Chapter 8

Respiratory Studies
8. RESPIRATORY STUDIES

8.i Introduction

Oxygen consumption is a very sensitive physiological process and the changes in respiratory activity have been used as indicators in toxicant-exposed animal (Sharp et al., 1979). Heavy metal salts constitute a very serious type of pollution in fresh-water, because they are stable compounds and are not readily removed by oxidation, precipitation or other means and affect the activity of the animals (Chinnaya, 1971). Studies on oxygen consumption will be more helpful and can be used as an index to assess the physiological stress of the animals. A few relevant works of inspiration were by Thurberg et al. (1973); Lingaraja et al. (1980); Khan and Sarojini (1989), Tulasi et al. (1987); Sakuntala (1992); Varghese et al. (1992) and Vijayaraman (1993).

The gills are the sites of respiration and transport system involved in osmoregulation and it has been confirmed that accumulation of metal ions within them may have an effect on these functions (Vemberg and O'Hara, 1972; Thurberg et al., 1973; Jones, 1975). Physiological damage may be just as important as mortality. Even a pollutant that does not affect a particular process under normal unstressed conditions, may affect the ability of an animal to adjust to changing environmental conditions, hence, it may ultimately decrease its chance for survival (Thurberg et al., 1973). Vemberg and Vemberg (1972) reported such an effect on fiddler crab, Uca Pugilator, exposed to mercury. Methyl-mercury is more toxic and is rapidly accumulated in fish than in inorganic forms (Paulose, 1989).

Cadmium ion is accumulated in the gills, gastrointestinal tracts and kidneys of fishes (Ohmono et al., 1972). Gokhale and Borgaoankar (1985), reported that the respiratory rate was related to different size and sex groups in crabs Ilyoplax gangetica, exposed to sublethal concentration of cadmium.
Besides, being an essential element, Zn has been shown to be toxic to aquatic organisms (Skidmore, 1964; Bryan, 1971; Waldichuk, 1974; Coombs, 1980). Lethal concentration of zinc induces cytological damage to the gills of fish (Skidmore and Tovell, 1972; Crespo et al., 1981) and the physiological cause of death may be related to the breakdown of respiratory and osmo-regulatory processes. Osmoregulation, among other physiological processes, is actually affected by heavy metals (Bouguegneau and Gilles, 1979; Crespo, 1984). Jones (1964) believed that gill damage by copious secretions of mucus restricted the respiration and were responsible for death in fresh-water fish. Lloyd (1960) reported that in 20 mg/l of zinc, a cytological breakdown of the gill epithelium of trout occurred in about 2 hours. At about 4mg/l zinc, gill lamella became swollen before death.

The lethal action of dissolved salts of heavy metals on fish and shell-fish has been investigated by many workers (Skidmore, 1970 review). It is known from the review, that the death of the fish placed in solutions of salts of heavy metals results, not from internal poisoning but from an interaction between the metallic ion and the mucus secreted by the gills, whereby a film or coagulated mucus is formed on the gill membranes, impairing their respiratory efficiency to such a degree that the fish is asphyxiated.

In the present investigation, when the crabs were exposed to heavy metals (Hg, Cd and Zn), their gills recorded markable accumulation of metals (See Chapter-Bioaccumulation). Under conditions of stress, the respiratory activity may undergo subtle variations and alterations in the respiratory surfaces which would lead to change in oxygen uptake, which eventually creates a physiological imbalance in the organism (Wolvekamp and Waterman, 1960). Such physiological imbalances caused due to metals may be useful to detect the water pollution and also to elucidate the metabolic biochemistry of the organism.
With this view, to understand the effect of mercury, cadmium and zinc on the oxygen consumption, the experiments have been carried out in the selected two species of the paddy field crabs.

8.ii. Materials and Methods

Respiratory studies were carried out, using a flow through chamber (Lingaraja et al., 1980). The crabs were allowed to acclimatize before the experiments and were exposed to sublethal levels of heavy metals as described for the previous studies.

