INTRODUCTION

The off-target movement of chemicals used in industry and agriculture is usually unavoidable; these chemicals get into natural water and may cause significant tissue damage in fish (McKim et al., 1970; Reichenbach-Klinke, 1972; Horvath and Stammer, 1979; Ferri and Macha, 1980; Rojik et al., 1983; Benedeczky et al., 1984). Heavy metals produce toxic effects on the tissues and alter the physiological functioning of various systems of animals. Passow et al. (1961) reported that the toxic effects of metals and chemicals may result from their binding with biologically active enzymes and other proteins. Alteration in enzymic and metabolic processes has been observed in animals treated with heavy metals (Webb, 1966; Sastry and Gupta, 1978b, c).

Verma et al. (1979b) reported that a pollution monitoring method using enzyme inducement or enzyme depression in fish or other organisms have been proposed for studying polluted environments. Enzymes getting into the blood after cell necrosis of certain organs may be used to indicate the tissue damage. (Kristofferson et al., 1974; Nemcsok et al., 1981; Nemcsok and Boross, 1982). Bouck et al. (1975) observed that the activity of serum enzymes are likely to reflect the metabolic state of fish in much the same way as they do in mammals. Thus it can be expected that toxic agents or factors which lead to chronic
impairment of the animal's metabolism will cause changes, usually increases, of the activities of some serum enzymes (Wieser and Hinterleitner, 1980). Some of these responses are likely to be of a more general nature, i.e., indicating the organism's answer to a situation of stress brought about by a general deterioration of water quality (Morgan et al., 1973; Oikari and Soivio, 1977).

Chronic cadmium poisoning manifested as Itai-Itai disease leading to high blood levels of glutamic oxaloacetic transaminase, alkaline phosphatase and lactate dehydrogenase were observed by Nogawa et al. (1979). According to Shaikh and Smith (1986), the above observation is representative of an advanced picture of cadmium poisoning. Aspartate and alanine transaminases not only function as link enzymes between the protein and carbohydrate metabolisms, but also serve as an indicator of altered physiological or stress condition (Knox and Greengard, 1965). The GOT and GPT activities in the blood and tissues are altered under certain clinical conditions and, therefore, these enzymes have considerable diagnostic value in humans; however, their diagnostic significance in fish is not clearly understood (Gill et al., 1991).

Monitoring of liver enzymes leakage in the blood has proved to be a very useful tool in hepatotoxic studies (Kaplan and Szabo, 1983). The observations on rainbow trout, *Salmo gairdneri* (Racicot et al., 1975; Pfeifer et al., 1977;
Stathman et al., 1978), English sole, *Parophrys vetulus* (Casillas et al., 1983) and grey mullet, *Mugil auratus* (Ozretic and Krajnovic-Ozretic, 1993) suggest that alteration of GOT and GPT activity in plasma could be applied as helpful tables of liver toxicity.

Christensen et al. (1982) reported that toxic chemical pollutants often affect the activity of enzymes atleast to some degree and hence enzymes are used as logical candidates in biomonitoring. Increased serum levels of LDH and aminotransferases have been reported in a variety of hepatic diseases and tissue injuries, such as myocardial infarction (Moss et al., 1986) and thus serve as markers of injury. The enzyme LDH is a pivotal enzyme between the glycolytic pathway and tricarboxylic acid cycle and any changes in protein and carbohydrate metabolism might cause changes in LDH activity (Abston and Yarbrough, 1976).

LDH has been used for demonstrating tissue damage in fish for a long time (Nemcsok and Boross, 1982). Asztalos and Nemcsok (1985) have reported that LDH isoenzymes have been proved to be a suitable basis for the identification of the damaged organ in human clinical diagnosis. The harmful effects of cadmium is attributed to its effects on sulfhydryl groups of enzymes, especially dehydrogenases (Belies, 1975). According to Gupta (1987), dehydrogenases are the enzymes involved in the energy release by the biological oxidation of food stuff inside the mitochondria,
and also in the production of reduced potential (NADPH) required in the biosynthetic and detoxification mechanisms.

There is not much work on the effect of cadmium toxicity on the plasma enzyme activity of fish _Cyprinus carpio_ in relation to water hardness which are a highly reliable indicator of aquatic pollution. Hence, in the present study, the same has been investigated.
MATERIAL AND METHODS

Blood samples of fish from control, treatment-I and II were collected. Plasma used for enzyme activity studies was separated as outlined in the Material and Methods section of Chapter-II and III.

