Chapter No. 4
ENZYME STUDIES
INTRODUCTION

Rapid population growth, unplanned towns and random growth of industry are common features of any developing country. Whereas slums, chimneys emitting poisonous gases and deafening noise fall to the lot of the city dweller, soil contamination and river and subsoil water pollution due to the use of fertilizers and pesticides are quite common in villages and small towns. Today, fortunately, all sections of society are aware of the problem of environmental pollution. There is a growing need to tackle the problem through a variety of measures such as legal, scientific, technical, economic and educational. Different legal and administrative methods have been introduced in almost all countries. The basic aim of legislation is to achieve air and water quality within acceptable levels.

Heavy metals are recognized as one of the most hazardous environmental pollutants and are toxic to many organisms. The contamination of aquatic environment by toxic metals is still an important problem. Freshwater biota especially gastropod molluscs which play an important role in aquatic food webs have proved to be very sensitive to heavy metals showing detrimental effects at low concentrations. These effects may be caused by heavy metal accumulation in the animal body. Therefore the study of different
metabolisms of the animal becomes an important pre-condition in monitoring heavy metal pollution. Also some gastropods play an important role as intermediate host of helminth parasites. Metal salts like copper sulphate are used as molluscicides. In the physiological activities of the organisms heavy metal pollution has great significance. Unless and until we understand the enzyme systems involved in vital processes and their vulnerability in different organisms it is difficult to prove toxic effects of pollutants.

Living cells are the seat of a number of chemical reactions. The chemical reactions of the organic compounds proceed at a very fast rate in a living cell even at lower temperature and at ordinary atmospheric pressure. This indicates that the reactions proceeding in a cell are influenced by catalytic action. Such catalysts which act inside the body or inside the body cells are termed as enzymes which, in other words, are biological catalysts. The enzymes increase the rate of chemical reactions taking place within living cells without themselves suffering any overall changes.

Enzymes, the catalysts of biological systems, are remarkable molecular devices that determine the pattern of chemical transformations. They also mediate the transformation of different forms of energy. The most striking characteristics of enzymes are their catalytic power and specificity. Furthermore, the actions of
many enzymes are regulated. Nearly all known enzymes are proteins. The discovery of catalytically active RNA molecules, however, indicates that proteins do not have an absolute monopoly on catalysis.

Proteins as a class of macromolecules are highly effective in catalyzing diverse chemical reactions because of their capacity to specifically bind a very wide range of molecules. By utilizing the full repertoire of intermolecular forces, enzymes bring substrates together in an optimal orientation, the prelude to making and breaking chemical bonds. In essence, they catalyze reactions by stabilizing transition states, the highest energy species in reaction pathways. By doing this selectively, an enzyme determines which one of several potential chemical reactions actually occurs. Enzymes can also act as molecular switches in regulating catalytic activity and transforming energy because of their capacity to couple the actions of separate binding sites.

Today enzymes still form a major subject for academic research. They are investigated in hospitals as an aid to diagnosis and because of their specificity of action are of great value as analytical reagents. Enzymes are still widely used in industry, continuing and extending many processes which have been used since the dawn of history.
Amylase and invertase are those enzymes which catalyse hydrolysis of compounds. Protease brings about hydrolysis of proteins and the related compounds. Lipases are a kind of esterases which act upon triglycerides and related compounds. Elements required in very small traces included Cu and Zn. Cu is an essential part of several enzymes such as cytochrome oxidase.

Digestion renders food absorbable through the alimentary canal by breaking the food material into simple molecular components through enzymatic action. Such enzymes are secreted by specialized cells of the digestive tract and hepatopancreas. Heavy metals are known for their strong attraction to biological tissues. Metal ions once absorbed into body are capable of reacting with a variety of binding sites and thus disturbing the normal physiology of an organism leading to toxicity.

Ghosh (1961) reported amylase, lipase and protease from the salivary gland and digestive diverticula in Lamellidens stagnalis. Bisswas and Ghosh (1968) elaborated amylolytic, proteolytic and lipolytic enzymes in the digestive gland of Viviparus bengalensis during first hour of digestion. Summer (1969) demonstrated the presence of these enzymes in lysosomes and food vacuoles of the digestive gland of Mytilus edulis and Helix aspersa.
Some workers have studied lipase activity in relation to aestivation in *Pila globosa* (Krishnamurthy and Brahmanandanam, 1970). The correlation between digestive enzymes and diet has been established but specific characterisation of different enzymes of different animals presents many interesting and puzzling questions (Prosser, 1973). Many workers have investigated the digestive enzymes of pelecypods. They were reported from digestive diverticula and digestive gland of several lamellibranchs.

Lomte (1973) reported, the most powerful carbohydrate digesting enzymes amylase, invertase, lactase and maltase in *Parreysia corrugata*.

Kreb's citric acid cycle is the final and common pathway for the oxidation of carbohydrates, proteins, lipids since glucose, amino acids and fatty acids are metabolised to acetyl CoA and then acetyl Co-A is oxidised to CO$_2$ and water through a series of metabolic steps. The oxidation of acetyl CoA reduces equivalents in the form of electrons. These electrons are released due to activity of a group of specific enzymes known as dehydrogenases. This dehydrogenase catalyses the formation of high energy phosphate bond through a process of oxidative phosphorylation (Campbell, 1973). The energy thus generated is most important for the organism. Any disruption or alteration in the activity of these
enzymes of the citric acid cycle, may therefore, disturb the entire physiological equilibrium resulting in complications of various nature.

Exposure to metals might in some way alter the enzyme and change its response in co-factors, temperature, pH and also its Michaelis' constant (Jackim, 1974). Amylase and lipase activities in the hemolymph of Biomphalaria glabrata, were studied by Rodrick et al. (1974).

