CHAPTER-IV

DISCUSSION
DISCUSSION

BIOCHEMICAL STUDY:

The present study deals with the change in biochemical contents (Soluble protein, glycogen and cholesterol) in liver, kidney and gills of teleost fish Heteropneustes fossilis, under the influence of sublethal concentration of lead nitrate, lead nitrate + zeolite and zeolite only to study the lead toxicity and to observe whether zeolite can be used to protect lead toxicity and improve their condition. For biochemical study, soluble protein (Lowry method), glycogen (Anthron method) and cholesterol (Libermann-Burchard method) contents in liver, kidney and gills have been estimated Plummer (1979). The observations were recorded, calculated for mean value, standard error and probability and summarized in tables (1 to 18).

I. ACUTE STUDY:

For short term study, 120 fish were divided into four equal groups, Group 1 worked as control, group 2, 3 and 4 were exposed to sublethal concentration of Pb(NO₃)₂ (60 mgL⁻¹day⁻¹); Pb(NO₃)₂+Zeolite (each 60 mgL⁻¹day⁻¹) and only zeolite (60 mgL⁻¹day⁻¹) respectively. After 7, 14, 21, 28 and 35 days of exposure, fishes were sacrificed, their liver, kidney and gills were removed and processed for the estimation of soluble protein, glycogen and cholesterol contents. All experiments were repeated 3 times and average values were summarized in tables 1 to 9.
(a) Soluble protein contents in liver, kidney and gills:

In control fishes, soluble protein contents in liver, kidney and gills were almost similar to that of initial control fish. A slight increase in the soluble protein contents with increasing age has been observed. As evident from table No. 1, 2 and 3, sublethal concentration of Pb(NO₃)₂ (60 mgL⁻¹day⁻¹) causes significant decrease in the soluble protein contents of all three experimental tissues. In case of liver, kidney and gills, the maximum decrease observed after 7 days of exposure i.e. 29.3, 28.9 and 28.9 percent respectively, while the minimum decrease was observed after 35 days of exposure in liver, kidney and gills i.e. 21.2, 20.3 and 20.9 percent respectively. The reason behind minimum decrease after 35 days of exposure is probably due to the resistance developed in the fish against lead toxicity. The overall decrease in the protein contents is probably due to enzyme inhibition, responsible for protein synthesis Sastri and Gupta (1978). Liver is the most important target organ for lead and cadmium toxicity Holcombe et al. (1976) and Dallinger et al. (1978). According to Holcombe et al. (1976), Patterson and Settle (1977), Hodson (1978a), Varanasi and Gmur (1978), Bollinberg and Johansen (1979) and Hodson et al. (1982), accumulation of lead is maximum in liver, kidney, gills and bones. The toxic effects of lead poisoning in fishes have been reviewed from time to time with reference to haematological and biological variables Jackim (1973), Johansson-Sjoebeck and Larsson (1979) and Haux and Larsson (1982). Lead is unique in its physiological toxicity and a series
of responses due to lead intoxication have been well characterised by Davies et al. (1976) and Hodson et al. (1984). Exposure of fish to high concentration of aqueous lead can elicit several biochemical responses Spry et al. (1981) and Hodson et al. (1984). According to Spry Wiener (1991), lead inhibit a part of haemoglobin synthetic pathway. Mass Oliva (1989) and Rajanna et al. (1990) reported that metal binds strongly with sulphahydryl groups to form mercaptides, inhibiting large number of enzymes containing functional thiol groups as also observed with other heavy metals such as cadmium Paier et al. (1993), Mercury Visser et al. (1976) and gold Mandel (1995). Mercury cause depletion in soluble protein, glycogen, RNA content and hepatic enzymes Shakorri et al. (1994). Mercuric chloride treatment significantly decreases total blood serum protein and increase free amino acids (FAA), indicate break down of total blood serum proteins which ultimately leaves to increase in the FAA pool Farah Aziz and Shakoori (1992).