ESTIMATION OF PLASMA GLUTAMATE OXALACETATE TRANSAMINASE (GOT)

Plasma GOT activity was estimated by 2,4-DNPH method of Reitman and Frankel (1957) using Diagnostic Reagent Kit supplied by Span Diagnostics Pvt. Ltd., Surat, India.

Principle

GOT catalyses the following reaction:

\[ \alpha-Ketoglutarate + L-Aspartate \rightarrow L-Glutamate + Oxalacetate \]

Oxalacetate so formed is coupled with 2,4-Dinitrophenyl hydrazine (2,4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium.

Reagents

Reagent 1: Buffered Aspartate - \( \alpha \)-KG Substrate, pH 7.4
Reagent 2: DNPH Colour Reagent
Reagent 3: Sodium hydroxide, 4N
Reagent 4: Working Pyruvate Standard, 2 mM

Preparation of working solution

Solution I: One ml of Reagent-3 was diluted to 10 ml with distilled water.
Preparation of Standard Curve

To five test tubes serially numbered 1 to 5 representing 0, 24, 61, 114 and 190 Units/ml enzyme activity, prescribed volumes of Reagent - 1, Reagent - 4, distilled water and Reagent - 2 were added. The content of the test tubes were mixed well and allowed to stand at room temperature for 20 min., after which 2.5 ml of Solution - I was added to all the tubes. The contents were mixed well by inversion and allowed to stand at room temperature for 10 min. and then the O.D. of all the five tubes were measured against distilled water (Blank) at 505 nm using spectrophotometer (Spectronic - 20, Baush and Lomb, USA). The standard graph was plotted by taking enzyme activity (Units/ml) on X-axis and O.D. on Y-axis (Fig. 15).

Procedure

For the estimation of GOT activity in plasma, 0.25 ml of Reagent - 1 was added to a test tube and incubated at 37°C for 5 min. Then 0.05 ml of plasma was added, mixed well and incubated at 37°C for 60 min., after which 0.25 ml of Reagent-2 was added. The contents were mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution -I was added mixed well and the contents were allowed to stand at room temperature for 10 min. The O.D. value was measured against distilled water at 505 nm using spectrophotometer (Spectronic - 20, Baush and Lomb, USA).
Fig. 15. Standard graph for glutamate oxalacetate transaminase (GOT) activity.
Calculation

The O.D. values were marked on the Y-axis of the standard curve and it was extrapolated to the corresponding enzyme activity on the X-axis.

ESTIMATION OF PLASMA GLUTAMATE PYRUVATE TRANSAMINASE (GPT)

Plasma GPT activity was estimated by 2,4-DNPH method of Reitman and Frankel (1957) using Diagnostic Reagent Kit supplied by Span Diagnostics Pvt. Ltd., Surat, India.

Principle

GPT catalyses the following reaction:
\[ \alpha - \text{Ketoglutarate} + L- \text{Alanine} \rightarrow L- \text{Glutamate} + \text{Pyruvate} \]

Pyruvate so formed is coupled with 2,4-Dinitrophenyl hydrazine (2,4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium.

Reagents

Reagent 1: Buffered Alanine \( \alpha - \text{KG} \) Substrate, pH 7.4
Reagent 2: DNPH Colour Reagent
Reagent 3: Sodium hydroxide, 4N
Reagent 4: Working Pyruvate Standard, 2 mM

Preparation of working solution

Solution I: One ml of Reagent-3 was diluted to 10 ml with distilled water.
**Preparation of Standard Curve**

To five test tubes serially numbered 1 to 5 representing 0, 28, 57, 97 and 150 Units/ml enzyme activity, prescribed volumes of Reagent - 1, Reagent - 4, distilled water and Reagent - 2 were added. The contents of the test tubes were mixed well and allowed to stand at room temperature for 20 min., after which 2.5 ml of Solution - I was added to all the tubes. The contents were mixed well by inversion and allowed to stand at room temperature for 10 min. and then the O.D. of all the five tubes were measured against distilled water (Blank) at 505 nm using spectrophotometer (Spectronic - 20, Baush and Lomb, USA). The standard graph was plotted by taking enzyme activity (Units/ ml) on X-axis and O.D. on Y-axis (Fig. 16).