Many workers have observed the effect of environmental pollutants on animals. Hinton and Koenig (1975) noted inhibition of different enzymes due to exposure to methyl mercuric chloride. Cheng et al. (1976) studied lipase activity in the hemolymph of Biomphalaria glabrata. Banerjee et al. (1978) reported elevation of different enzymes after exposure to metals in the air breathing fish, Clarias batrachus and in the non-breathing one Tilapia mossambica.

Kabeer Ahmed et al. (1978) studied the pesticidal impact on enzyme activity in foot, mantle and hepatopancreas of snail, Pila globosa. The activities of amylase and lipase in the whole hemolymph of Mya arenaria were determined by Rodrick and Gary (1979). Sastry et al. (1979) reported the effect of mercuric chloride on the activities of a few digestive enzymes of teleost fish, Channa punctatus.
Vasil Eva et al. (1980) noted Cu effect on organism, tissues and some enzymatic system of the bivalve, Glycymeris yessoensis. A change in lipase activity was noted by Swami et al. (1983) in Lamellidens marginalis when treated with flodit and metacid.


Mal and Venkateshwar (1987) studied the influence of Cu on some of the lipolytic enzymes of freshwater snail, Lymnaea luteola. The accumulation of Hg and Cd and their effects on few enzymes in Notopterus notopterus were observed in kidney and brain after 24, 48, 72 and 96 hours by Chand et al. (1988). The effect of heavy metals on amylase activity is also important as the ability of the mussel’s digestive gland to accumulate these metals is well known (Lakshmanan and Nambisan, 1989).

Teo et al. (1990) noted some properties of the sucrose from the digestive gland of the green mussel, Perna viridis. The
most potent inhibitor of sucrose was HgCl$_2$ followed by CuSO$_4$, CdCl$_2$, PbCl$_2$ and ZnCl$_2$.

A number of digestive enzymes in the green mussel, *Perna viridis* have been reported (Teo and Sabapathy, 1990) and L-amylase is believed to have a higher activity than others. The effect of acute Pb poisoning on the enzymes concerned with the membrane transport neurotransmission and energy metabolism in selected tissues of rosy barb, *Barbus conchonius* a freshwater fish have been studied by Gill *et. al.* (1991). Different digestive enzymes in the green mussel, *Perna viridis* were studied by Teo and Lim (1993). Ingle *et. al.* (1994) studied transaminases and phophatases after exposing the animals in *Lamellidens corrianus* to the same concentrations of iron and manganese found in the patalganga river. Significant variations were observed in the activities of transaminases and phosphatases in different tissues.

Kulkarni *et. al.* (1997) observed by the specific activities of acid and alkaline phosphatases, aspartate and alanine amino transferases and ATPase. They found alterations in various degrees in gills, hepatopancreas and adductor muscles of the intertidal clam *Gifrarium divaricatum* after exposure to 10 and 25 µg/l of copper for a month.
The review of literature shows investigative lacunae with regard to effects of heavy metals on digestive respiratory and oxidative processes of aquatic animals especially where freshwater molluscs are concerned. Hence an attempt has been made to study the effect of heavy metals on freshwater bivalve, {\textit{Parysia cylindrica}} with respect to changes in the level of digestive enzymes.
MATERIAL AND METHODS

The bivalves, *Parreysia cylindrica* were collected from the Girna dam area near Chalisgaon, and were acclimatized to laboratory conditions for 3 days prior to subjecting them to experiments. Two groups of active bivalves were formed, one of the two groups was considered as experimental and exposed to pollutants like NiCl₂, CdCl₂ and PbCl₂ for 24, 48, 72 and 96 hrs. for acute exposure and 7 days, 14 days and 21 days for chronic exposure. Another group not treated with pollutants was considered as control.

The concentrations used for acute exposure were 0.547 ppm NiCl₂, 2.184 ppm CdCl₂ and 12.0284 ppm PbCl₂ for 96 hours. For chronic exposure the concentrations were 0.106 ppm NiCl₂, 0.436 ppm CdCl₂ and 2.405 ppm PbCl₂ for 21 days.

For digestive enzymes such as amylase, invertase, protease and lipase the animals were dissected and the digestive diverticula were taken out cleaned and homogenised in ice cold distilled water. After brief centrifugation clear supernatant of the tissue was used for enzyme assay. In all the experiments, 1% homogenate (extract) of digestive gland prepared in glass distilled water was used. Half of this extract was boiled for half an hour to
destroy the enzyme activity and this boiled extract was used as control for all the experiments.

QUALITATIVE ASSAY OF DIGESTIVE ENZYMES IN DIGESTIVE GLAND:

Action of digestive enzymes on carbohydrates, proteins and fats was determined by carrying out experiments with the substrates such as starch, sucrose, peptone and olive oil.

TEST FOR AMYLASE

5 ml. of homogenised tissue were taken in microtubes. Boiled homogenate was taken in a separate tube. In each of the control and experimental tubes two drops of 1% boiled starch solution were added. A few drops of toluene were added to cover this reaction mixture which was allowed to incubate for 24 hours, at room temperature. After incubation, potassium iodide, iodine test was performed. The solution does not turn blue indicating the presence of amylase.

TEST FOR INVERTASE:

To 5 ml of extract, 5 ml of 1% sucrose were added. The mixture was incubated for 20 hours at 30°C. After incubation a drop of Fehlings' A and then a drop of Fehlings' B were added to the mixture A red precipitate appears indicating the presence of invertase.
TEST FOR PROTEASE:

To 5 ml of extract, 5 ml of 1% peptone were added. The mixture was incubated for 20 hours at 30°C. After incubation a drop of phenolphthalein was added which developed pink colour. Disappearance of pink colour after some time indicated the presence of protease.

