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ACUTE TOXICITY DETERMINATION OF WSF OF DIESEL FUEL TO THE FRESHWATER TELEOST, *CHANNA PUNCTATUS* (BLOCH)

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ABSTRACT:

The present study reflects the results of the acute bioassay tests performed to determine the 96 hr LC50 of the water soluble fractions (WSF) of diesel fuel to the freshwater teleost *Channa punctatus* and the behavioural responses of the fish at different concentrations of the WSF of diesel. The LC_{50} was determined as 4.85 ppm following a new method (Dede and Kaglo, 2001). Within the first 24 hr and within the concentration range of 6.0 ppm to 30.00 ppm the mortality ranged between 5-50%. In 48 hours within the same concentration range, a mortality range of 10-70% was observed. Similarly, 35-90% and 55-100% mortality occurred within the same concentration range in 72 and 96 hrs respectively. Upon exposure to different lethal concentrations a shift from normal behaviour was exhibited by the fish. The signs of distress in the form of surfacing, uncoordinated movement, increased operculur activity, escaping tendency, muscle twitching, excessive mucus secretion, inflammation of gill lamellae and poor response to external stimuli were exhibited by the experimental fish. The results led us to conclude that under confinement, even low concentration of WSF of petroleum hydrocarbon (diesel) could be lethal to *Channa punctatus* and may adversely affect aquatic eco-balance.

KEY WORDS : WSF, Diesel, 96hr LC_{50} Fish Behaviour, *Channa punctatus*.

INTRODUCTION:

In past few decades, much emphasis has been laid on enhancing fish productivity on account of their high nutritional value as major source of dietary protein and several byproducts. The present stock of fish is inadequate to meet the demand of the country as it forms only 25% of the 16million tons target (Pandey, 2004) Despite this, the fishes are being continuously exposed to ever increasing number of contaminants which adversely affect their health, growth, maturity, reproductive ability and metabolic pool (Jha,2004) . Amongst different types of aquatic pollutants petroleum products need scientific attention in context of aquatic toxicology. The deleterious effects of petroleum products are the consequences of fouling and intake of water soluble and insoluble hydrocarbons by aquatic biota (NAP, 2003). Petroleum hydrocarbons, on entering water bodies, affect biota interfering with the normal functioning of an organism and its ability to live in harmony with the environment. Besides , the animal species at higher level of biological organization may also be harmed via food web.

The absorption and accumulation of soluble petroleum hydrocarbons in fish tissues are extremely rapid, up to 10 to 100 times higher than in water (Anyakora et al, 2005; Ramchandran et al, 2006). Fish can, therefore, be used as bio indicators of petroleum contamination . The toxicity of petroleum products on the freshwater system and their biota are still to be explored especially in Indian context. The main source of inland water contamination from petroleum products is the small and continuous leakages from underground bulk storage tanks ultimately reaching ground water and rivers (Tiburtius et al,2005).

In India, most of the petroleum industries and refineries have been established along the coastal and riverine areas and their effluents are discharged into these water bodies. Besides, substantial volume enters the freshwater system through leakage and rupture of oil ducts and pipes, Thereby creating every possibility of their absorption and accumulation in aquatic organisms including fishes. Scanning of pertinent literature reveals that barring a few (Borah and Yadav, 1996 and Kakkar et al; 2011) no work has been performed on impact of petroleum products in freshwater Indian fishes. Keeping this in view, The present study was under taken, which forms only a part of our extensive study and is confined to determination of 96 hr LC_{50} of water soluble fraction (WSF) of diesel to the freshwater air -breathing teleost, *Channa punctatus* (Bloch) by a new arithmetic method and also fish behaviour at different lethal concentration of WSF of diesel.