**Procedure**

For the estimation of GPT activity in plasma, 0.25 ml of Reagent - 1 was added to a test tube and incubated at 37°C for 5 min. Then 0.05 ml of plasma was added, mixed well and incubated at 37°C for 30 min., after which 0.25 ml of Reagent - 2 was added. The contents were mixed well and allowed to stand at room temperature for 20 min. 2.5 ml of Solution -I was added, mixed well and the contents were allowed to stand at room temperature for 10 min. The O.D. value was measured against distilled water at 505 nm using spectrophotometer (Spectronic - 20, Baush and Lomb, USA).
Fig. 16. Standard graph for glutamate pyruvate transaminase (GPT) activity.
Calculation

The O.D. values were marked on the Y-axis of the standard curve and it was extrapolated to the corresponding enzyme activity on the X-axis.

ESTIMATION OF PLASMA LACTATE DEHYDROGENASE (LDH)

LDH activity in plasma was estimated by 2,4-DNPH method (King, 1959) using Diagnostic Reagent Kit supplied by Span Diagnostics Pvt. Ltd., Surat, India.

Principle

Lactate dehydrogenase catalyses the following reaction:

\[
\text{LDH} \quad \text{Lactate} + \text{NAD}^+ \xrightarrow{\text{H+}} \text{Pyruvate} + \text{NADH}
\]

Products so formed are coupled with 2,4-Dinitrophenyl hydrazone (2,4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium.

Reagents

Reagent 1 : Buffered Lactate Substrate, pH 10.0
Reagent 2(A) : NAD for Test
Reagent 2(B) : NAD for Graph
Reagent 3 : DNPH Colour Reagent
Reagent 4 : NADH
Reagent 5 : Sodium hydroxide, 4N
Reagent 6 : Working Pyruvate Standard, 1 mM
Preparation of Working Solutions

Solution I (A) : Just before use each vial of Reagent - 2(A) (NAD for Test) was reconstituted with 0.3 ml of distilled water.

Solution I (B) : Just before use, each vial of Reagent - 2(B) (NAD for Graph) was reconstituted with 1.4 ml of distilled water.

Solution II : Just before use, each vial of Reagent - 4 (NADH) was reconstituted with 1.2 ml of Reagent-1 (Buffered Substrate).

Solution III : Reagent - 5 (Sodium hydroxide, 4N) was diluted 1 to 10 with distilled water

Preparation of Standard Curve

Seven test tubes were taken and serially numbered from 1 to 7. They represented 0, 167, 333, 500, 667, 833 and 1000 IU/litre of enzyme activity. Prescribed volumes of Reagent - 6, Solution - II, Reagent - 1, Solution I(B), distilled water and Reagent - 3 were added to each of the tubes, mixed well and incubated at 37°C for 5 min. Then 5.0 ml of Solution -III was added to each of the tubes. The contents were mixed well by inversion and allowed to stand at room temperature for 5 min. The O.D. of the contents of the tubes from 2 to 7 were measured against that of tube 1 (Blank) at 440 nm using spectrophotometer (Spectronic - 20, Baush and Lomb, USA). The standard curve was plotted by taking enzyme activity (IU/litre) on the X-axis and O.D. on the Y-axis (Fig. 17).
Fig. 17. Standard graph for lactate dehydrogenase (LDH) activity.
FIG. 17

Enzyme activity (IU/litre)

Optical density

0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.001
0.0009
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
**Procedure**

For the estimation of LDH in plasma, two test tubes marked as "Control" (C) and "Test" (T) were taken. To the 'Control' and 'Test' tubes, 0.5 ml of Reagent - 1 was added. To the tube marked 'Test' 0.05 ml diluted plasma was added and to the tube marked 'Control', 0.01 ml of distilled water was added. The contents in the tubes were mixed well and incubated at 37°C for 5 min. Then 0.1 ml of Solution - I (A) was added to the 'Test' tube, mixed well and both the 'Control' and 'Test' tubes were incubated for 37°C for 15 min.

The tubes were taken out and 0.5 ml of Reagent - 3 was added to both the tubes. Immediately 0.05 ml of diluted plasma was added to the 'Control' tube alone. Once again the contents in both the tubes were mixed well and incubated at 37°C for 15 min., after which the tubes were taken out and 5.0 ml of Solution - III was added to both the tubes. The contents were mixed well by inversion and allowed to stand at room temperature for 5 min. The O.D. of 'Control' and 'Test' were measured against distilled water using spectrophotometer (Spectronic - 20, Baush and Lomb, USA).

**Calculation**

The nett O.D. of the Test = O.D. Test - O.D. Control

The nett O.D. of the test was marked on Y-axis of the standard curve and it was extrapolated to the corresponding enzyme activity on the X - axis.
RESULTS

Changes in plasma glutamate oxalacetate transaminase activity of *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment-I and II are given in Table 27 and Fig. 18. At the end of first week of treatment, the enzyme activity from both the treatments showed an elevation over that of the control. In treatment-I, the fish showed 20.34 per cent increase, while in treatment-II they exhibited 13.45 per cent elevation in their enzyme activity.