TEST FOR LIPASE:

To 5 ml of extract, 1 ml of olive oil and small amount of sodium turocholate were added. The mixture was incubated for 20 hours at 30°C. After incubation 2 drops of phenol red were added to the mixture. The colour was observed at regular intervals. A change in colour from red to yellow indicated the presence of lipase.

ENZYME DETERMINATION METHODS

ESTIMATION OF AMYLASE AND INVERTASE:

The amylase and invertase activity was determined as described by Noelting and Bernfold (1948). The reaction mixture consists of 0.5 ml substrate (2%), 1.5 ml phosphatase buffer (pH 7.5) and 0.5 ml tissue homogenate (10% W/V). The reaction mixture was incubated for one hr. at 37°C. The enzyme activity was terminated by adding 2 ml of 3, 5-dinitrosalicylic acid reagent and
then heated in boiling water bath for 5 min. After cooling the optical, density was recorded at 530 µm. For amylase activity starch solution was used as substrate and for invertase activity sucrose solution was used as substrate. The activities of amylase and invertase were estimated by taking known amounts of maltose and glucose with the same procedure for calibration curve.

ESTIMATION OF PROTEASE:

The protease activity was determined by following Sorenson's formaldehyde titration method as modified by Prosser and Vanweel (1958). The reaction mixture contained 0.3 ml gelatin (3%) 1.0 ml Phosphatase buffer (pH 7.5) and 1.0 ml tissue homogenate (10% W/V). The reaction mixture was incubated for one hr. at 37°C. The enzyme activity was terminated by keeping it in boiling water bath for 5 min. Then equal amount of neutral formaldehyde was added and titrated against KOH (0.1 N) solution by using alcoholic phenolphthalein (0.5%) as an indicator. The difference between boiled and unboiled tissue homogenate gave the amount of protease activity. The amount of amino acids liberated in terms of ml of KOH (0.1 N) solution was taken as an index of enzyme activity.

ESTIMATION OF LIPASE:

The lipase activity was determined by the method of Sinha (1976) based on titrimetric estimation of liberated fatty acids from
substrate during enzyme action. The reaction mixture consists of 1.0 ml olive oil, 1.0 ml phosphate buffer (pH 8) and 1.0 ml tissue homogenate (10% W/V). The reaction mixture was incubated for one hr. at 37°C with frequent shaking. The enzyme activity was terminated by boiling reaction mixture in water bath. The lypolytic activity was determined by titrating the reaction mixture with NaOH (0.1 N) solution after adding 3.0 ml of alcohol (95%) using 0.5% alcoholic phenolphthalein as an indicator. The difference between the volume of NaOH (0.1N) solution utilised in unboiled and boiled homogenate containing reaction mixture indicated the lipase activity.
OBSERVATIONS AND RESULTS

The effect of different heavy metals (NiCl₂, CdCl₂ and PbCl₂) on the digestive enzymes after acute and chronic exposure of the freshwater bivalve, Parreysia cylindrica were studied. The experimental findings obtained are summarised in Tables 1 to 4.

AMYLASE:

The amylase activity in the control bivalves, P. cylindrica ranged from 6.805 to 6.3136 mg of maltose/gm protein/hr at 37⁰C. In the present investigation all the heavy metals showed decrease in amylase activity.

The acute exposure to NiCl₂ showed amylase activity of 4.319 mg of maltose/gm protein/hr at the end of 96 hours whereas CdCl₂ and PbCl₂ exhibited decreased amylase activity which was 4.599 and 4.931 mg of maltose/gm protein/hr respectively after 96 hours. Further it was evident from the table (1) that the decreased activity was time dependent. There was decrease in amylase activity at 24, 48, 72 and 96 hours exposure in NiCl₂, CdCl₂ and PbCl₂. The calculated values (experimental data) of F-ratio (24, 48, 72 and 96 hours) 2693.33, 4477.69, 11137.14 and 14202.85 in NiCl₂ respectively, (24, 48, 72 and 96 hr.) 2027.82, 7903.22, 7600 and 9793.33 in CdCl₂ respectively and 24, 48, 72 and 96 hours 1074.51,
3028.33, 2119.09 and 6372.00 in PbCl₂ respectively are greater than the table value which is 11.3 for the corresponding degrees of freedom at 1% level of significance. This indicates that the treatment showed significant differences. The maximum percentage decrease was upto 31.5856% (96 hours of NiCl₂ stress).

Changes in the amylase activity of the bivalve, *Parreysia cylindrica* after chronic exposure to heavy metals are summarised in Table -1. The amylase activity in the control bivalve was 5.742 mg of maltose/gm protein/hr at the end of 21 days. NiCl₂ showed a decrease in activity and it was 4.405 mg of maltose/gm protein/hr while CdCl₂ and PbCl₂ showed 4.708 and 5.051 mg of maltose/gm protein/hr respectively after 21 days exposure. Among the three heavy metals NiCl₂ was found to be more potent inhibitor of amylase than CdCl₂ and PbCl₂. The calculated values (experimental data) of F-ratio (7, 14, and 21 days) 1715.45, 4071.66 and 6384.28 in NiCl₂ respectively (7, 14, and 21 days) 1423, 3060 and 3820 in CdCl₂ respectively (7, 14 and 21 days) 707, 3413.33 and 1707.14 in PbCl₂ respectively are greater than the table value which is 11.3 for the corresponding degrees of freedom at 1% level of significance. This indicates that the treatment showed significant differences. The maximum percentage decrease was upto 23.2845% after 21 days exposure to NiCl₂.
INVERTASE:

