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# Toxicity Evaluation of a Few PAHs on a Local Freshwater Fish—*Rasbora daniconius*

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***Abstract***

*Polycyclic Aromatic Hydrocarbons (PAHs) have become ubiquitous pollutants of water resulting mainly from petroleum products. These are absorbed into the living systems and converted into secondary metabolites which show bioaccumulation in the living systems and have several deleterious effects on them. The present study attempts to evaluate toxicity of two such PAHs— Naphthalene and Anthracene—on the freshwater fish, Rasbora daniconius, using pure PAHs dissolved in dichloromethane. $LC_{50}$ values were seen in the range of 3.6–4.4 in case of Naphthalene and 0.5–2.4 in case of Anthracene after exposure of fish to these PAHs for 24–96 h. Studies indicated that Anthracene is comparatively more toxic to fish Rasbora than Naphthalene. This paper discusses in detail the toxicity of few PAHs on freshwater fish R. daniconius and the morphological changes observed.*

**Keywords:** *bioaccumulation, bioassay, polycyclic aromatic hydrocarbons (PAHs), Rasbora daniconius, toxicity*

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## 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 Polycyclic Aromatic Hydrocarbons (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; 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 exists 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 river and estuarine systems [7].

PAHs have deleterious effects on fish species [8]. Water quality is impaired and also the aquatic organisms suffer when PAH-laden wastewater 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. PAHs are produced by most oil refineries and gas installations and significant quantities of associated water are generated. This wastewater has to be disposed off. 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 No Observed Effect Concentration (NOEC) based on 96 h acute toxicity have been calculated for the benthic amphipod [9].

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 [10].

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* [11]. 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 is 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 that is, Anthracene and Naphthalene, 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 and Naphthalene using the local freshwater fish *Rasbora daniconius.*

## MATERIALS AND METHODS

The test fish, *R. daniconius,* were procured from a freshwater lake. Methods for measuring the toxicity of industrial effluents/chemicals on fish were followed as cited in the literature [12, 13]. The fishes were acclimatized for 10 days in an aerated and dechlorinated tap water (dilution water) at ambient temperature. Analysis of dilution water was carried out as per standard method [14] (AHPA-1998) and the characteristics of dilution water are shown in Table 1.

***Table 1**: 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 | 126 |
| *All the values are expressed as mg/l except temperature and pH.* | |

Once the fishes were acclimatized, acute toxicity tests were initiated. During acute toxicity evaluation, the test water was changed daily, and fresh dose of PAH was introduced into it. Solutions of PAHs—Naphthalene and Anthracene—were prepared using dichloromethane and the same amount of dichloromethane was added in the control also. Ten fishes for each concentration were used in ten litre aquariums and mortality rate was monitored every 24 h for a period of 96 hours.

***Table 2:*** *$LC_{50}$, NOEC, 95% Confidence Limit, Slope Function and Regression Values for Anthracene to Rasbora daniconius.*

| Exposure period | Parameter | Values |
|---|---|---|
| 24 h | $LC_{50}$ (mg/l) | 2.4 |
| | NOEC (mg/l) | 0.25 |
| | 95% Confidence Interval | 0.8–7.2 |
| | Slope function | Y=7.857 x 26.19 |
| | Regression ($R^2$) | 0.9429 |
| 48 h | $LC_{50}$ (mg/l) | 1.3 |
| | NOEC (mg/l) | 0.15 |
| | 95% Confidence Interval | 0.325–5.2 |
| | Slope function | Y=9.0659 x 21.154 |
| | Regression ($R^2$) | 0.9821 |
| 72 h | $LC_{50}$ (mg/l) | 0.8 |
| | NOEC (mg/l) | 0.10 |
| | 95% Confidence Interval | 0.156–4.08 |
| | Slope function | Y=9.6154 x 16.667 |
| | Regression ($R^2$) | 0.9947 |
| 96 h | $LC_{50}$ (mg/l) | 0.5 |
| | NOEC (mg/l) | 0.05 |
| | 95% Confidence Interval | 0.083–3.0 |
| | Slope function | Y=10 x -10 |
| | Regression ($R^2$) | 1.0 |