The enzyme activity showed further increase in the treated fish after second week treatment, giving 26.58 and 15.30 per cent elevation over that of the control in treatment-I and II, respectively. The maximum per cent increase in the plasma GOT activity was exhibited by the experimental fish at the end of third week in both the treatments. Later on, the elevation in the enzyme activity of treated fish started showing a gradual reduction in the case of treatment-I, whereas in treatment-II there was a sudden and steep fall.

DMRT analysis of plasma GOT activity in the plasma of fish *Cyprinus carpio* var. *communis* (Table 28) in control, treatment-I and II for first week revealed that all the three significantly varied from one another. A similar trend was noted during second, third, fourth and fifth week treatment.
Table 27. Changes in the plasma glutamate oxalacetate transaminase (GOT) activity in *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment-I and II

<table>
<thead>
<tr>
<th>Duration of treatment (in weeks)</th>
<th>Glutamime oxalacetate transaminase activity (Units/ml)</th>
<th>Control</th>
<th>Treatment-I</th>
<th>Per cent change</th>
<th>Treatment-II</th>
<th>Per cent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td>1104.000 ± 14.400</td>
<td>1328.500 ± 10.302</td>
<td>+ 20.34</td>
<td>1252.500 ± 9.000</td>
<td>+ 13.45</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>1095.000 ± 11.940</td>
<td>1386.000 ± 14.550</td>
<td>+ 26.58</td>
<td>1262.500 ± 5.361</td>
<td>+ 15.30</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>1101.750 ± 10.301</td>
<td>1434.000 ± 11.880</td>
<td>+ 30.16</td>
<td>1338.750 ± 18.475</td>
<td>+ 21.51</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>1092.188 ± 5.741</td>
<td>1271.000 ± 4.897</td>
<td>+ 16.37</td>
<td>1127.500 ± 6.773</td>
<td>+ 3.23</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>1098.235 ± 1.925</td>
<td>1250.000 ± 4.798</td>
<td>+ 13.82</td>
<td>1128.546 ± 11.509</td>
<td>+ 2.76</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of five individual observations

+ Denotes per cent increase over control
Fig. 18. Bar-diagram showing changes in the plasma glutamate oxalacetate transaminase (GOT) activity in *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment-I and II.
DURATION OF TREATMENT (in weeks)

Plasma GOT activity (Units/ml)

Control  Treatment - I  Treatment - II

FIG. 18
Table 28. DMRT table for glutamate oxalacetate transaminase (GOT) activity in the plasma of *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment-I and II

<table>
<thead>
<tr>
<th>Duration of treatment (in weeks)</th>
<th>Control</th>
<th>Treatment-I</th>
<th>Treatment-II</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1104.000&lt;sup&gt;CA&lt;/sup&gt; ± 14.400</td>
<td>1328.500&lt;sup&gt;aC&lt;/sup&gt; ± 10.302</td>
<td>1252.500&lt;sup&gt;bB&lt;/sup&gt; ± 9.000</td>
<td>1228.333&lt;sup&gt;II&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>1095.000&lt;sup&gt;CA&lt;/sup&gt; ± 11.940</td>
<td>1386.000&lt;sup&gt;aB&lt;/sup&gt; ± 14.550</td>
<td>1262.500&lt;sup&gt;bB&lt;/sup&gt; ± 5.361</td>
<td>1247.833&lt;sup&gt;II&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>1101.750&lt;sup&gt;CA&lt;/sup&gt; ± 10.301</td>
<td>1434.000&lt;sup&gt;aA&lt;/sup&gt; ± 11.880</td>
<td>1338.750&lt;sup&gt;bA&lt;/sup&gt; ± 18.475</td>
<td>1291.500&lt;sup&gt;I&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>1092.188&lt;sup&gt;CA&lt;/sup&gt; ± 5.741</td>
<td>1271.000&lt;sup&gt;aD&lt;/sup&gt; ± 4.897</td>
<td>1127.500&lt;sup&gt;bC&lt;/sup&gt; ± 6.773</td>
<td>1163.563&lt;sup&gt;III&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>1098.235&lt;sup&gt;bA&lt;/sup&gt; ± 1.925</td>
<td>1250.000&lt;sup&gt;aD&lt;/sup&gt; ± 4.798</td>
<td>1128.546&lt;sup&gt;bC&lt;/sup&gt; ± 11.509</td>
<td>1158.927&lt;sup&gt;III&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>1098.234&lt;sup&gt;6&lt;/sup&gt;</td>
<td>1333.900&lt;sup&gt;0&lt;/sup&gt;</td>
<td>1221.9592&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Small letters are represented for comparison of row means (Different treatments for a particular period)
Capital letters are represented for comparison of column means (Different periods for a particular treatment)
Roman letters are represented for comparison of period means
Greek letters are represented for comparison of treatment means
Means of similar letters are on par
Degrees of freedom at p < 0.05
When the control group alone was tested for different exposure periods, the GOT activity was similar. On analysis, the GOT activity from treatment-I indicated that first, second and third week were significantly different among themselves and also from fourth and fifth week, and the latter two were similar. In treatment-II, first and second week, fourth and fifth week were similar, but varied significantly from the rest of their counterparts.