When fishes were exposed to \((\text{PbNO}_3)_2\) \((60 \text{ mgL}^{-1} \text{ day}^{-1})\) along with zeolite \((60 \text{ mgL}^{-1} \text{ day}^{-1})\), the values of protein contents in liver, kidney and gills improved significantly. According to table 1, 2 and 3, the maximum improvement in soluble protein contents in liver, kidney and gills has been observed to be 6.82, 6.95 and 7.1 percent after the exposure of 35, 35 and 7 days respectively. The minimum improvement was observed to be 3.09, 5.76 and 4.54 percent after 21, 21 and 35 days of exposure in liver, kidney and gills respectively. When
experimental fishes were exposed to zeolite only (60 mgL⁻¹day⁻¹) the picture improved further as summarized in table No. 1, 2 and 3. In each case the values of soluble protein contents were observed to be more than the control values. The range of improvement due to zeolite exposure has been 0.53-0.77, 1.6-13.6 and 0.66-0.85 mg/g in liver, kidney and gills respectively.

Zeolites (natural and synthetic) are ion exchangers. The sodium of zeolites is exchanged by lead ions in their molecular sieve Jain et al. (1996, 1997). The possible reaction is shown in fig No. III b-1. The improvement in the values of soluble protein in the experimental tissues exposed only to zeolite, suggests that zeolite is not toxic rather beneficial to the biological system. Zeolites are also used as feed additives in case of cattles. Chelation therapy for metal ion toxicity has also been suggested by Sharma (1995). There are many metal chelators and other means used for remediation of metal toxicity Graziano et al. (1985), Klavassen (1985), Chisom (1970, 1971), Friedheim et al. (1978) and Hammond (1971). Depuration of lead in gill, liver and kidney in Brooktrout has been observed, 12 weeks after transfer to control water Holcombe et al. (1976) and Stripps et al. (1990). The effect of dietary supplementation of iron and ascorbic acid have been reported in the treatment of lead toxicity Suzuki and Yoshida (1979). The chelators, 2, 3-dimercaptopropane-1-sulphonic acid (DMPS) and meso 2, 3-dimercaptosuccinic acid (DMSA) have been used in the treatment of lead poisoning Apshian, (1983), Friedheim et al. (1978) Chisom and Thomes,
(1985) and Graziano et al. (1985). A potential lead antagonist, diethyl dithiocarbonate (DDC) has also been suggested by Gale et al. (1986). L-cysteine and N-acetyl-L-cysteine (NAC) are also promising antidotes for other heavy metal intoxications Llobet et al. (1985, 1986). EDTA (Ethylene Diamine tetra acetic acid di sodium salt) has been reported as chelating agent for copper and zinc toxicity to frog tadpoles Khangrot et al. (1984).

(b) Glycogen contents in liver, kidney and gills:

In control fishes, glycogen contents in liver, kidney and gills observed are almost similar to that of initial control fish. A slight increase in the glycogen contents with increasing age has been observed. As evident from table no. 4, 5 and 6, sublethal concentration of Pb(NO₃)₂ (60 mgL⁻¹day⁻¹) causes significant decrease in the glycogen contents of all three experimental tissues. In case of liver, kidney and gills the maximum decrease in glycogen contents has been observed after 7 days of exposure i.e. 28.9, 29.08 and 29.9 percent respectively while it was minimum after 35 days of exposure i.e. 19.2, 20.0 and 20.2 percent respectively. The reason behind minimum decrease after 35 days of exposure is perhaps due to the development of a little resistance to lead toxicity. In the present study, lead nitrate has been found to inhibit glycogen contents, might be due to increased glycogenolysis as reported by Shaffi (1979). Depression of enzyme activity due to lead nitrate has been reported by Johansson-sjoheek and Larsson (1979). Due to lead toxicity, embryonic abnormalities have
been reported by Ozoh (1979), suggestive of abnormal biochemical contents. Chronic toxicity of lead increases with increasing lead concentrations in the tissues Hodson et al. (1982) and Hodson (1986). Exposure of fish to high concentration of aqueous lead can elicit several biochemical responses Spry et al., (1981), Hodson et al. (1984). According to Mass Oliva (1989) and Rajanna et al. (1990), metal binds strongly with sulphahydryl groups to form mercaptides, inhibiting large number of enzymes containing functional thiol groups as also observed with other heavy metals such as cadmium Paier et al. (1993), mercury Visser et al. (1976) and gold Mandel (1995). Mercury also cause depletion in glycogen contents and hepatic enzymes Shakorri et al. (1994). Cadmium causes marked depletion in glycogen contents in liver and muscles Jinde and Niimi, (1984). When fishes exposed to Pb(NO₃)₂ (60 mgL⁻¹ day⁻¹) along with zeolite (60 mgL⁻¹ day⁻¹), the values of glycogen contents in liver, kidney and gills improved significantly. In case of liver, kidney and gills the maximum improvement in glycogen contents has been observed to be 10.4, 13.7 and 17.8 percent after the exposure of 21, 21 and 14 days respectively. The values of improvement were minimum i.e. 7.64, 10.9 and 14.1 percent after 7, 28 and 28 days of exposure in liver, kidney and gills respectively. When experimental fishes were exposed to only zeolite, the picture further improved as summarized in table no. 4, 5 and 6. In every case, the values of glycogen contents were observed more in comparison to control values. The range of improvement due to only zeolite
exposure has been 0.92-1.97, 1.56-2.76 and 2.34-4.13 mg/g in liver, kidney and gills respectively. Biochemical effects due to lead toxicity and protective action of zeolite has already been reported by Jain et al. (1995). Zeolite (sodium aluminium silicates), natural and synthetic are ion exchanges. The sodium of zeolites present within their molecular sieve are exchanged with lead ions Jain et al. (1996, 1997) The possible reaction is shown in Fig.no.IIIb-1. The improvement in glycogen contents in the experimental tissues exposed only to zeolite suggest that zeolites are not toxic rather can be used for remediation of metal toxicity, as they are also used as feed additives in case of Cattles Jain et al. (1996). There are many metal chelaters and other means used for remediation of metal toxicity Graziano et al. (1985), Klaassen (1985), Chisom (1970,1971), Triedheim et al. (1978) and Hammond (1971). Depollution effect of pistia against lead has been reported by Raj (1995).

The chelators 2,3-dimercaptopropane 1-sulfonic acid and meso 2,3 dimercaptosuccinic acid (DMSA) have been used in the treatment of lead poisoning. Apshian (1983), Friedheim et al. (1978) Chisom and Thomas (1985), Graziano et al. (1985). A potential lead antagonist diethylthiocarbamate (DDC) has also been suggested by Gale et al. (1986), L-cysteine and N-acetyl-L-cysteine (NAC) are also promising antidote for other heavy metal intoxications (Llobet et al. (1985, 1986) EDTA (Ethylene Diamine Tetra Acetic acid di sodium salt) has been reported as chelating agent for copper and Zinc toxicity to frog tadpoles.
Khangrot et al. (1984). Electrochemical monitoring of heavy metal bioleaching by Thiobacillus ferrooxidus has been worked out by Takamtsu and Mangrich (1997). Biogeochemical means for enviromental decontamination were suggested by Adriano (1997). Decreased liver glycogen due to cadmium toxicity in channa punctatus has been reported by Duvale and Shah (1981). Cadmium increased glycogenolytic activity in liver and muscle of Channa punctatus, may be mediated through hormonal or neuromuscular changes as reported my Jinde and Niimi (1984). Biogeochemical means for environmentals decontamination were suggested by Adriano (1997).