***Table 3:*** *$LC_{50}$, NOEC, 95% Confidence Limit, Slope Function and Regression Values for Naphthalene to Rasbora daniconius.*

| Exposure period | Parameter | Values |
|---|---|---|
| 24 h | $LC_{50}$ (mg/l) | 4.4 |
| | NOEC (mg/l) | 3.4 |
| | 95% Confidence Interval | 3.35–5.764 |
| | Slope function | Y=10.545x-9.090 |
| | Regression ($R^2$) | 0.986 |
| 48 h | $LC_{50}$ (mg/l) | 4.2 |
| | NOEC (mg/l) | 3.2 |
| | 95% Confidence Interval | 3.11–5.67 |
| | Slope function | Y=10.66x-10.62 |
| | Regression ($R^2$) | 0.981 |
| 72 h | $LC_{50}$ (mg/l) | 4.0 |
| | NOEC (mg/l) | 3.0 |
| | 95% Confidence Interval | 3.44–4.64 |
| | Slope function | Y=11.45x - 14 |
| | Regression ($R^2$) | 0.994 |
| 96 h | $LC_{50}$ mg/l | 3.6 |
| | NOEC mg/l | 2.8 |
| | 95% Confidence Interval | 2.88–4.50 |
| | Slope function | Y=12.33x -15 |
| | Regression ($R^2$) | 0.992 |

Percent mortality was plotted against the PAH concentration on a probability paper and $LC_{50}$ values were graphically derived. Using Litchfield and Wilcoxon method [15], 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 Tables 2 and 3, respectively.

## RESULTS AND DISCUSSION

The freshwater fish *R. daniconius* 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, 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 concentration, that is above 2.0 mg/l. A unique feature at higher dose of 4.0 and 5.0 mg/l of Anthracene was observed wherein a red patch appeared in the area of the heart on death (Figures 1 & 2). This may be due to respiratory problems leading to a lower supply of oxygen.


***Fig. 1:*** *Dead Fish Rasbora Showing Morphological Change—a Red Patch in the Cardiac Area.*

  

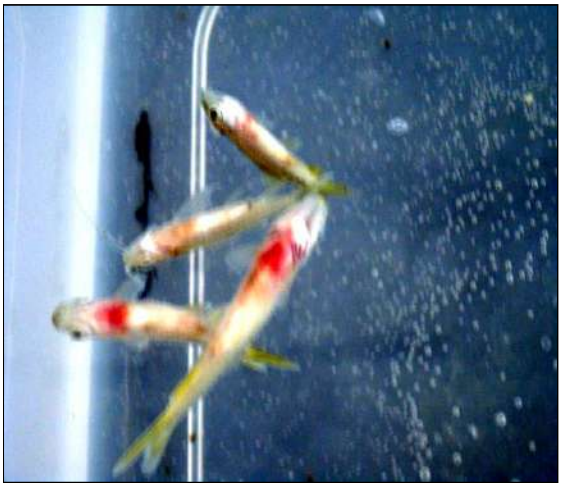

***Fig. 2:*** *Fish Rasbora Showing Red Patch in Cardiac Area on Exposure to PAH in Aquarium.*

Static bioassay tests indicate that the toxicity of Anthracene to *R. daniconius* is a function of the Chloride molecule present and the exposure period. Naphthalene appeared less toxic to fish as compared to Anthracene, as indicated by the $LC_{50}$ values. Moreover, fish did not show restlessness for long period except in the initial stages of addition of Naphthalene. Loss of balance was observed at a higher concentration of Naphthalene, but after few hours, they became normal. In a few cases, the curling of caudal fin was very prominent. In case of Anthracene, bulging of eyeballs was very conspicuous, while in case of Naphthalene, such condition was not observed. Bulging of stomach was observed in case of both PAHs studied. At high concentrations of both PAHs studied, 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. Regression values ranged between 0.9429 and 0.9947 in case of Anthracene and between 0.9810 and 0.9940 in case of Naphthalene, 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 wastewater discharge and the prediction of environmental effects—both acute and chronic toxic levels have to be determined to conserve aquatic life.

## CONCLUSION

Results obtained from the studies indicated that PAH Anthracene is comparatively more toxic than Naphthalene to the fish, *R. daniconius*. 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 compounds. However, these results may be useful for preliminary evaluation of the toxic effect of Anthracene and Naphthalene on the wastewaters containing these PAHs. Short-term bioassay results also help us in calculating sub lethal doses to carry out detailed bioaccumulation studies using chronic bioassays.

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