Analysis of period means irrespective of their treatments indicated the same trend like that of treatment-II. Analysis of treatment means irrespective of their periods revealed that all the three treatments, viz., control, treatment-I and II significantly differed from one another. Analysis of the data points out that the GOT activity increased upto third week and declined thereafter. GOT activity increased in both the treatments but the increase was less in treatment-II.

Table 29 and Fig. 19 give the results on the changes in plasma glutamate pyruvate transaminase activity in *Cyprinus carpio* var. *communis* treated with sublethal levels of cadmium nitrate from treatment-I and II. The plasma GPT activity of fish from treatment-I and II recorded marked increase over that of their control. The maximum increase in the enzyme activity (83.92 %) was exhibited by fish from treatment-I at the end of third week and the least increase (15.93 %) was noted at the end of fifth week of
Table 29. Changes in the plasma glutamate pyruvate transaminase (GPT) activity in *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment-I and II

<table>
<thead>
<tr>
<th>Duration of treatment (in weeks)</th>
<th>Glutamate pyruvate transaminase activity (Units/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment-I</td>
</tr>
<tr>
<td>I</td>
<td>752.400</td>
<td>949.000</td>
</tr>
<tr>
<td></td>
<td>± 8.112</td>
<td>± 10.253</td>
</tr>
<tr>
<td>II</td>
<td>747.750</td>
<td>1069.000</td>
</tr>
<tr>
<td></td>
<td>± 6.965</td>
<td>± 11.788</td>
</tr>
<tr>
<td>III</td>
<td>751.688</td>
<td>1382.500</td>
</tr>
<tr>
<td></td>
<td>± 6.009</td>
<td>± 54.140</td>
</tr>
<tr>
<td>IV</td>
<td>746.110</td>
<td>879.500</td>
</tr>
<tr>
<td></td>
<td>± 3.349</td>
<td>± 0.632</td>
</tr>
<tr>
<td>V</td>
<td>752.673</td>
<td>872.600</td>
</tr>
<tr>
<td></td>
<td>± 5.944</td>
<td>± 0.417</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of five individual observations

+ Denotes per cent increase over control
Fig. 19. Bar-diagram showing changes in the plasma glutamate pyruvate transaminase (GPT) activity in *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment-I and II.
FIG. 19

DURATION OF TREATMENT (in weeks)

Plasma GPT activity (units/ml)
treatment. In the case of treatment-II also, the GPT activity recorded a gradual increase from first week onwards, leading to a maximum elevation (51.44 %) at the end of third week. In subsequent treatments, the enzyme activity decreased suddenly and drastically.

Statistical analysis on the changes in the plasma GPT activity in *Cyprinus carpio* var. *communis* exposed to sublethal concentration of cadmium nitrate from treatments - I and II by DMRT gave the following results (Table 30). The GPT activity observed after first week was found to be significantly different from each other. A similar trend was noted in all the exposure periods except fifth week, where control and treatment-II were similar, indicating a protective role of calcium.

When control group alone was tested, the GPT activity during all the exposure periods was similar. In treatment-I, it was significantly different during all the exposure periods except fourth and fifth week, which was similar. A similar trend was noted in treatment-II also. Taking the GPT activity from different periods irrespective of the treatments, the following trends were observed. The GPT activity after first, second and third week was significantly different from that of the other periods. The GPT activity of fish after fourth and fifth week was found
Table 30. DMRT table for glutamate pyruvate transaminase (GPT) activity in the plasma of *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment -I and II.