The invertase activity in control animals after 24, 48, 72 and 96 hours of exposure was 5.436, 5.364, 5.313 and 5.241 mg of glucose/gm protein/hr respectively. In case of NiCl₂ the invertase showed a continuous decrease in activity at 24, 48, 72 and 96 hours of exposure. The values were 4.471, 4.161, 3.823 and 3.643 mg of glucose/gm/protein/hr respectively. In case of CdCl₂ invertase activity decreased which was 4.665, 4.456, 4.219 and 3.974 mg of glucose/gm protein/hr at 24, 48, 72 and 96 hours respectively. PbCl₂ showed a decrease in activity and it was 4.932, 4.744, 4.471 and 4.226 mg of glucose/gm protein/hr at 24, 48, 72 and 96 hours respectively. The calculated values (experimental data) of F-ratio (24, 48, 72 and 96 hr) 2115.45, 3011.66, 7932.85 and 5322.5 in NiCl₂ respectively (24, 48, 72 and 96 hr.) 1855, 1870, 4277.14 and 5735.7 in CdCl₂ respectively and (24, 48, 72 and 96 hr) 396.875, 685.0, 2534.28 and 4295 in PbCl₂ respectively are greater than the table value which is 11.3 for the corresponding degrees of freedom at 1% level of significance. This indicates that the treatment showed significant differences. The maximum percentage decrease was upto 30.49% after 96 hours exposure to NiCl₂.

Changes in the invertase activity of the bivalve, Parreysia cylindrica after chronic exposure to heavy metals are summarised
in Table-2. The control animals showed the following values at 7, 14 and 21 days of exposure. The values were 5.328, 5.306 and 5.270 mg of glucose/gm protein/hr. A constant depletion in invertase activity was observed after the stress of heavy metals. NiCl₂ stress decreased the activity more than CdCl₂ and PbCl₂. NiCl₂ showed a decrease in activity at 7, 14, and 21 days. The values were 4.356, 3.996 and 3.664 mg of glucose/gm protein/hr respectively. In case of CdCl₂ invertase activity decreased which was 4.536, 4.183 and 3.8952 mg of glucose/gm protein/hr at 7, 14, and 21 days respectively. PbCl₂ showed a decrease in activity and it was 4.752, 4.356 and 4.010 mg of glucose/gm protein/hr at 7, 14, and 21 days respectively. The calculated values (experimental data) of F-ratio (7, 14 and 21 days) 2371, 5366.25 and 10741.66 in NiCl₂ respectively (7, 14 and 21 days) 1568, 5256.66 and 7880 in CdCl₂ respectively and (7, 14 and 21 days) 1381.66, 4518.00 and 5668.57 in PbCl₂ respectively are greater than the table value which is 11.3 for the corresponding degree of freedom at 1% level of significance. This indicates that the treatment showed significant differences. The maximum percentage decrease was up to 30.4743% after 21 days exposure to NiCl₂.
PROTEASE:

The protease activity in the control bivalves, *P. cylindrica* ranged from 5.16 to 5.04 mg of amino acids/gm protein/hr at 37°C; NiCl₂ was found to be more potent in inhibiting protease activity. NiCl₂ showed protease activity as 3.20, 3.14, 3.06 and 2.94 mg of amino acids/gm protein/hr respectively after 96 hours. CdCl₂ showed a decrease in activity at 24, 48, 72 and 96 hours of exposure. The values were 3.86, 3.78, 3.72 and 3.64 mg of amino acids/gm protein/hr respectively. In case of PbCl₂ the protease activity decreased which was 4.26, 4.20, 4.10 and 3.92 mg of amino acids/gm protein/hr at 24, 48, 72 and 96 hours respectively. The calculated values (experimental data) of F-ratio (24, 48, 72 and 96 hrs) 2401.00, 3333.33, 4080.04 and 3675.00 in NiCl₂ respectively (24, 48, 72 and 96 hrs.) 1408.33, 0924.08, 1027.55 and 0890.90 in CdCl₂ respectively (24, 48, 72 and 96 hrs.) 675.00, 552.25, 686.00 and 1254.04 in PbCl₂ respectively are greater than the table value which is 11.3 for the corresponding degrees of freedom at 1% level of significance. This indicates that the treatment showed significant differences. The maximum percentage decrease was up to 41.6666% (96 hours of NiCl₂ stress).

Changes in protease activity after chronic exposure are summarised in Table-3. The control animals showed protease activity ranging from 5.08 to 4.90 mg of amino acids/gm protein/
hr. In the present investigation NiCl₂ stress showed more decrease in protease activity than CdCl₂ and PbCl₂ after chronic exposure. The values were 3.08, 2.98 and 2.86 mg of amino acids/gm protein/hr for NiCl₂ respectively at the end of 21 days. In case of CdCl₂ protease activity decreased which was 3.80, 3.72 and 3.60 mg of amino acids/gm protein/hr at 7, 14 and 21 days respectively. PbCl₂ showed a decrease in activity and it was 4.14, 4.06 and 3.92 mg of amino acids/gm protein/hr at 7, 14 and 21 days respectively. The calculated values (experimental data) of F-ratio (7, 14, and 21 days) 1428.57, 2121.8 and 2601.00 in NiCl₂ respectively (7, 14, and 21 days) 1170.28, 871.2 and 563.33 in CdCl₂ respectively (7, 14 and 21 days) 441.8, 46.545 and 400.16 in PbCl₂ respectively are greater than the table value which is 11.3 for the corresponding degrees of freedom at 1% level of significance. This indicates that the treatment showed significant differences. The maximum percentage decrease was up to 41.6326% after 21 days exposure to NiCl₂.

