CHAPTER IV

BEHAVIORAL EFFECTS
Introduction-

The physical, chemical and biological components of the environment play an important role in manifestation of biological responses to pollutants. The toxicity of a pollutant depends upon many factors, such as animal weight, developmental stages, period of exposure, temperature, pH, hardness of water and dissolved content of the medium. The effects of pollutants are generally characterized on survival, reproduction or growth of the animal due to physiological alterations taking place. The response of an animal to a toxic medium is important, since it reflects the internal changes taking place within it (Pathan et al, 2009). Being unable to escape fishes are the most sensitive to the presence of aquatic pollutants. Presence of environmental stressors, reduce the ability of fish to function normally, both physiologically and behaviorally.

(Courtesy Scott and Sloman, 2004).
Many physiological and environmental factors influence the performance of normal behavior by fish. After appropriate environmental stimuli are perceived, sensory information is generally integrated centrally. Secondary physiological responses may also ensue, and together central and peripheral changes in physiology determine the behavioural responses to stimuli. Behavioural changes may then feedback to influence subsequent physiological processes and environmental stimuli (Scott and Sloman, 2004).

Behavior is a major link between the organism and its environment (Little, 2002). It is both a result and determinant of molecular, physiological, and ecological aspects of toxicology; therefore it provides insight into various levels of biological organization (Scott and Sloman, 2004). Behavioral responses also reflect an organism’s ecological fitness and their abilities to avoid predators, select prey items, and reproduce (Little, 2002). Alterations in any of these behaviors, coupled with changes in physiology may alter population stability (Scott and Sloman, 2004).

Since behavior is not a random process, but rather a highly structured and predictable sequence of activities designed to ensure maximal fitness and survival (i.e., success) of the individual (and species), behavioral endpoints serve as valuable tools to discern and evaluate effects of exposure to environmental stressors. Behavioral endpoints that integrate endogenous and exogenous factors can link biochemical and physiological processes, thus providing insights into individual- and community-level effects of environmental contamination. Most importantly, alterations in behavior represent an integrated, whole-organism response. These altered responses, in turn, may be associated with reduced fitness and survival, resulting in adverse consequences at the population level (Kane et al, 2004). Thus, behavior may be considered a promising tool in ecotoxicology.
Since behavior links physiological function with ecological processes, behavioral indicators of toxicity also appear ideal for assessing the effects of aquatic pollutants on fish populations (Scott and Sloman, 2004). The presence of these pollutants cause various degrees of effects, depending on the species of the affected fish (Rao and Murty, 1982), its sex, age, size, general physical condition, exposure time and the concentration of the pollutants (Stross and Haines, 1979); type of toxicant used and environmental factors (Fafioye, 2001). Such effects may be influenced by water characteristics, such as hardness, alkalinity and hydrogen ion concentration (Rand, 1985).

Behavioral abnormalities in various fish species on exposure to pollutants have been reported by several workers. Feeding of pollutant contaminated phytoplanktons and zooplanktons cause damaged peripheral organs and reduction in swimming activities in fishes (Sprague, 1971). Exposure to different concentrations of cadmium causes frequent surfacing with irregular opercular movements and loss of equilibrium in *Tilapia mossambica* (Ghatak and Konar, 1990). Fafioye (1990) reported erratic swimming in *Oreochromis niloticus* and *Sarotherodon galilaeus* fingerlings exposed to Gammalin 20, Gramazone, Primextra and Sandox. Similarly, Adeogun (1994) also noted that the extracts of *Raphia hookeri* caused erratic behavior, darting up and down the water column and swimming alternatively on lateral and ventral sides in *Oreochromis niloticus* and *Clarias gariepinus*. Schooling behaviors of Fathead minnows, *Pimphales promelas* and Rainbow trout, *Ochoryn mykiss*, were disrupted when they were exposed to Keltane, Bisulton, Pydrin, Dursban and Permethrin (Holcombe et al., 1982). *C. gariepinus* and *O. niloticus* exposed to aqueous and ethanolic extracts of *Parkia biglobosa* and *Raphia vinfera* experienced overturning times, which declined with increase in the concentration (Fafioye, 2001). Holcombe et al. (1976) observed hyperactivity, erratic swimming and loss of
equilibrium in Brook trout, *Salvelinus frontalis* in response to lead treatment. Besides, loss of equilibrium, frequent surfacing and sinking, burst of erratic swimming and gradual onset of inactivity in Rainbow trout, *Salmo gairdneri* on exposure to mercury is also reported (Macleod and Pessah, 1973).