<table>
<thead>
<tr>
<th>Duration of treatment (in weeks)</th>
<th>Control</th>
<th>Treatment-I</th>
<th>Treatment-II</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>752.400&lt;sup&gt;CA&lt;/sup&gt; ± 8.112</td>
<td>949.000&lt;sup&gt;AC&lt;/sup&gt; ± 10.253</td>
<td>877.500&lt;sup&gt;bC&lt;/sup&gt; ± 4.497</td>
<td>859.633&lt;sup&gt;III&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>747.750&lt;sup&gt;CA&lt;/sup&gt; ± 6.965</td>
<td>1069.000&lt;sup&gt;AB&lt;/sup&gt; ± 11.788</td>
<td>982.500&lt;sup&gt;bB&lt;/sup&gt; ± 7.268</td>
<td>933.083&lt;sup&gt;II&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>751.688&lt;sup&gt;CA&lt;/sup&gt; ± 6.009</td>
<td>1382.500&lt;sup&gt;AA&lt;/sup&gt; ± 54.140</td>
<td>1138.375&lt;sup&gt;bA&lt;/sup&gt; ± 13.508</td>
<td>1090.854&lt;sup&gt;I&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>746.110&lt;sup&gt;CA&lt;/sup&gt; ± 3.349</td>
<td>879.500&lt;sup&gt;AD&lt;/sup&gt; ± 0.632</td>
<td>800.250&lt;sup&gt;bD&lt;/sup&gt; ± 4.531</td>
<td>808.620&lt;sup&gt;IV&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>752.673&lt;sup&gt;bA&lt;/sup&gt; ± 5.944</td>
<td>872.600&lt;sup&gt;AD&lt;/sup&gt; ± 0.417</td>
<td>800.000&lt;sup&gt;bD&lt;/sup&gt; ± 2.450</td>
<td>808.424&lt;sup&gt;IV&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>750.124&lt;sup&gt;b&lt;/sup&gt; ± 12.140</td>
<td>1030.5200&lt;sup&gt;a&lt;/sup&gt; ± 2.450</td>
<td>919.7250&lt;sup&gt;a&lt;/sup&gt; ± 2.450</td>
<td>919.7250&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Small letters are represented for comparison of row means (Different treatments for a particular period)
Capital letters are represented for comparison of column means (Different periods for a particular treatment)
Roman letters are represented for comparison of period means
Greek letters are represented for comparison of treatment means
Means of similar letters are on par
Degrees of freedom at p < 0.05
to be similar, but they were significantly different for the other periods.

Considering the GPT activity in the plasma of fish from different treatments irrespective of periods, treatment-I and II differed significantly from control, which also differed among themselves. In general, GPT activity was steeply enhanced up to third week and thereafter showed a declining trend. Analysis of treatments showed higher GPT activity in treatment-I than treatment-II which may be due to the higher toxicity of cadmium nitrate in soft water.

Changes in the plasma lactate dehydrogenase activity in *Cyprinus carpio* var. *communis* treated with sublethal levels of cadmium nitrate from treatment-I and II are given in Table 31 and Fig. 20. It is interesting to note that the plasma LDH activity of fish declined to 49.62 per cent in treatment-I and 11.05 per cent in treatment-II at the end of first week. The data revealed that there was a steep increase in the enzyme activity of fish after second week, giving 104.92 per cent and 31.07 per cent from treatment-I and II, respectively. The plasma LDH activity steadily increased leading to a maximum increase of 190.32 per cent after fourth week in the case of treatment-I. On the other hand, the maximum increase in the LDH activity of fish was 89.85 per cent after third week from treatment-II.
Table 31. Changes in the plasma lactate dehydrogenase (LDH) activity in *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment-I and II

<table>
<thead>
<tr>
<th>Duration of treatment (in weeks)</th>
<th>Lactate dehydrogenase activity (IU/Litre)</th>
<th>Per cent change</th>
<th>Per cent change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment-I</td>
<td>Treatment-II</td>
</tr>
<tr>
<td>I</td>
<td>5032.000 ± 8.319</td>
<td>2535.000 ± 12.807</td>
<td>4476.000 ± 12.442</td>
</tr>
<tr>
<td>II</td>
<td>4983.000 ± 13.683</td>
<td>10211.000 ± 21.374</td>
<td>6531.000 ± 21.973</td>
</tr>
<tr>
<td>III</td>
<td>5006.000 ± 16.638</td>
<td>13480.000 ± 22.804</td>
<td>9504.000 ± 28.546</td>
</tr>
<tr>
<td>IV</td>
<td>5022.000 ± 9.859</td>
<td>14580.000 ± 22.804</td>
<td>8322.000 ± 28.546</td>
</tr>
<tr>
<td>V</td>
<td>4994.000 ± 17.344</td>
<td>12580.000 ± 26.834</td>
<td>8934.000 ± 30.673</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of five individual observations