Two animals were introduced one in each respiratory chamber, containing 2 different test concentrations (TU₁ and TU₂). Controls were maintained simultaneously. The flow rate was adjusted in such a way as to replace the water in the respiratory as well as in control chambers, within half an hour. Six consecutive samplings were made at regular intervals of half an hour, to measure the oxygen consumption in the control and test crabs. The respiratory studies were conducted for the 1st, 7th, 14th and 21st days of exposures. The dissolved oxygen was estimated following Winkler's method (Strickland and Parson, 1972). The rate of \( \text{O}_2 \) consumption was expressed in \( \text{M}10^2/\text{g/wet wt./hr.} \) Student's 't' test (absolute) was used to examine the significance of difference among the test concentrations and the control on different exposure days.

8.iii Results

The rate of oxygen consumption of \( S. \text{Spinigera} \) and \( P. \text{atkinsonianum} \) exposed to the three heavy metals, mercury, cadmium and zinc individually is shown in Figs. 47 and 48.

Mercury

The oxygen consumption increased markedly over the control when the crabs were exposed to sublethal concentrations of mercury. The increase in oxygen consumption was found to be dose dependent. The percentage increase over the control was 46.3 and...
59.56 on the first day of exposure. However there was a noticeable decrease and in the
21st day of exposure the oxygen consumption decreased below the control. The
percentage decrease was 18.4 and 20.1 in the crab, *P. atkinsonianum* while *S. spinigera*
was found to be more tolerant to mercury than *P. atkinsonianum*. The percentage increase
over the control was found to be 34.5 and 34 on the first day and decrease on the
21st day was 24 and 42.5 percentage. A uniform trend of decrease was found after the
first day of exposure to 21st day of exposure to mercury in both the species. There is
a significant difference in the 1st and 21st days of exposure for both the species.
Tables 37 and 38.

**Cadmium**

The results revealed that cadmium reduced the oxygen consumption in both
the species. The reduction in oxygen consumption increased with increase in the test
concentration and also duration. However, there was a slight elevation in oxygen
consumption in the first day of exposure in both the species, exposed to cadmium. The
percentage increase in the first day was 7.95 and 13.25 in *P. atkinsonianum* and 3.89 and
12.85 in *S. spinigera*. But remarkable decrease over the control from 7th day onwards
was noticed until the 21st day when the percentage decrease below the control was
noted to be 20.9 and 34.5 in *P. atkinsonianum* and 31.56 and 41.6 in *S. spinigera*. The
percentage change over control in oxygen consumption is clearly shown in Figs. 47 and
48 for the two species of crabs. Tables 37 and 38 show 't' values indicating significant
changes in oxygen consumption of the crabs exposed to cadmium on various days.

**Zinc**

An increase in oxygen consumption over control in the first day was noted
in both the crabs exposed to zinc. But decreasing trend was noticed from the 7th day
onwards. The percentage increase over control was 4.26 and 10.6 in *P. atkinsonianum* and
2.04 and 11.34 in *S. spinigera*. 21st day showed a significant decrease in the oxygen
consumption (Figs. 47 and 48). The percentage decrease below the control was 18.4 and
25.8 in *P.atkinsonianum* while in *S. spinigera* it was 26.9 and 33.55 percent. Significant changes were observed in zinc exposed crabs as shown in Tables 37 and 38.

8.iv Discussion

In the present study, both the crabs *S. spinigera* and *P.atkinsonianum* show a notable elevation in the uptake of oxygen on the 1st day of exposure with all the three metals individually. Later, there is a gradual decline in their consumption rate. This clearly indicates that the increasing accumulation of metals reduces the rate of oxygen consumption.