LIPASE:

Control animals exhibited lipase activity ranging from 4.10 to 3.90 units/gm protein/hr NiCl₂ which showed more inhibition in lipase activity as compared to CdCl₂ and PbCl₂. The values were 2.78, 3.36 and 3.50 units/gm protein/hr for NiCl₂, CdCl₂ and PbCl₂ at the end of 96 hours. The calculated values of F-ratio (24, 48, 72
and 96 hours) 112.1538, 450.0, 416.0 and 522.66 in \( \text{NiCl}_2 \) respectively (24, 48, 72 and 96 hrs.) 72.25, 72.00, 135.2 and 112.15 in \( \text{CdCl}_2 \) respectively (24, 48, 72 and 96 hrs.) 36.0, 50.0, 64.8 and 53.33 in \( \text{PbCl}_2 \) respectively are greater than the table value which is 11.3 for the corresponding degrees of freedom at 1% level of significance. This indicates that the treatment showed significant differences. The maximum percentage decrease was upto 28.7179% (96 hours of \( \text{NiCl}_2 \) stress).

Changes in the lipase activity after chronic exposure are summarised in Table-4. The control animals showed lipase activity which decreased from 3.84, to 3.74 units/gm protein/hr. Among the three metals tested \( \text{NiCl}_2 \) was found to be more potent in inhibiting lipase activity than \( \text{CdCl}_2 \) and \( \text{PbCl}_2 \). The lipase activity for \( \text{NiCl}_2 \), \( \text{CdCl}_2 \) and \( \text{PbCl}_2 \) was 2.72, 3.04 and 3.26 units/gm, protein/hr at the end of 21 days. All the values of F-ratio were statistically significant at \( P < 1\% \) level of significance. The maximum percentage decrease was upto 27.2727% after 21 days exposure to \( \text{NiCl}_2 \).
Table 1

Changes in the *Amylase* activity of the bivalve, *Parreysia cylindrica* after acute and chronic exposure to the heavy metals NiCl₂, CdCl₂ and PbCl₂.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Exposure period</th>
<th>Name of Pollutant.</th>
<th>Control</th>
<th>NiCl₂</th>
<th>CdCl₂</th>
<th>PbCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 24 hrs.</td>
<td>6.805 ± 0.0524</td>
<td>5.302 ± 0.0228</td>
<td>5.439 ± 0.0289</td>
<td>5.651 ± 0.0457</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>P &lt; 1% S + 22.0867</td>
<td>P &lt; 1% S + 20.0734</td>
<td>P &lt; 1% S + 16.9581</td>
<td></td>
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</tr>
<tr>
<td>2. 48 hrs.</td>
<td>6.708 ± 0.0213</td>
<td>5.182 ± 0.0388</td>
<td>5.308 ± 0.0213</td>
<td>5.502 ± 0.0384</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &lt; 1% S + 22.7489</td>
<td>P &lt; 1% S + 20.8706</td>
<td>P &lt; 1% S + 17.9785</td>
<td></td>
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<tr>
<td>3. 72 hrs.</td>
<td>6.617 ± 0.0227</td>
<td>4.851 ± 0.0213</td>
<td>5.108 ± 0.0213</td>
<td>5.251 ± 0.0520</td>
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<tr>
<td></td>
<td>P &lt; 1% S + 26.6888</td>
<td>P &lt; 1% S + 22.8048</td>
<td>P &lt; 1% S + 20.6437</td>
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<tr>
<td>4. 96 hrs.</td>
<td>6.3136 ± 0.0254</td>
<td>4.319 ± 0.0212</td>
<td>4.599 ± 0.0254</td>
<td>4.931 ± 0.0227</td>
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</tr>
<tr>
<td></td>
<td>P &lt; 1% S + 31.5856</td>
<td>P &lt; 1% S + 27.1503</td>
<td>P &lt; 1% S + 21.8913</td>
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<tr>
<td>5. 7 days</td>
<td>6.039 ± 0.0340</td>
<td>5.171 ± 0.0259</td>
<td>5.285 ± 0.0180</td>
<td>5.508 ± 0.0213</td>
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<tr>
<td></td>
<td>P &lt; 1% S + 14.3732</td>
<td>P &lt; 1% S + 12.4855</td>
<td>P &lt; 1% S + 08.7928</td>
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<tr>
<td>6. 14 days</td>
<td>5.879 ± 0.0212</td>
<td>4.891 ± 0.0213</td>
<td>5.022 ± 0.0212</td>
<td>5.239 ± 0.0142</td>
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<tr>
<td></td>
<td>P &lt; 1% S + 16.8055</td>
<td>P &lt; 1% S + 14.5773</td>
<td>P &lt; 1% S + 10.8862</td>
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<tr>
<td>7. 21 days</td>
<td>5.742 ± 0.0254</td>
<td>4.405 ± 0.0212</td>
<td>4.708 ± 0.0213</td>
<td>5.051 ± 0.0213</td>
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<tr>
<td></td>
<td>P &lt; 1% S + 23.2845</td>
<td>P &lt; 1% S + 18.0076</td>
<td>P &lt; 1% S + 12.0341</td>
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</table>

Enzyme activity is expressed as mg of maltose/mg of protein / hr. at 37°C temperature.
Each value is the mean of 5 observations ± S.D.
Values are significant at P < 1%
+ indicate % variation over control.
Anova table was used for calculation.
Changes in the *Invertase* activity of the bivalve, *Parreysia cylindrica* after acute and chronic exposure to the heavy metals NiCl₂, CdCl₂ and PbCl₂.