Lewis and Lewis (1971) reported surfacing, restlessness, failure to school, sluggishness and loss of equilibrium in Golden shiner *Notemigonus crysoleveus* when exposed to 5 ppm of copper. *Heteropneustes fossilis, Entroplus maculatus* upon exposure to copper, mercury and selenium showed irregular erratic swimming, frequent surfacing, gulping of air, revolving, convulsions and accelerated ventilation with rapid opercular and mouth movements (Veena et al., 1997). Ikpi et al (2003) observed the loss of skin coloration in *O.niloticus* fingerlings exposed to different concentrations of textile mill effluents.

For ecologically relevant monitoring of environmental contamination, many researchers have proposed using behavioral indicators in fish (Atchison et al., 1987). The performance of normal behavior by individual fish follows specific physiological sequences, which are triggered by external stimuli acting via neural networks (Weber and Spieler, 1994). Disruption of these sequences before completion is likely to result in detrimental behavioral alterations. Inappropriate behavioral responses to environmental and physiological stimuli due to toxic effects of aquatic contaminants can have severe implications for survival (Weber and Spieler, 1994).

Several authors have considered effects of numerous anthropogenic pollutants on various fish behaviors (Marcucella and Abramson, 1978; Rand, 1985; Atchison et al., 1987; Little and Finger, 1990; Beitingler, 1990; Doving, 1991; Blaxter and Hallers- Tjabbes, 1992; Scherer, 1992; Little et al., 1993; Atchison et al., 1996; Kasumyan, 2001). Damaged peripheral
organs and reduction in swimming activities have been reported in fish feeding on pollutant contaminated phytoplanktons (Sprague, 1971). Anita et al (2010) observed acute toxicity, oxygen consumption and behavioral changes in the three major carps, *Labeo rohita* (Ham.) *Catla catla* (Ham.) and *Cirrhinus mrigala* (Ham.) exposed to Fenevolerate and observed anomalous behavior like surfacing movement ,irregular and erratic and darting swimming ,hyper excitability ,loss of equilibrium and hitting to the wall of the test tank and finally sinking to the bottom, just before death. Pathan et al (2009) studied the toxicity and behavioral changes in the freshwater fish *Rasbora daniconius* exposed to paper mill effluents and observed erratic swimming, jerky movements, rapid opercular movements, leaping out of water and thick mucus covering over the whole body surface during experiments.

*Channa striatus* have been found to exhibit altered behavioral responses, increased opercular movements and decreased bottom dwelling activity upon exposure to fertilizer wastewater (Yadav et al ,2007). A decrease in surface activities, including, linear movements, distance travelling, jumping, equilibrium, movement of fins were also observed. The movements of eyes were observed to be very slow. The opercular movement was reported to increase considerably with increase in the wastewater concentration. The hypoxic condition in fish was found to cause increase in the breathing rate. Patil and David (2008) in their study on behavior and respiratory dysfunction as an index of Malathion toxicity in *Labeo rohita* clearly reported that while the control fish were active with controlled and coordinated movements, the toxicant exposed fish exhibited irregular, erratic,darting movements and loss of equilibrium due to inhibition of Ache activity, thereby, leading to accumulation of acetylcholine in cholinergic synapses and ending up with hyper stimulation. These findings are in corroboration with those of Murshigeri and David, 2005 and others viz., Dube and Hosetti, 2010, Rao et al., 2003 and Parma.
de Croux et al., 2002. Pandey et al., (2009) reported erratic swimming increased surfacing, decreased rate of opercular movements, copious mucus secretion, reduced agility and inability to maintain normal posture and balance with increasing exposure time in test fishes exposed to Dimethoate.

