627
4.5000	20	12	0.6000	0.6000	0.6351

Chi - Square for Heterogeneity (calculated) = 0.359

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 5.991

Mu = 0.612980 Sigma = 0.116487

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-0.262224	1.427208	(-3.059552, 2.535103)
Slope	8.584655	2.448371	(3.785848, 13.383462)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

Point	Exposure Conc.	95% Confidence Limits	
		Lower	Upper
LC/EC 1.00	2.198	1.087	2.702
LC/EC 5.00	2.639	1.638	3.053
LC/EC 10.00	2.909	2.032	3.266
LC/EC 15.00	3.106	2.345	3.427
LC/EC 50.00	4.102	3.788	4.762
LC/EC 85.00	5.416	4.696	8.625
LC/EC 90.00	5.785	4.910	9.988
LC/EC 95.00	6.377	5.239	12.428
LC/EC 99.00	7.655	5.907	18.758

The concentrations of both total hydrocarbons and total aromatics shown to reduce growth and/or survival range from about 0.2 to 10 ppm. In the present study different mortality rates were found at different concentrations (Table 1). It is evident from the Table 1 that with the increase of concentrations of the mixture of grease and engine wash oil, the mortality rates of the test fishes increased at a given exposure time and at a given concentration, the mortality rates increased with increase of exposure time. The present experiment indicates that the extent of

Table 5: Output from EPA Probit Analysis Program (Version 1.5) for the Exposure of *Pangasius sutchi* to the Mixture of Grease and Engine Wash Oil for 96 H

EPA Probit Analysis Program Used for Calculating LC/EC Values (Version 1.5)

Conc.	No. Exposed	No. Resp.	Observed Proportion Resp.	Proportion Responding Adjusted for Controls	Predicted Proportion Responding
3.0000	20	4	0.2000	0.2000	0.2033
3.5000	20	10	0.5000	0.5000	0.4785
4.0000	20	14	0.7000	0.7000	0.7318
4.5000	20	18	0.9000	0.9000	0.8870

Chi - Square for Heterogeneity (calculated) = 0.175

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 5.991

Mu = 0.548723 Sigma = 0.086286

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-1.359387	1.443945	(-4.189520, 1.470746)
Slope	11.589428	2.549115	(6.593163, 16.585693)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

Point	Exposure Conc.	95% Confidence Limits	
		Lower	Upper
LC/EC 1.00	2.228	1.518	2.609
LC/EC 5.00	2.551	1.920	2.877
LC/EC 10.00	2.742	2.174	3.034
LC/EC 15.00	2.879	2.362	3.764
LC/EC 50.00	3.538	3.275	3.764
LC/EC 85.00	4.347	4.036	5.066
LC/EC 90.00	4.564	4.193	5.496
LC/EC 95.00	4.905	4.428	6.214
LC/EC 99.00	5.616	4.887	7.850

toxic effect of grease and engine wash oil also depend on time.

As the time increased the mortality rates at different concentrations of test compounds also increased and for different exposure time the LC_{50} values were different which was in agreement with other findings (Samuel *et al*., 2008; Osman *et al*., 2009; Abdel-Moneim *et al*., 2008; Chukwn and Lawal, 2010).

In the present study the values of LC_1, LC_5, LC_{15}, LC_{50}, LC_{85}, LC_{90}, LC_{95}, LC_{99} varied inversely with the increase of exposure time. It is evident from the present study that the mixture of grease and engine wash oil was toxic to *Pangasius sutchi*, the extent of toxicity depends on the amount of doses and length of the exposure period. Although during the present investigation the concentrations of toxicants and duration of exposure were given priority to test the extent of toxicity but the possible influence of other factors such as temperature, pH, dissolved oxygen, etc. on the toxicity of grease and engine wash oil can not, however, be denied. Bender (1969) stated that malathion is toxic below pH and loses its toxicity in alkaline pH, as it undergoes hydrolysis at pH 7 with few exception. The toxicity of organochlorine compounds is not affected by changes in the hydrogen ion concentration as reviewed by Pickering *et al.* (1962). Mexa carbamate, a carbamate compound, was 38 times more toxic to bluegills at pH 9.5 than at pH 7.5. The 96 hours LC_{50} being 22.9 mg/l at pH 7.5 and 0.6 mg/l at pH 9.5. Suspended particles, both living and nonliving, alter the toxicity of a compound by influencing the bio-availability of compound. Pickering *et al* (1962)) showed that the toxicity of zinc to bluegills and hydrogen sulphide to goldfish were higher when the oxygen concentration was lower. From the above discussion, the recorded water parameters in the present study may be a resourceful pre-requisite of toxicity test as the toxicity of a compound by environmental factors like temperature, pH load may modify the result of an experiment at a reasonable extent.

CONCLUSION

The test fish is too much hardy than other fishes. Such hardy fish is susceptible to grease and engine wash oil at different concentrations. Therefore it reveals that the mixture of grease and engine wash oil may change the fish community if these are ultimately find their way to the water body by direct discharge or rain run off. Physiological stress may affect the immunology and progeny of species that can be eventually reduced the total population after a certain period.

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