<table>
<thead>
<tr>
<th>Sr. No.</th>
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<th>Control</th>
<th>NiCl₂</th>
<th>CdCl₂</th>
<th>PbCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>± 0.0269</td>
<td>± 0.0176</td>
<td>± 0.0394</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NiCl₂</td>
<td>± 0.0269</td>
<td>± 0.0176</td>
<td>± 0.0394</td>
<td>± 0.0352</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdCl₂</td>
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<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PbCl₂</td>
<td>+17.7520</td>
<td>+14.1832</td>
<td>+09.2715</td>
<td>+11.5585</td>
</tr>
<tr>
<td>2.</td>
<td>48 hrs.</td>
<td>Control</td>
<td>5.364</td>
<td>4.1616</td>
<td>4.456</td>
<td>4.744</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NiCl₂</td>
<td>± 0.0288</td>
<td>± 0.0269</td>
<td>± 0.0352</td>
<td>± 0.0269</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdCl₂</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PbCl₂</td>
<td>+22.4161</td>
<td>+16.9276</td>
<td>+11.5585</td>
<td>+15.8479</td>
</tr>
<tr>
<td>3.</td>
<td>72 hrs.</td>
<td>Control</td>
<td>5.313</td>
<td>3.823</td>
<td>4.219</td>
<td>4.471</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NiCl₂</td>
<td>± 0.0269</td>
<td>± 0.0269</td>
<td>± 0.0269</td>
<td>± 0.0269</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdCl₂</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PbCl₂</td>
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<td>+20.5910</td>
<td>+15.8479</td>
<td>+19.3665</td>
</tr>
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<td></td>
<td>NiCl₂</td>
<td>± 0.0352</td>
<td>± 0.0176</td>
<td>± 0.0176</td>
<td>± 0.0176</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td>+24.1747</td>
<td>+19.3665</td>
<td>+23.9089</td>
</tr>
<tr>
<td>5.</td>
<td>7 days</td>
<td>Control</td>
<td>5.328</td>
<td>4.356</td>
<td>4.536</td>
<td>4.752</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NiCl₂</td>
<td>± 0.0321</td>
<td>± 0.0321</td>
<td>± 0.0227</td>
<td>± 0.0227</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdCl₂</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PbCl₂</td>
<td>+18.2432</td>
<td>+14.8648</td>
<td>+10.8108</td>
<td>+17.9042</td>
</tr>
<tr>
<td>6.</td>
<td>14 days</td>
<td>Control</td>
<td>5.306</td>
<td>3.996</td>
<td>4.183</td>
<td>4.356</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NiCl₂</td>
<td>± 0.0321</td>
<td>± 0.0269</td>
<td>± 0.0227</td>
<td>± 0.0227</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdCl₂</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PbCl₂</td>
<td>+24.6890</td>
<td>+21.1647</td>
<td>+17.9042</td>
<td>+23.9089</td>
</tr>
<tr>
<td>7.</td>
<td>21 days</td>
<td>Control</td>
<td>5.270</td>
<td>3.664</td>
<td>3.8952</td>
<td>4.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NiCl₂</td>
<td>± 0.0269</td>
<td>± 0.0269</td>
<td>± 0.0288</td>
<td>± 0.0288</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CdCl₂</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PbCl₂</td>
<td>+30.4743</td>
<td>+26.0910</td>
<td>+23.9089</td>
<td>+23.9089</td>
</tr>
</tbody>
</table>

Enzyme activity is expressed as mg of glucose/mg of protein/hr. at 37°C temperature. Each value is the mean of 5 observations ± S.D. Values are significant at P < 1% + indicate % variation over control. Anova table was used for calculation.
Table: 3

Changes in the *Protease* activity of the bivalve, *Parreysia cylindrica* after acute and chronic exposure to the heavy metals NiCl₂, CdCl₂ and PbCl₂.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Exposure period</th>
<th>Control</th>
<th>NiCl₂</th>
<th>CdCl₂</th>
<th>PbCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.16 ± 0.0489</td>
<td>3.2 ± 0.0632</td>
<td>3.86 ± 0.0489</td>
<td>4.26 ± 0.0489</td>
</tr>
<tr>
<td>1.</td>
<td>24 hrs.</td>
<td>P &lt; 1% S + 37.9844</td>
<td>P &lt; 1% S + 25.1937</td>
<td>P &lt; 1% S + 17.4418</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>48 hrs.</td>
<td>5.14 ± 0.0489</td>
<td>3.14 ± 0.0489</td>
<td>3.78 ± 0.0748</td>
<td>4.20 ± 0.0632</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 1% S + 38.9105</td>
<td>P &lt; 1% S + 26.4591</td>
<td>P &lt; 1% S + 18.2879</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>72 hrs.</td>
<td>5.08 ± 0.04</td>
<td>3.06 ± 0.0489</td>
<td>3.72 ± 0.0748</td>
<td>4.1 ± 0.0632</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 1% S + 39.7637</td>
<td>P &lt; 1% S + 26.7716</td>
<td>P &lt; 1% S + 19.2913</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>96 hrs.</td>
<td>5.04 ± 0.0489</td>
<td>2.94 ± 0.0489</td>
<td>3.64 ± 0.08</td>
<td>3.92 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 1% S + 41.6666</td>
<td>P &lt; 1% S + 27.7777</td>
<td>P &lt; 1% S + 22.2222</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>7 days</td>
<td>5.08 ± 0.04</td>
<td>3.08 ± 0.0979</td>
<td>3.80 ± 0.0632</td>
<td>4.14 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 1% S + 39.3700</td>
<td>P &lt; 1% S + 25.1968</td>
<td>P &lt; 1% S + 25.1968</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>14 days</td>
<td>5.04 ± 0.0489</td>
<td>2.98 ± 0.0748</td>
<td>3.72 ± 0.0748</td>
<td>4.06 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 1% S + 40.8730</td>
<td>P &lt; 1% S + 26.1904</td>
<td>P &lt; 1% S + 19.4444</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>21 days</td>
<td>4.90 ± 0.0632</td>
<td>2.86 ± 0.0489</td>
<td>3.60 ± 0.0894</td>
<td>3.92 ± 0.0748</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 1% S + 41.6326</td>
<td>P &lt; 1% S + 26.5306</td>
<td>P &lt; 1% S + 20.0000</td>
<td></td>
</tr>
</tbody>
</table>

