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Acute Toxicity of Anthracene on a local fresh water fish – *Puntius ticto* (Ham.)

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Abstract
Polycyclic Aromatic Hydrocarbons (PAHs) have become ubiquitous pollutants of water resulting mainly from petroleum products. These are get accumulated in tissues and are converted into secondary metabolites which show bioaccumulation in the living systems and have several deleterious effects on them. They further get biomagnified in the food chain. This study attempts to evaluate toxicity of one such PAH, Anthracene, on the fresh water fish, *Puntius ticto*, using pure Anthracene dissolved in dichloromethane. LC50 values were seen in the range of 5-2.4 after exposure of fish for 24 to 96 hours. Statistical evaluation carried out showed regression values in the range of 0.9945 to 0.9918, indicating high correlation between the mortality rate and concentration of Anthracene. This paper discusses in detail the toxicity of Anthracene on fresh water fish *Puntius ticto* and the behavioral changes observed.
Key words: PAHs; bioassay; toxicity; *Puntius ticto*.

Introduction

In the past few decades, industrial and domestic effluent toxicity evaluation has become much more magnified in the field of ecotoxicology. Fossil fuel consumption has increased many folds due to increased industrialization, population growth and man's greed for comfort. Oil refineries add to the woes of pollution due to inefficient combustion of carbonaceous matter and PAHs ultimately reach the aquatic environment.[1] Apart from these factors, industrial and domestic sewage effluents, surface run-off from land, deposition of airborne particulates and spillage of petroleum and petroleum products adds to the aquatic pollution.

PAHs are common environmental pollutants found in automobile emissions, tobacco smoke; charcoal broiled food and chimney soot. Many of these pollutants are an important class of environmental pollutants that are known to exert carcinogenic and immunotoxic effects on aquatic organisms.[2]

PAHs are relatively inert compounds that are metabolized and the metabolites are the actual agents which are mutagenic and carcinogenic in nature.[3] PAHs are ubiquitous trace elements of marine and freshwater environmental pollutants that are potent mutagens and carcinogens in nature.[4] Literature reports fish kills and also accumulation of PAHs by fish *Tilapia mossambicus*.[5] Reports are available on the effects of PAH on fish due to oil spills. [6]

In recent years, more attention is being given to acute toxicity evaluation of different industrial waste waters. Fish bioassay is widely used to study the toxicity of different xenobiotics. PAH as a xenobiotic persists in nature for long period of time and gets bioaccumulated in aquatic biota and in fish. Residues of PAH were found in aquatic species from few rivers and estuarine systems.[7, 8]

PAHs have deleterious effects on fish species.[9] Water quality is impaired and also the aquatic organisms suffer when PAH laden waste water is discharged into the water bodies. At this juncture, it is necessary to carry out fish toxicity/ bioassays to assess the toxicity levels. Fish bioassay is widely used to study the toxicity of xenobiotics. Bioassay results provide baseline information in the formulation of strategy for controlled release and dilution of treated effluents into the receiving water bodies.

Over the years, oil spills have been associated with damage to aquatic organisms and also to the marine birds. Oil spills are the main source of PAH in the aquatic environment. Polycyclic Aromatic Hydrocarbons are produced by most oil refineries and gas installations and significant quantities of associated water are generated. This waste water requires treatment prior to its discharge. This water if not treated properly, leads to pollution in the receiving waters. PAHs also find their way into the marine environment due to large scale oil spills.

Report on acute toxicity of seven PAHs namely acenaphthalene, chrysene, fluoranthene, fluorine, naphthalene, phenanthrene and pyrene on benthic amphipod is available, where LC_{50} and NOEC based on 96 hrs acute toxicity have been calculated for the benthic amphipod. [10]

Literature on acute toxicity bioassay tests for evaluating LC_{50} concentration of PAHs on fish is very scanty. Few studies on acute toxicity of retene to two freshwater species of Zebrafish and rainbow trout are reported. In real sense, the studies were for a longer period of 14 days, and larval stages of the fish were used. [11]

Fish bioassay to evaluate the toxicity of benzo[a] pyrene to teleost *Fundulus heteroclitus* has been quoted in literature but these tests were of long duration of 21 days and the fish were fed with pyrene contaminated *Nereis*.[12] Studies were more focused on the effects due to ingestion of PAH contaminated food.

As such, literature on LC_{50} values obtained from short term 96 hour acute toxicity tests on fish due to PAHs are very few. Majority of the work pertains to long time chronic effects of contaminated soils and water on fish.

To understand the effects of exposure of fish to PAH concentrations present in waters polluted due to oil spills, detailed laboratory studies by acute toxicity tests were made to arrive at LC_{50} values using pure PAH i.e. Anthracene, which would give an insight into the toxicity levels of PAH.

Fewer studies have been carried out in fish to determine such acute toxicity. Considering the PAH pollution due to natural disasters, it was envisaged to evaluate acute toxicity of Anthracene using the local fresh water fish *Puntius ticto*.