Normally, when an animal is exposed to a pollutant, due to initial restless activity, there is a transitory excitation in the metabolic rate. Similarly, the initial stress of accumulation of metals shoots up the level of consumption of oxygen and later the consumption level is declined. Such trend of oxygen consumption has been reported in *Scylla serrata* with Hg, Cd and Zn (Narayanan, 1989). Likewise, Sakuntala (1992) reported an immediate increase in the rate of consumption and its depression during the subsequent period of exposure to sub-lethal concentrations of lead and chromium in the crab, *Spiralotelphusa hydrodrama*. Further, the present findings are in agreement with the reports of Raymont and Shields (1963); O’Hara (1971) and Thurberg *et al.* (1974).

Depledge (1984) reported 100% mortality in 24-48 hours of exposure with mercury in *Carcinus maenas*. This was associated with the loss of osmoregulatory ability of these crabs. Thurberg *et al.* (1973) has reported the changes caused in the oxygen consumption in juvenile and adult in two species of estuarine crabs exposed to heavy metals. Gokhale and Borgaonkar (1985) reported that, respiratory rate was related to different size and sex group crabs, *Ilyoplax gangetica*, exposed to sublethal concentration of cadmium.

The present investigation clearly showed that the rate of oxygen consumption in the crab *S. spinigera* decreased with increase in concentration of cadmium. Similar
findings were observed by Thurberg et al. (1973), in the crabs, *Carcinus maenas* and *Cancer irroratus*, where cadmium reduced the rate of oxygen consumption in both the species. Exposure to heavy metals produced respiratory disturbance and reduced oxygen uptake in marine crab, *Uca pugilator* (Vernberg and Vernberg, 1972); estuarine crab, *C. maenas* and *C. irroratus* (Thurberg et al., 1973) and terrestrial crabs *B. guerini* (Reddy, 1980); *O. senex senex* (Bharanikumar, 1986) and *B. cunicularis* (Sarojini et al., 1989).

The decrease may be related with inhibition of enzyme activity (Vernberg and Vernberg, 1972; Tucker and Matte, 1980). Physiological, histological and ultra structural studies have shown that metal ions interfere with respiration by disrupting the structure of the gill cells. (Bengali and PatU, 1984; and Torreblanca et al., 1988). Bubel (1976) and Papanathanassiou and King (1983) reported that mitochondria were the most affected organelles by metals, hence there is a reduction in respiratory rate. The reduced oxygen consumption in the cadmium-exposed mud crab, *Eurypanopeus depressus* by Collier et al., (1973) confirms the present result. Reish (1978) noticed a decrease in the respiratory rate and enzyme alterations when grass shrimp was exposed to sublethal levels of cadmium for 30-60 days. Reduced oxygen consumption was also noticed by Engel and Fowler (1979) in *Crassostrea virginica* after 14 days of exposure to cadmium.

Increasing concentration of zinc decreased the rate of respiration with TU\(_1\) and TU\(_2\) levels. The fresh water crab, *B. cunicularis* showed significant alteration in oxygen consumption as the exposure period to the heavy metals increased (Khan and Sarojini, 1989), which was observed with TU\(_1\) and TU\(_2\) in the crab, *S.spinigera* exposed to zinc. In fish, the gill appears to be the primary organ for zinc accumulation (Lovegrove and Eddy, 1982; Skidmore, 1972). Damage to the gill tissue in acutely exposed fish interferes with respiration and causes tissue hypoxia (Skidmore, 1970; Burton et al., 1972; and Hughes, 1973).

Recently, when the crabs, *B. cunicularis*, exposed to mercury, copper and zinc, Varghese et al. (1992) reported, a decrease in O\(_2\) consumption at the higher concentration
of the accumulated metals. They related much decline in $O_2$ consumption with the reduced efficiency of gill, gill damages, altered membrane physiology and poor transport of $O_2$ due to the stress in the affinity of respiratory pigment haemocyanin.