Hence, summarizing the various behavioral anomalies in fish exposed to various toxicants, in general, include initial increase in opercular movements followed by steady decrease with increased duration of exposure (Shiva kumar and David, 2004), gulping air at the surface, swimming at the water surface, disrupted shoaling behavior and easy predation (Ural and Simsek, 2006). Gulping of air may help to avoid contact of toxic medium. Surfacing phenomenon might be a demand of higher oxygen level during the exposure period (Katja et al., 2005). Finally, fish sunk to the bottom with the least opercula movements and die with their mouth opened. The increased oxygen consumption in Labeo rohita and Catla catla under sublethal concentrations of both the toxicants is in corroboration with the increased consumption of oxygen in trout exposed to permethrin (Haya, 1989) and P. pugio larvae exposed to fenvalerate for twenty four hours (McKenney and Hamakar, 1984). Reddy et al. (1977) reported an elevation in oxygen up-take during first two hours of exposure followed by decrease in subsequent hours in Channa striatus exposed to cypermethrin. Similar trend was reported in L. rohita (Raju, 1991) and C. punctatus (Jeevapradha, 1990) exposed to Cypermethrin. Bradbury et al. (1986) stated that the greater decrease in the rate of oxygen consumption in the fish Cirrhinus mrigala may be due to internal action of the pesticide, as toxicant altering the metabolic cycle at subcellular level. Similar observations were also reported by Mushigeri and David (2003) and Jadhav and Sontakka (1977). The decrease in oxygen consumption at sub lethal concentrations of the toxicant indicates lowered energy requirements, which in turn indicates pronounced
haematological changes (Tilak and Satyavardhan, 2002). Similar reduction in oxygen consumption has been reported in *Channa striatus* exposed to organophosphate pesticide (Natarajan, 1981), *O. mossambicus* due to organ Chlorine intoxication (Vasanthi and Ramasamy, 1987), *M. cupanus* following Carbamide treatment (Arunachalam and Palanichamy, 1982), and *C. punctata* exposed to Carbaryl (Tilak, 1979).

**Results and discussion** –

Behavioral changes in *C.punctatus* in response to short and long term exposure to low and high concentration of phenolic effluent are depicted in Table 4.1 and 4.2.

Fishes exposed to low concentrations of phenolic effluent showed no obvious change and normal behavior in the first 48 hours in 0.1 and 0.15%. However, those exposed to 0.2% effluent showed rapid opercular and mouth movements. Those exposed to 0.25% effluent showed increased surfacing with fast, erratic, darting swimming movement. Heavy mucous secretion was shown in both cases during 48 hours and beyond.

In response to long term exposure to high concentration of phenolic effluent (10, 20 and 30%), fishes exhibited erratic swimming, jerky movements, respiratory distress and a tendency to escape out of the toxic medium in the first week after exposure. In the second and third weeks, those exposed to 10% effluent exhibited heavy mucous secretion which increased day by day. On the other hand, the fishes exposed to 20 and 30% effluent exhibited violent movements, dashing against the wall of the aquarium with no heed for self injury. By the third week, their bodies and organs, particularly, gills and liver turned pale. The fishes assumed an erect posture, with the body aligned on the caudal fin, showing loss of equilibrium. The normal surfacing behavior was replaced by jerky movements towards the lateral side. The operculum
beat rapidly depicting respiratory distress. A heavy protective coat of mucous secretion was found throughout.

Review of literature shows that the various behavioral anomalies in fish exposed to different toxicants, in general, include initial increase in opercular movements followed by steady decrease with increased duration of exposure (Shiva kumar and David, 2004), gulping air at the surface, swimming at the water surface, disrupted shoaling behavior and easy predation (Ural and Simsek, 2006). Gulping of air may help to avoid contact of toxic medium. The present author agrees with Katja et al., 2005 that the increased surfacing phenomenon visible in case of fishes exposed to low concentration of effluent might be due to a higher oxygen demand during the exposure period.

