Effects of Naphthalene Exposure on Blood Serum Enzyme Activities in the Crab *Scylla serrata* (Forskal)

B G KULKARNI & V B MASUREKAR
Department of Zoology, The Institute of Science, Bombay 400032

Received 1 March 1984; revised received 12 April 1984

Juvenile *S. serrata* were exposed to 1.25, 2.5 and 5 mg l\(^{-1}\) concentrations of naphthalene. Specific activities of lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase in blood serum were determined after 1.2, 6 and 9 weeks of exposure. Changes in enzyme specific activities found after 1.2 and 6 weeks of exposure were attributable to naphthalene. Elevation in the activities of the enzymes was highly significant after 45 d exposure to naphthalene at the two higher concentrations.

Toxic effects of water-borne hydrocarbons on marine organisms are of significance in relation to petroleum contamination of marine waters. It is evident that aromatic hydrocarbons like naphthalenes are highly toxic to marine animals\(^{1-4}\). Acute toxicities of petroleum products and their accumulation and depuration by marine organisms have been studied but less attention has been paid to study the physiological responses of marine organisms to such pollutants. Of the many physiological processes of animals, studies of variations in specific enzyme activities yield relevant information on the use of changed enzyme activities, as indicators of environmental stress\(^{5,6}\).

In this paper the effects of sublethal concentrations of naphthalene on the specific activities of lactate dehydrogenase (LDH; EC 1.1.1.27), aspartate aminotransferase (AAT; EC 2.6.1.1) and alanine aminotransferase (AIAT; EC 2.6.1.2) in blood serum of intertidal crab *Scylla serrata* (Forskal) are reported.

Juvenile crabs of intermoult stage and more or less of uniform size (4.5 to 5.5 cm across) were collected from Bassein creek. The seawater in which they were acclimated in the laboratory for 7 d had temperature 27 to 29°C, pH 7.6 to 8, dissolved oxygen 6.3 to 8.5 mg l\(^{-1}\) and salinity 30 to 32 \(\times 10^{-3}\); these conditions were same as during experimentation. After acclimation the crabs were exposed to naphthalene concentrations of 1.25, 2.5 and 5 mg l\(^{-1}\) in glass aquaria each containing 3 l of seawater and 10 crabs. These 3 concentrations were selected with reference to 96 h LC\(_{50}\) value of naphthalene (17 mg l\(^{-1}\)) for intermoult crabs\(^{4}\). Acetone was used as the solvent for preparing stock solution of naphthalene. Aliquots of this solution were added to the seawater in the experimental tanks so as to get the desired concentrations of the toxicant. In case of control experiments, acetone alone was added to the seawater. The level of naphthalene in each tank was kept more or less constant by changing the water every day and adding the requisite amount of naphthalene stock. The crabs were fed clam flesh on alternate days.

At the end of each experimental period (7, 15 and 45 d) blood of the crabs was removed by inserting a hypodermic needle into the arthrodial membrane at the base of the leg. This method ensures practically no damage to the animal, thus enabling samples to be taken from the same animal on successive days. To obtain serum the blood was transferred to polyethylene vials and frozen. Upon thawing the clots were slightly broken and stirred with a glass rod. Each sample thus obtained was carefully decanted into another vial and aliquots of the serum were used for determination of the enzyme activities. The specific activities of LDH\(^{7}\), AAT\(^{8}\) and AIAT\(^{9}\) were determined. Protein content was estimated\(^{10}\) using bovine serum albumin as the standard.

Activities of the 3 enzymes in blood serum were elevated following exposure to naphthalene (Table 1). This elevation was most significant after 45 d of exposure to naphthalene at concentrations 2.5 and 5 mg l\(^{-1}\).

The observed changes in the enzyme activities might be due to their leakage from damaged tissues like the hepatopancreas\(^{11}\). This is consistent with other reports\(^{12,13}\) that altered serum aminotransferase activity may be used as an indicator of the hepatocellular damage due to toxicants. Stimulation in the activity of LDH indicates disturbances in the cellular oxidative process. This change might appear to favour a less efficient anaerobic metabolism in crabs in response to the environmental stress, probably due to the inability of tissues to derive sufficient oxygen for the normal metabolic functions. The suggested lack of proper oxygen supply to the tissues may be due to damage to the gills, as such damage is noticed in naphthalene treated crabs\(^{11}\). This is in agreement with Hodson's\(^{14}\) view that aquatic pollutants cause gill damage leading to tissue hypoxia and probably death.
Table 1—Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AAT), Alanine Aminotransferase (AIAT) Levels in Blood Serum of Control and Naphthalene Treated Crabs

(Values are mean ± of 5 determinations. Values in parentheses indicate percent increase)

<table>
<thead>
<tr>
<th>Exposure period</th>
<th>Control</th>
<th>Naphthalene conc. mg 1⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.25</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>LDH</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>68.48 ± 15.96</td>
<td>62.30 ± 6.40</td>
</tr>
<tr>
<td>15</td>
<td>64.09 ± 10.32</td>
<td>60.07 ± 15.85</td>
</tr>
<tr>
<td>45</td>
<td>69.12 ± 14.0</td>
<td>70.69 ± 9.63</td>
</tr>
<tr>
<td></td>
<td>AAT</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>20.53 ± 2.07</td>
<td>20.74 ± 3.04</td>
</tr>
<tr>
<td>15</td>
<td>22.06 ± 2.41</td>
<td>20.68 ± 1.48</td>
</tr>
<tr>
<td>45</td>
<td>23.91 ± 4.18</td>
<td>42.36 ± 4.41</td>
</tr>
<tr>
<td></td>
<td>AIAT</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>23.07 ± 1.49</td>
<td>23.86 ± 1.34</td>
</tr>
<tr>
<td>15</td>
<td>23.86 ± 1.34</td>
<td>23.47 ± 2.31</td>
</tr>
<tr>
<td>45</td>
<td>24.92 ± 3.19</td>
<td>29.88 ± 8.02</td>
</tr>
</tbody>
</table>

*P = 0.01
Activity expressed as: LDH - μ mole product formed·min⁻¹·mg⁻¹ protein
AAT - μ mole product formed·(60 min)⁻¹·mg⁻¹ protein
AIAT - μ mole product formed·(30 min)⁻¹·mg⁻¹ protein

Enhancement in the activity of AAT and AIAT in serum of treated crabs might be due to imposition of stress on these crabs during naphthalene exposure. It has been suggested that stress condition in general induces an elevation in the activities of aminotransferase and it is likely that the toxic stress could be responsible for their elevation as observed in the present study. This elevation of aminotransferase also indicated protein mobilization. Protein mobilization due to increase in aminotransferase activity has also been suggested in shrimp due to their exposure to crude oil, in Pila globosa (Swainson) due to stress of malathion exposure and in tilapia Sarotherodon mossambicus (Peters) because of naphthalene exposure.

References