+ Denotes per cent increase over control

- Denotes per cent decrease over control
Fig. 20. Bar-diagram showing changes in the plasma lactate dehydrogenase (LDH) activity in *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment-I and II.
FIG. 20

DURATION OF TREATMENT (in weeks)

- Control
- Treatment - I
- Treatment - II

Plasam LDH (IU/litre)

FIG. 20
Subsequent exposure in both the treatments indicated a gradual decrease in the plasma LDH activity up to fifth week.

The DMRT analysis on plasma LDH activity of *Cyprinus carpio* var. *communis* from treatment-I and II is given in Table 32. The plasma LDH levels of fish after first week was found to differ significantly from each other in control, treatment-I and II groups. Similar observations were also noted for second, third, fourth and fifth week exposures. In control groups, the LDH activity after first, second, third, fourth and fifth week was similar. But in treatment-I and II, the enzyme activity was significantly different during all the exposure periods viz., first to fifth week.

Considering the means for different periods irrespective of treatments, the enzyme activity was similar during third and fourth week, while it was significantly different from that of first, second and fifth week. When the LDH activity in the plasma of fish was analysed for different treatments irrespective of their periods, treatment-I and II were significantly different from control and also among themselves.
Table 32. DMRT table for lactate dehydrogenase (LDH) activity in the plasma of *Cyprinus carpio* var. *communis* treated with sublethal concentration of cadmium nitrate from treatment -I and II

<table>
<thead>
<tr>
<th>Duration of treatment (in weeks)</th>
<th>Control</th>
<th>Treatment-I</th>
<th>Treatment-II</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5032.000aA</td>
<td>2535.000cE</td>
<td>4476.000bB</td>
<td>4014.333 IV</td>
</tr>
<tr>
<td></td>
<td>± 8.319</td>
<td>± 12.807</td>
<td>± 12.442</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>4983.000cA</td>
<td>10211.000aD</td>
<td>6531.000bD</td>
<td>7241.667 III</td>
</tr>
<tr>
<td>III</td>
<td>5006.000cA</td>
<td>13480.000aB</td>
<td>9504.000bA</td>
<td>9330.000 I</td>
</tr>
<tr>
<td></td>
<td>± 16.638</td>
<td>± 22.804</td>
<td>± 28.546</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>5022.000cA</td>
<td>14580.000aA</td>
<td>8322.000bC</td>
<td>9308.000 I</td>
</tr>
<tr>
<td></td>
<td>± 9.859</td>
<td>± 22.804</td>
<td>± 5.359</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>4994.000cA</td>
<td>12580.000aC</td>
<td>8934.000bB</td>
<td>8836.000 II</td>
</tr>
<tr>
<td></td>
<td>± 17.344</td>
<td>± 26.834</td>
<td>± 30.673</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5007.4000e</td>
<td>10677.2000a</td>
<td>7553.4000g</td>
<td></td>
</tr>
</tbody>
</table>

Small letters are represented for comparison of row means (Different treatments for a particular period)
Capital letters are represented for comparison of column means (Different periods for a particular treatment)
Roman letters are represented for comparison of period means
Greek letters are represented for comparison of treatment means
Means of similar letters are on par
Degrees of freedom at p < 0.05
DISCUSSION

Christensen et al. (1982) reported that toxic chemical pollutants often affect the activity of enzymes at least to some degree and hence enzymes are used as logical candidates in biomonitoring. Elevation in the plasma GOT and GPT level in fish under toxic stress has been observed by many authors (Wieser and Hinterleitner, 1980; Asztalos et al., 1990; Rao et al., 1990; Renqing, 1990; Gill et al., 1991; Reddy and Venugopal, 1991; Neskovic et al., 1993). The studies of Harper et al. (1978) show that transamination and transdeamination reactions are prominent under stress condition. According to Subba Rao and Rajender (1990), GOT and GPT are two key enzymes known for their role in the utilization of proteins and carbohydrates.

Increase in the levels of serum enzymes can be attributed to the efflux of enzymes from damaged liver/organs into circulation (Kiran et al., 1991). Samuel and Sastry (1989) are of the opinion that the increase in GOT and GPT activity in heart, liver and kidney of Channa punctatus may be due to the damage of organs. According to Rao et al. (1990) and Gill et al. (1991), hepatocellular disorders cause plasma GOT and GPT levels to go up. Similar observation was made by Sasinovich and Voronina (1972), Boyd (1983), Kulkarni (1990) and Reddy et al. (1992) who reported that the increase of GOT activity
could be as a result of general tissue damage, particularly liver, muscle and heart.