Enzyme activity is expressed as mg of amino acid/mg of protein / hr. at 37°C temperature. Each value is the mean of 5 observations ± S.D. Values are significant at P < 1% * + indicate % variation over control. Anova table was used for calculation.
Table 4

Changes in the Lipase activity of the bivalve, Parreysia cylindrica after acute and chronic exposure to the heavy metals NiCl₂, CdCl₂ and PbCl₂.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Exposure period</th>
<th>Name of Pollutant.</th>
<th>Control</th>
<th>NiCl₂</th>
<th>CdCl₂</th>
<th>PbCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>± 0.0632</td>
<td>± 0.0748</td>
<td>± 0.0632</td>
</tr>
<tr>
<td>1</td>
<td>24 hrs.</td>
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<td>4.1</td>
<td>± 0.08</td>
<td>± 0.0498</td>
<td>± 0.0489</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>+13.1707</td>
<td>+8.2926</td>
<td>+5.8536</td>
</tr>
<tr>
<td>2</td>
<td>48 hrs.</td>
<td></td>
<td>4.08</td>
<td>± 0.04</td>
<td>± 0.0748</td>
<td>± 0.0748</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
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<td>+14.7058</td>
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<td>+07.3529</td>
</tr>
<tr>
<td>3</td>
<td>72 hrs.</td>
<td></td>
<td>4.04</td>
<td>± 0.0489</td>
<td>± 0.0748</td>
<td>± 0.0748</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
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<td>+25.7425</td>
<td>+12.8712</td>
<td>+08.9108</td>
</tr>
<tr>
<td>4</td>
<td>96 hrs.</td>
<td></td>
<td>3.9</td>
<td>± 0.0632</td>
<td>± 0.08</td>
<td>± 0.0894</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
<td>P &lt; 1% S</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>+28.7179</td>
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<td>+10.2564</td>
</tr>
<tr>
<td>5</td>
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<td>3.84</td>
<td>± 0.08</td>
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<td>± 0.0632</td>
</tr>
<tr>
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<td>P &lt; 1% S</td>
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<tr>
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</tr>
<tr>
<td>6</td>
<td>14 days</td>
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<td>3.80</td>
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<td>± 0.0489</td>
</tr>
<tr>
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</tr>
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<tr>
<td>7</td>
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<td>3.74</td>
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<td>± 0.0979</td>
<td>± 0.0489</td>
</tr>
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</tr>
<tr>
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<td></td>
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<td>+12.8342</td>
</tr>
</tbody>
</table>

Enzyme activity is expressed as units of lipase activity /mg of protein / hr. at 37°C temperature.
Each value is the mean of 5 observations ± S.D.
Values are significant at P < 1%
+ indicate % variation over control.
Anova table was used for calculation.
DISCUSSION

Digestion is a sequential process which renders food absorbable through the gut wall by breaking the food into simple molecular components through enzymatic action. Such enzymes are secreted by specialised cells of digestive tract. Digestion although dependent on enzymatic actions is determined by the functional organization and the structure of digestive system.

Important activities like energy synthesis, metabolism etc. are carried out by enzymes the active biocatalysts. The living cells are the centre of majority of enzyme catalisation reactions. When an energy imbalance occurs in the cell due to exposure to infective agents or toxic substances, enzymes leak through the membranes into circulating fluids. This causes their fluid level to be augmented above the normal level. It has been also agreed that some of the cytoplasmic enzymes are leaked out of the tissues due to damage to cells. They are often released in fluids resulting in the decreased enzyme activities in tissues and corresponding increase in the fluids.

A number of digestive enzymes were found to occur in the digestive gland particularly amylase, maltase, lactase, esterase, lipase, protease (Arvy, 1969). Toxicological evaluation of some
enzymes, modified starches and certain other substances was reported (WHO, 1972). The invertase activity was studied by Verma and Prasad (1972) in *Mylolobis dustulata*.

In the present investigation the amylase activity was significantly altered in the bivalve, *P. cylindrica* after the acute and chronic treatment of three heavy metal salts NiCl$_2$, CdCl$_2$ and PbCl$_2$. **Table-1**. Among the tested heavy metals, NiCl$_2$ was found to be the potent inhibitor of amylase activity as compared to CdCl$_2$ and PbCl$_2$. From the table it is also evident that the decrease in activity was time dependent. Lomte (1973) reported, the most powerful carbohydrate digesting enzymes amylase, invertase, lactase and maltase in *Parreysia corrugata*.

Caley and Jaensen (1973) noticed an increase in lipid concentration in tissues by the organophosphate pesticidal impact and suggested that this might be due to the inhibition of the lipase activity. Boucaud-Comou and Eve (1974) studied the activities of protease and amylase in *S. officinalis*. Cheng *et al.* (1975) studied the amylase and lipase activity in the hemolymph of *Crassostrea virginica* and *Mercenaria mercenaria*. Amylase only occurred in the whole hemolymph and serum of *C. virginica*. Since this mollusc possesses a crystalline style, the amylase is believed to have originated from this structure.
There is no doubt that the effect of certain metals is profound on the enzyme activity in aquatic organisms though the mode of action of these heavy metals has not been clearly outlined with regard to their mechanism of action on certain key enzymes which are responsible for the general energetics of an animal. There is a considerable amount of literature devoted to the study of organic pesticides concerning the enzyme system of various animals (Yap et al., 1975; Koundinya and Ramamurthi, 1978).