The changes in the respiratory activity of fish have been used by several investigators as indicators of response to environmental stress. The respiratory potential or oxygen consumption of an animal are the important physiological parameters to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general (Prosser and Brown, 1977). Since, aquatic organisms have their external surfaces and important organs such as gills almost entirely exposed to water, the effect of toxicants on the respiration is more pronounced. Pesticides enter into the fish mainly through gills and with the onset of symptoms of poisoning, the rate of oxygen consumption increases (Premdas and Anderson, 1963; Ferguson et al., 1966a). Holden (1973) observed that one of the earliest symptoms of acute pesticide poisoning is respiratory distress. This serves not only as a tool in evaluating the susceptibility or resistance potentiality of the animal, but also useful to correlate the behavior of the animal. Skidmore (1970) using cannulation techniques, found that zinc
reduced the oxygen level of blood leaving the gills. It reduced the efficiency of oxygen transport across the gill membrane, so that fish die of hypoxia. When fish were exposed to sub lethal and lethal concentrations of fenvalerate, several behavioral changes were observed which included swimming at the surface of water. This surfacing phenomenon was more in fish exposed to lethal concentration and sub lethal concentration over the control fish. Hyper excitation, loss of equilibrium, increased cough rate, flaring of gills, increase in production of mucus from the gills, darting movements, hitting against the walls of test tanks and curvature of spine were also noticed in all the three major carps. When exposed to lethal concentration, body surface acquired dark colour before their death which is one of the symptoms of toxicity. A film of mucus was observed all over the body and also on the gill.

The present author opines that the hypoxic condition in fish causes increase in the breathing rate, which in turn is caused by decreased efficiency in oxygen uptake. Reduction in bottom dwelling activities may be due to toxic stress on account of increased uptake of toxicants from the waste water. Similar responses due to toxicants have also been reported in *Gasterosteus aculeatus* (Jones, 1956) and *Mystus kelitus* (Singh and Singh, 1979; Stephen et al., 1987).

Ethological responses are the most sensitive parameters for measuring neurotoxicity in presence of toxicants (Doving, 1992). The loss of equilibrium noticed, in the present case, during long term exposure to higher concentration of effluent may be due to non functioning of the brain. This observation was also reported in *C. punctatus* exposed to in nickel and zinc by Saxena et al. (1981). Irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and hitting to the walls of the test tank before finally sinking to
the bottom just before death in the present cases, all reveal neurotoxicity. Increased mucus secretion is a protective method to counter the irritating effect of the toxicant.

**TABLES**

**Table.4.1 Behavioral changes in C.punctatus in response to short term exposure to low concentration of phenolic effluent.**

<table>
<thead>
<tr>
<th>HOURS</th>
<th>0.1 %</th>
<th>0.15 %</th>
<th>0.2 %</th>
<th>0.25 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>No obvious change.</td>
<td>Normal behavior.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>Heavy mucous secretion.</td>
<td>Rapid opercular and mouth movement.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>Heavy mucous secretion.</td>
<td>Surfacing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Heavy mucous secretion.</td>
<td>Surfacing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>144</td>
<td>Fast, erratic, darting swimming movement.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>168</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2. Behavioral changes in *C. punctatus* in response to long term exposure to high concentration of phenolic effluent.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
</tr>
</thead>
</table>
| 1     | Erratic swimming.  
       | Jerky movements  
       | Respiratory distress.  
       | Tendency to leap out of the toxic medium. |
| 2     | Heavy mucous secretion which increases day by day.  
       | Dash against aquarium wall. |
| 3-4   | Heavy mucous secretion which increases day by day  
       | Pallor (Body, gills and liver)  
       | Erect posture.  
       | Loss of equilibrium.  
       | Lateral movement with jerks.  
       | Rapid opercular movements.  
       | Respiratory distress.  
       | Heavy mucus secretion |