Materials and Methods

The test fish, *Puntius ticto* were procured from a fresh water lake. Methods for measuring the toxicity of industrial effluents/ chemicals on fish were followed as cited in literature.[13, 14] The fish were acclimatized for ten days in an aerated and dechlorinated tap water (dilution water) at ambient temperature. Analysis of dilution water was carried out as per standard method (AHPA-1998)[15] and the characteristics of dilution water are shown in Table I.

Once the fishes were acclimatized, acute toxicity tests were initiated. During acute toxicity evaluation, the test water was changed daily and fresh dose of Anthracene was introduced into it. Solution of Anthracene was prepared using Dichloromethane and the same amount of dichloromethane was added in the control also. Ten fishes for each concentration were used in 10 liter aquariums and mortality rate was monitored every 24 hours for a period of 96 hours. Characteristics of dilution water are shown in Table 1.

Percent mortality was plotted against the Anthracene concentration on a probability paper and LC50 values were graphically derived. Fig. 1 shows Graph indicating effect of Anthracene on fish mortality up to 96 hours.

Using Litchfield and Wilcoxon method[16], 95% confidence limit, both upper and lower limits were calculated. The Slope function (S) was calculated as indicated in the literature and regression (R^2) values were also calculated. Results obtained are indicated in Table II.

Results and Discussion

The fresh water fish *Puntius ticto* is supposed to be very sensitive. This was observed during the acute toxicity tests. It exhibited several types of reactions. For instance, when low Anthracene concentrations were added, fish exhibited distress during the initial stages. They became disorientated and began to swim upside down and also exhibited somersaulting (as shown in Fig.2), but gradually regained their posture and started swimming normally. But at higher dosage, fish settled at the bottom initially and came to the surface to gulp air very frequently. Opercular movements became very slow at higher concentrations.

Static bioassay tests indicate that the toxicity of Anthracene to *Puntius ticto* is a function of the Chloride molecule present and the exposure period. Also, bulging of eyeballs and abdomen were very conspicuous. The fish exhibited somersaulting immediately after the addition of chemicals.

Acute toxicity bioassay tests, the results of which are expressed as median lethal concentration (LC_{50}) provide the means to calculate the relative toxicity of industrial effluents of various chemicals and the relative tolerance of various fishes. The results obtained were subjected to statistical evaluation. Correlation coefficients, slope function and confidence interval were also calculated.

Table I: Characteristics of Dilution Water

Parameters	Values *
Temperature ° C	25-27
pH	7.5-8.2
Total Alkalinity as $CaCO_3$	156-190
Total Hardness as $CaCO_3$	142-172
Ca Hardness as $CaCO_3$	80-94
Mg Hardness as $CaCO_3$	62-78
Dissolved Oxygen	6.9-7.3
Calcium as Ca	32-38
Magnesium as Mg	14-18
Sodium as Na	36-38
Potassium as K	2-4
Chloride as Cl	126

(All the values are expressed as mg/L except temperature and pH.)

Regression values ranged between 0.9945 to 0.9918, indicating good correlation between fish mortality and PAH concentrations. Sensitivity of an organism to a toxic chemical/ effluent cannot be judged only by comparing LC_{50} of the chemicals/ effluents. The full range of lethal concentration (LC_0-LC_{100}) should be taken into consideration while assessing the susceptibility of organism to any toxic chemical/ effluent. Results obtained from bioassay studies will help the industry management to take necessary pollution control measures before the discharge of the effluents into the natural streams or water bodies. This would help in minimizing many ecotoxicological problems.

Results of fish bioassay, widely used to study the toxicity of xenobiotics, provide baseline information in formulation of strategies for controlled release of treated industrial effluents into receiving water bodies. For application of toxicity data in regulation of waste water discharge and the prediction of environmental effects- both acute and chronic, toxic levels have to be determined to conserve aquatic life.

Table II: LC_{50}, NOEC, 95% Confidence Limit, Slope function and regression values for Anthracene to *Puntius ticto*

Exposure Period	**Parameter Values**	**Observation**
24 hrs	LC_{50}mg/L	**3.4**
	NOEC mg/L	**1.5**
	95% Confidence Interval	**2.76-3.893**
	Slope function	**Y=11.515 x -13.383**
	Regression (R^2)	**0.9945**
48 hrs	LC_{50}mg/L	**2.8**
	NOEC mg/L	**1.0**
	95% Confidence Interval	**2.28-3.43**
	Slope function	**Y=12.667 x -12.222**
	Regression (R^2)	**0.9936**
72 hrs	LC_{50}mg/L	**2.2**
	NOEC mg/L	**0.5**
	95% Confidence Interval	**1.76-2.75**
	Slope function	**Y=13.167 x- 14.722**
	Regression (R^2)0.9917	**0.9917**
96 hrs	LC_{50}mg/L	**2.0**
	NOEC mg/L	**0.25**
	95% Confidence Interval	**1.538-2.5**
	Slope function	**Y=13.571x -6.0714**
	Regression (R^2)0.9918	**0.9918**