Sastry and Sharma (1978) have obtained elevation of acid phosphatase activity due to the increased phosphorylation or disintegration of the cells which normally elevate the lysosomal activity. Studies of amylase from crystalline style of short necked clam, *Ruditapes philippinarum* were noted by Hora et al. (1979). The enzyme was activated with chlorides of metals such as Na, Sr, Ca and Hg the highest activity being observed in the presence of 0.025M Sr Cl₂. Lipase was believed to be of cellular origin with hemolymph cells in *Mya arenaria* as reported by Rodrick and Gary (1979). Rosoiu et al. (1981) studied the partially purified L-amylase from the hepatopancreas and whole body of *M. arenaria*.

Starvation stress decreased the protease activity which was reported by Mandal and Roy (1981) in the insect *Schizodactylus monstrosus*. A depletion in the lipase activity was observed in the
bivalve, *Lamellidens marginalis*. When treated with Flodit and metacid (Swami *et al.* 1983). Amylase activity was observed in millipede, *Trigoniulus lumbricus* (Shukla, 1984) when exposed to DDT. It was found to be dose dependant. A change in amylase activity was dependent upon the type of pollutant and its exposure period.

In the present investigation, invertase activity was significantly inhibited in *Parreysia cylindrica*. However, among the tested heavy metals NiCl$_2$ was found to be the potent inhibitor of invertase activity as compared to CdCl$_2$ and PbCl$_2$. A significant decrease in invertase activity was noted by Alam (1984) in the *Viviparus bengalensis*. Similar observations have been reported by Lomte and Patil (1985) in the army worm *P. separata* when exposed to pollutants. Pesticidal impact caused depletion in the invertase activity in the armyworm, *M. (P.) separata* (Patil, 1986).

Mathews and Murleedharan (1986) also reported a significant drop in protease activity after 24, 48, and 72 hours exposure to prococence-II to the caster and semilooper *Achaoea janata*. Decreased digestive enzyme secretion due to sublethal doses of methyl parathion affects food utilization in *Spodoptera litura* (Vasanta and Chockalingam, 1986).
Nalina Sundari et al. (1986) reported depletion in the amylase, invertase and protease activity in Spodoptera litura when exposed to vinca rosea extract. Lomte and Patil (1989) observed a decrease in amylase activity in the army worm Mythimna (P.) separata, after pesticide treatment. Selvarani et al. (1989) studied the digestive enzymes of marine bivalves, Donax cuneatus and Perna viridis. Crystalline style of both bivalves showed strong amylase activity compared to that of the digestive diverticula indicating that the style is the main source of extracellular carbohydrate digestion in these bivalves.

Reddy et al. (1989) reported the influence of cadmium on certain aspects of carbohydrate metabolism in the tissues of the freshwater field crab, Barytelphusa guerini exposed to sublethal concentration of cadmium chloride.

A kinetic study of the L-amylase from the digestive gland of P. viridis was done by Sabapathy Uma and Teo (1990). They found that HgCl₂ was the most potent inhibitor of amylase activity, followed by CuSO₄, NiCl₂ and CdCl₂. Toshihiko Ogawa et al. (1990) showed similar inhibition pattern of enzyme in bacteria. All these results show a good correlation with the results obtained in Parreysia cylindrica after exposure to heavy metals.
In the present investigation the decrease in the activity of protease was observed when *Parreysia cylindrica* was exposed to heavy metals. Teo and Sabapathy (1990) noted a decrease in protease activity in *Perna viridis*. Similar findings were obtained by Sontakke (1992) in *Thiara tuberculata*. Bhamre (1993) in *Parreysia favidens*, Jadhav (1993) in *Corbicula striatella* and Patil (1993) in *Lamellidens marginalis*.

The lipase activity was inhibited due to treatment of heavy metals on *Parreysia cylindrica*, similar observations have been reported by Bhamre (1993) in *Corbicula striatella* and Patil (1993) in *Lamellidens marginalis*. Lipase activity in scallop hepatopancreas was reported by Itabashi *et al.* (1994).

Yan *et al.* (1996) observed the effects of metals on L-amylase activity in the digestive gland of the green mussel, *Perna viridis* L. Masarrat Sultana (1997), reported the effect of different heavy metals (Cu, Hg and Zn) on the enzymatic activity of the bivalve, *Lamellidens marginalis*. The activity of digestive enzymes such as amylase, invertase, protease and lipase after acute treatment showed continuous depletion in all the tested heavy metals. Similar findings were also obtained by Patil (1998) in *Lamellidens corrianus*. 
In the present investigation a depletion in the amylase, invertase, protease and lipase activity was observed in *Parreysia cylindrica*. The depletion in the enzymatic activity may be due to the damage caused by the heavy metals to the cells of the alimentary canal. The decrease in enzymatic activity of *Parreysia cylindrica*, was affected most by NiCl$_2$ and was followed by CdCl$_2$ and PbCl$_2$. 
SUMMARY

1. The effect of different heavy metals (NiCl₂, CdCl₂ and PbCl₂) on the enzymatic activity of the bivalve, *Parreysia cylindrica* was observed.

2. Digestive enzymes like amylase, invertase, protease and lipase were chosen for studying the impact of heavy metals. The effect of heavy metals varies with the type of metal used and the period of exposure.

3. The activity of amylase, invertase, protease and lipase showed continuous depletion in all the tested heavy metals.

4. The decrease in the enzymatic activity may be due to the damage caused by the heavy metals to the cells of the alimentary canal.
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*Aurangabad* 265-281.


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*Fisheries Science* (Tokyo) 60(3): 347.


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PATIL P.N. (1986): Impact of different pesticides on some physiological and Neuroendocrinological aspects of *Mythimna (Pseudoletia) separata*.
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