MATERIALS AND METHODS

The freshwater air-breathing teleost, *Channa punctatus* (Bloch), procured from local non-polluted ponds, of average length (15.0±2.5cm) and weight (38.0±2.5g) were the experimental animal model for this study. Their transportation maintenance, acclimation and feeding procedures were followed as per methods described elsewhere (Jha and Jha, 2011). Running tap water (average temperature 24.5^{0} C ; pH - 7.6 mgl^{-1} Dissolved solids - 12.3 mgl^{-1} , Suspended soilds -25.2 mgl^{-1} , Dissolved oxygen - 6.8mgl^{-1} Free Co_2- 1.8 mgl^{-1}, Biochemical oxygen demand - 3.0 mgl^{-1}, total hardness $CaCo_3$ -152.5 mgl^{-1} and total alkalintiy as $CaCo_3$ mgl-1) was used in all the experiments. The refined petroleum products (diesel) was obtained from local filling station. The water soluble fraction (WSF) of diesel was used as test chemical for determination of 96 hr LC_{50} to the experimental animal. The feeding was stopped 24 hr before the bioassay tests.

Preparation of water soluble Fraction (WSF) of diesel Fuel:

One liter (1 part) of the fresh diesel fuel was diluted with 4-litres (4-parts) of running tap water in a 6 litre flask. The diesel - water mixture was slowly stirred for 24 hrs with Gallenkamp magnetic stirrer to enhance the dissolution of the WSF of diesel fuel in water. The mixture was lift to stand for 3 hours before it was poured into separating funnel to obtain a clear oil-water interphase. Thereafter, it was allowed to stand overnight . The lower layer of water containing the WSF of the diesel was decanted several times into containers unfil sufficient quantity was obtained for the experiment (Afolabi et al; 1985; Dede and kaglo, 2001). This solution was treated as 100% stock solution.

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Static acute bioassays to determine 96 hr LC50

Six rectangular glass aquaria (A-F) of 30 liter capacity were taken. Each aquarium was filled with 20 - liters water and 20 numbers of fish were transferred randomly from acclimated aquaria to each of the six aquaria. Fish of aquaria B,C,D,E and F were exposed to graded levels of WSF of diesel fuel in concentrations of 6.0 ppm ; 9.5 ppm ; 14.75ppm; 20.25 ppm and 30.00 ppm respectively while aquarium A served for the control group of fish with no diesel fuel. The fish were observed for 96 hrs record the mortality percentage and fish behaviour. Fish were treated dead when they no longer responded to prodding. Dead fish were removed immediately as soon as detected . Tables were framed and the numbers of dead fish were recorded against the time of their death as suggested by Sprague (1975). The data were subjected to determination of LC_{50} of the WSF of diesel to the test fish with the Arithmetic method of Dede and Kaglo (2001).

RESULTS AND DISCUSSION

The result of the toxicity determination are shown in Table 1-4, based on which Table-5 was framed to determine LC_{50} value. As evident from Table-1 within 24 hrs the no. of fishes died was 1,3,5,8 and 10 at WSF concentration of 6.0; 9.5; 14.75; 20.25 and 30.0 ppm respectively. The no. of dead fish in the same toxicant concentration ranges were 2, 6, 8, 9 and 14 respectively (Table-2). Again, the observed no. of deaths within 72 hrs were 7, 11, 13, 16 and 18 in exposure concentration of 6.0; 9.5; 14.75; 20.25 and 30.0 ppm respectively (Table-3). Similarly, the death toll was even higher during 96 hrs at each of the above exposure concentrations which were observed to be 11, 15, 18, 19 and 20 (100% mortality) respectively (Table-4).

No death during 96hr test occurred in the control group (Aquarium A).

Table 5 was framed based on the number of dead fish in each group against the time of death and dose difference. This was used for Lc50 calculation with the help of the formula :-

$$LC_{50} = LC_{100} - \frac{\sum Mean\ Death \times concentration\ difference}{No.of\ fish\ per\ group}$$

As is evident from Table- 5, LC_{100} was 30.0 ppm; Summation Mean death X Dose difference was 503.245 and the number of fish was by employing the above formula :-

$$LC_{50} = 30 - \frac{503.245}{20} = 30\text{-}25.15 = 4.85 \text{ ppm.}$$

Hence 96hr LC_{50} of WSF of diesel = 4.85 ppm.

Upon exposure to different lethal concentrations, a shift from normal behaviour was exhibited by the fist. The fish showed signs of distress such as restlessness, frequent surfacing increased opercula activity, escaping tendency muscle twitching, excessive mucus secretion, information of gill lamellae and poor response to external stimuli.