LC_{50}: Lethal Concentration 50 NOEC: No Observed Effect Concentration

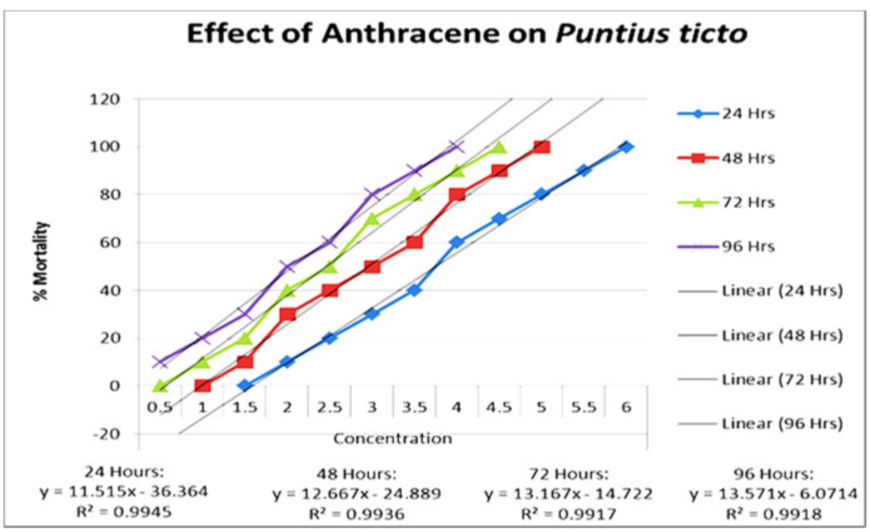

Figure 1. Graph showing increasing effect of Anthracene on fish mortality up to 96 hours.

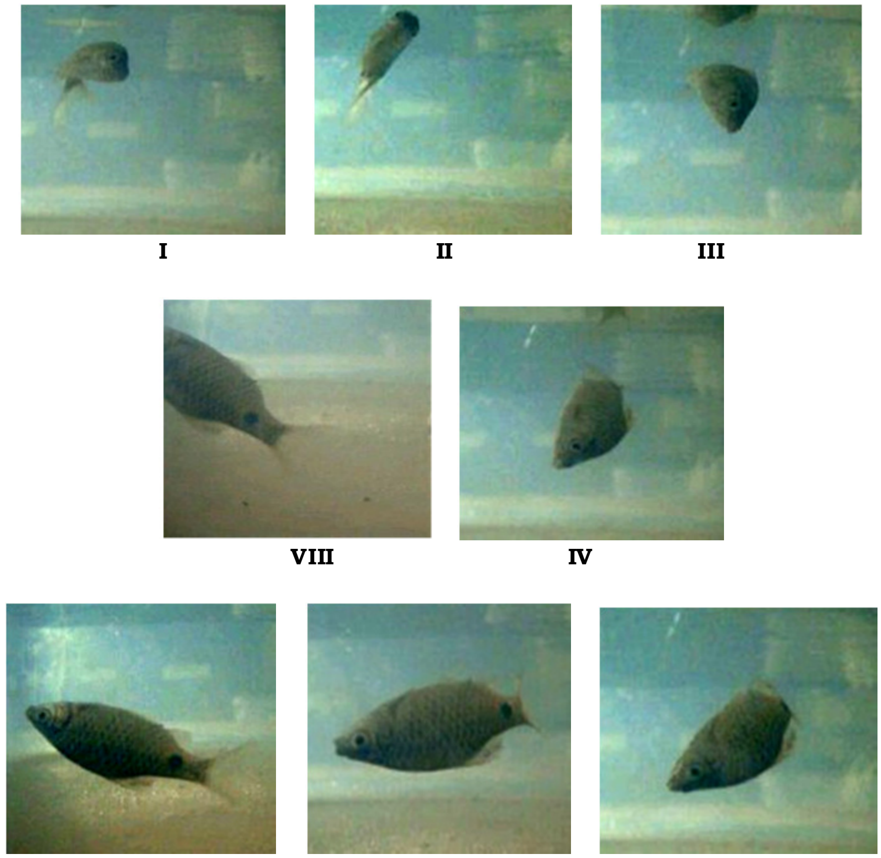

Figure 2. Fish exhibiting somersaulting on exposure to PAHs.

Conclusion

Results obtained from the studies indicated that PAH Anthracene is toxic to the fish, *Puntius ticto*. The results of this investigation provide long term safe levels of toxicants for fish and other aquatic life which can be estimated by using short term acute toxicity bioassays.

Studies were carried out only with pure compound. Effects may increase manifold in case of derivatives of Anthracene. However, these results may be useful for preliminary evaluation of the toxic effect of Anthracene on the wastewaters containing this PAH. Short term bioassay results also help in calculating sub lethal doses to carry out detail bioaccumulation studies using chronic bioassays.

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