The result of the histological changes of the gills due to metals of the present investigation suggests that the accumulation of metals induces gill damages and hampering or reduction in respiratory activity, which is being confirmed from the results of this chapter. To have further elucidation, as suggested by Hughes (1973), respiratory studies require further details at all levels of respiratory chain from the gill membrane to the cellular mechanisms of particular organ systems. Hence the present respiratory results influenced to investigate the histopathological studies of gill tissues.
Table 37  
't' values for the difference in respiratory rate of the crab, *P. atkinsonianum*  
between control and TU₁ and TU₂ test concentrations

<table>
<thead>
<tr>
<th>Metals</th>
<th>Con</th>
<th>1st day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg</td>
<td>TU₁</td>
<td>20.1464**</td>
<td>7.24329**</td>
<td>0.6097</td>
<td>4.3069**</td>
</tr>
<tr>
<td></td>
<td>TU₂</td>
<td>2.9511*</td>
<td>13.991**</td>
<td>0.0000</td>
<td>4.7636**</td>
</tr>
<tr>
<td>Cd</td>
<td>TU₁</td>
<td>5.4749**</td>
<td>3.0297*</td>
<td>9.8388**</td>
<td>4.8759**</td>
</tr>
<tr>
<td></td>
<td>TU₂</td>
<td>4.9015**</td>
<td>9.0982**</td>
<td>17.0415**</td>
<td>8.1176**</td>
</tr>
<tr>
<td>Zn</td>
<td>TU₁</td>
<td>3.0816*</td>
<td>1.1987</td>
<td>9.2276**</td>
<td>4.2567**</td>
</tr>
<tr>
<td></td>
<td>TU₂</td>
<td>7.6600**</td>
<td>10.194**</td>
<td>17.4557**</td>
<td>5.9711**</td>
</tr>
</tbody>
</table>

Significance ** at 1%, * at 5% level
Table 38  't' values for the difference in respiratory rate of the crab, *S. spinigera* between control and TU<sub>1</sub> and TU<sub>2</sub> test concentrations.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Con</th>
<th>1st day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg</td>
<td>TU&lt;sub&gt;1&lt;/sub&gt;</td>
<td>3.9416**</td>
<td>0.1544</td>
<td>10.0816**</td>
<td>11.4729**</td>
</tr>
<tr>
<td></td>
<td>TU&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.9294**</td>
<td>0.1101</td>
<td>8.9512**</td>
<td>12.6494**</td>
</tr>
<tr>
<td>Cd</td>
<td>TU&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.3616</td>
<td>2.2206</td>
<td>17.8677**</td>
<td>19.8907**</td>
</tr>
<tr>
<td></td>
<td>TU&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1.4728</td>
<td>3.1042*</td>
<td>28.5029**</td>
<td>24.0966**</td>
</tr>
<tr>
<td>Zn</td>
<td>TU&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.3500</td>
<td>1.4222</td>
<td>52.8628**</td>
<td>16.1883**</td>
</tr>
<tr>
<td></td>
<td>TU&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1.3500</td>
<td>3.0000*</td>
<td>25.1439**</td>
<td>20.2000**</td>
</tr>
</tbody>
</table>

Significance ** at 1%, * at 5% level
Fig. 47  Oxygen Consumption in *P. atkinsonianum*
Exposed to Heavy Metals

% Change over control

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1st Day</th>
<th>7th Day</th>
<th>14th Day</th>
<th>21st Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg (TU1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hg (TU2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd (TU1)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Zn (TU1)</td>
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<td></td>
</tr>
<tr>
<td>Zn (TU2)</td>
<td></td>
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</tbody>
</table>
Fig. 48
Oxygen Consumption in *S. spinigerae*
Exposed to Heavy Metals

% Change over control

1st Day  7th Day  14th Day  21st Day

- Hg (TU1)  - Hg (TU2)  - Cd (TU1)  - Zn (TU1)  - Zn (TU2)
- Cd (TU2)