Besides, opening and closure of mouth and opercular covering with partial extension of fins, peeling of skin and fading of natural colouration, unco-ordinated movement and gulping of air were the other behavioural changes noticed during this study. These changes were more prominent at higher lethal doses. Again, during first hour of exposure the fish showed hyperactivity w × ch gradually reduced to hypoaction or lethargic behaviour.

The results of the present study are in conformity with those of Kakkar *et al;* (2011); Fayeofori (2012) and Islam et al (2013). In our investigation, within first 24hr. and within the concentration range of 6.0 ppm to 3.00 ppm the mortality ranged between 5-50%. In 48 hr. within the same concentration range mortality was observed between 10-70% which increased to 35-90% and 55-100% within 72 hr and 96 hr respectively. This clearly reflects that toxicity of WSF of diesel fuel is a function of does and duration and there is a positive correlation between concentration of WSF and duration of exposure. Mortality recorded even at low concentration agrees with earlier reports on water soluble fraction of hydrocarbons on aquatic organisms (Dede and Kaglo; 2001; Fafioye; 2006). The death of the fish as a result of acute exposure of WSF of diesel may be attributed to changed membrane permeability as WSF is reported to accumulate in lipidice compartments like cellular membrane (Di Tore *et al.*: 2001); clogging of gills by mucus leading to death (Jha & Pandey, 1990) and depletion of dissolved oxygen (Rodrigues *et al;* 2010). Similarly, the abnormal behaviour exhibited by the fish during acute bioassay tests reflect the expression of sings of distress. The observed uncoordinated movement and also of balance may be associated with nerve inhibiting action of test chemical due to accumulation of Ach content at nerve ends and neuromast organs, thus disrupting the synaptic transmission of nerve impulses along neurons (Islam *et al*; 2013). The excessive mucous secretion and body dispigmentation may be due to impairment of pituitary gland induced by diesel fuel causing changes in number and area of mucous gland and chromatophoers (Pandey *et al*; 1990).

It can, therefore, be reasonably concluded that even low concentrations of the WSF of diesel fuel in captivity of confinement, could be lethal to fish and thereby affect the bio diversity of the species.

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Table-1
24 Hr. Toxicity Assessment of WSF of diesel on *channa punctatus*

Conc. (ppm)	No. survey	% Alive	% Dead
0	20	100	0
6.0	19	95	5
9.5	17	85	15
14.75	15	75	25
20.25	12	60	40
30.00	10	50	50

Table-2
48 Hr. Toxicity Assessment of WSF of diesel on *channa punctatus*

Conc. (ppm)	No. survey	% Alive	% Dead
0	20	100	0
6.0	18	90	10
9.5	14	70	30
14.75	12	60	40
20.25	11	55	45
30.00	6	30	70

Table-3
72 Hr. Toxicity Assessment of WSF of diesel on *channa punctatus*

Conc. (ppm)	No. survey	% Alive	% Dead
0	20	100	0
6.0	13	65	35
9.5	9	45	55
14.75	7	35	65
20.25	4	20	80
30.00	2	10	90

Table-4
96 Hr. Toxicity Assessment of WSF of diesel on *channa punctatus*

Conc. (ppm)	No. survey	% Alive	% Dead
0	20	100	0
6.0	9	45	55
9.5	5	25	75
14.75	2	10	90
20.25	1	0.5	95
30.00	0	0	100

Table-5.Lc $_{50}$ determination of 96 hour by method of Dede and Kaglo (2001)

Conc. (ppm)	Conc difference	No of alive	No of death	Mean death	Mean death x dose difference
0 controll	0	20	10.	…..	…………..
6.0	6.0	9	11	10.8	64.80
9.5	3.5	5	15	14.5	50.75
14.75	5.25	2	18	17.5	91.875
20.25	5.50	1	19	18.8	103.40
30.0	10.25	0	20	19.0	194.75

$$LC_{50} = LC_{100} - \frac{\sum Mean\ Death \times concentration\ difference}{No. of\ fish\ per\ group}$$

$$LC_{50} = 30 - \frac{503.245}{20} = 30\text{-}25.15 = 4.85 \text{ ppm.}$$

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