Aminotransferases are key enzymes of nitrogen metabolism and are also important in energy mobilization (Calabrese et al., 1977). Wieser and Hinterleitner (1980) suggested that levels of sGOT and sGPT activity in Salmo gairdneri may serve as good indicators of certain types of organic water pollution. Transaminases are stress mediated enzymes and also serve as indicators of stress (Knox and Greengard, 1963; Prasada Rao and Ramana Rao, 1984). Elevated transaminases could be taken as a measure of compensatory mechanism as a consequence to impaired carbohydrate metabolism (Reddy and Venugopal, 1991).

According to Prasada Rao and Ramana Rao (1984), the GOT/GPT ratios give an assessment of the nature of the carbohydrate metabolism being favoured by the tissues. To conserve rapid depletion of the tissue glycogen content, the intermediary metabolic enzyme systems, namely the transaminases, are accelerated to promote gluconeogenesis, possibly to meet the increased energy demands under sustained and prolonged toxic stress (Rao et al., 1990). GPT activity levels also indicate the degree of adaptability (Chaplin et al., 1967).

The increase of GOT and GPT activity in the plasma of fish during sublethal treatment in the present study indicates preponderance of anaerobic nature of carbohydrate
metabolism in fish, possibly to meet the increased energy demands under sustained and prolonged toxic stress. Probably the damage of cells from gills, heart and liver and impaired protein and carbohydrate metabolism may be the reason for increased transaminases activity in the present study. The above point may find support from the work of Rao et al. (1990).

Toxicity of pesticides (Dragomirescu et al., 1979; Sastry and Siddiqui, 1984; Babu et al., 1988), metals (Singh et al., 1974; Sastry and Sharma, 1980; Reddy et al., 1989) and dye effluents (Ramesh and Manavalaramanujam, 1993) inhibited lactate dehydrogenase activity in fish.

The enzyme LDH is generally associated with cellular metabolic activity which is present in most animal tissues and is involved in the interconversion of pyruvic acid to lactic acid; it serves as a pivotal enzyme between the glycolytic pathway and tricarboxylic acid cycle and any damage in protein and carbohydrate metabolism might cause changes in LDH activity (Abston and Yarbrough, 1976). Gupta (1987) reported that inhibition or stimulation in enzyme activity was found to be dose and duration dependent. Inhibition of LDH activity in fish may be due to the change in the mitochondrial membrane function (Zalme et al., 1976) or due to impaired glycolysis (Sastry and Siddiqui, 1984). Rajeswari et al. (1990) reported that the decrease in NAD+ dependent LDH may lead to a metabolic shift from aerobiosis
to anaerobiosis during cadmium exposure. Probably in the present study, the inhibition of LDH activity after first week of treatment may be due to impaired carbohydrate metabolism which may find support from the above workers.

The magnitude of the increase in the LDH activity of blood sera shows the specific tissue damage and the degree of damage (Asztalos and Nemcsok, 1985). In the present study, the significant increase in LDH activity may be due to damage of organs by cadmium nitrate which may find support from the work of Nemcsok et al. (1985). The increase in LDH activity might be due to disruption of respiratory epithelium resulting in tissue hypoxia leading to shift in oxidative metabolism to anaerobic glycolysis. Similar studies are made in fish by Gill et al. (1990) and Suresh et al. (1993b). Dependence on glycolysis increased in fish, Labeo rohita, on exposure to copper as evidenced by increased LDH activity in liver and muscle; this type of metabolic reorganization is strategic (Radhakrishnaiah et al., 1992).

The above results suggest that cadmium nitrate, a non-essential heavy metal, which is washed into the aquatic ecosystem in small quantities is capable of inducing alterations in protein and carbohydrate metabolism as evident from the alterations in the enzyme activities. However, addition of calcium to the water tend to decrease the toxic effects of cadmium to some extent which is clear
from the lesser degree of increase in the enzyme activity in treatment-II of the present study. The data on the bioaccumulation of cadmium given in Chapter-II of this thesis clearly indicates that when external calcium was high, the metal uptake by fish was low. Probably, reduced bioaccumulation of cadmium by fish from treatment - II might be the reason for the reduced elevation in enzyme activity when compared to that of fish from treatment - I.