(c) Cholesterol contents in liver, kidney and gills:

In control set of fishes, cholesterol contents in the liver, kidney and gills were similar to that of initial control fish. A slight increase in the cholesterol contents was observed with increasing age. As evident from table no. 7, 8 and 9, sublethal concentration of Pb(NO₃)₂ (60 mgL⁻¹ day⁻¹) causes significant increase in the cholesterol of all three experimental tissues. In case of liver, kidney and gills the maximum increase was observed after 35, 28 and 35 days of exposure i.e. 11.33, 11.99 and 5.32 percent respectively, while the minimum decrease observed after 7 days of exposure i.e. 6.82, 7.71 and 1.72 percent respectively.

Liver is the most important target for lead and cadmium Holcombe et al. (1976) and Dallinger et al. (1978). Lead accumulation in bones, scales, gills, kidney and liver has been reported by Holcombe et al. (1976), Patterson and Settle
(1977), Hodson (1978a), Varanasi and Gmur (1978), Bollinberg and Johansen (1979) and Hodson et al. (1982). Various aspects of lead toxicity in the fishes have been reviewed by Jackim (1973), Johansson-Sjobeck and Larsson (1979) and Haux and Larsson (1982). Unique physiological toxicity of lead has been well characterised by Davies et al. (1976) and Hodson et al. (1984). Deleterious biochemical effects due to aqueous lead in fishes have been reported by Spry et al. (1981) and Hodson et al. (1984). In lead workers, cholesterol contents and unsaturated fatty acids were comparatively higher as reported by Matteo valentino et al. (1991), Karai et al. (1982), Apostoli et al. (1985) and Donaldason (1985).

When fishes were exposed to Pb(NO$_3$)$_2$ (60 mgL$^{-1}$day$^{-1}$) along with zeolite (60 mgL$^{-1}$day$^{-1}$) the values of cholesterol contents in liver, kidney and gills improved significantly toward normal. In case of liver, kidney and gills, the maximum improvement in cholesterol contents have been observed to be 7.0, 8.12 and 2.29 percent after the exposure of 35 days, while it was minimum i.e. 5.73, 7.65 and 1.19 percent after 7, 28 and 7 days of exposure respectively. When experimental fishes were exposed to only zeolite (60 mgL$^{-1}$day$^{-1}$), further improvement was observed as summarized in table no. 7, 8 and 9. In each case, the values of cholesterol contents were observed to be more than the control values. The range of improvement due to zeolite exposure has been 1.13-1.84, 0.00-1.09 and 0.85-1.37 percent in liver, kidney and gills respectively, prove its protective role without any side effects.
Ionic property of natural and synthetic zeolite has already been reported by Jain et al. (1995, 1996) and (1997) as illustrated in Fig.No.IIib-1. The improvement in the values of cholesterol contents in the experimental tissues exposed only to zeolite, suggests that zeolites are not toxic rather useful to biological system with reference to protect metal toxicity and other wise also. There are many metal chelators and other means used for remediation of metal toxicity Graziano et al. (1985), Klaassen, (1985), Chisolm, (1970, 1971); Friedheim et al. (1978), Hammond (1971), Bharadwaj, (1995) and Mathur (1995), Sharma, (1995). Depuration of lead in gill, liver and kidney in Brook trout has been reported 12 weeks after transfer to normal water Holcombe et al. (1976) and Strippes et al. (1990). The effect of dietary supplementation of iron and ascorbic acid in the treatment of lead poisoning has been reported by Apshian (1983), Friedheim et al. (1978), Chisolm and Thomas (1985) and Graziano et al. (1985). Potential lead antagonists diethyldithio carbonate (DDC) Gale et al. (1986); L-cystein and N-acetyl-L-cysteine (NAC) Llobet et al., (1985, 1986) and also EDTA (Ethylene Diamine tetra acetic acid di sodium salt) has been reported by Khangrot et al. (1984).

II. Chronic Study:

For long term study, 120 fishes were divided into four equal groups. Group 1 worked as control, group 2 exposed to sublethal concentration of Pb(NO₃)₂, (20 mgL⁻¹day⁻¹), group 3 exposed to Pb(NO₃)₂+Zeolite (each 20
mgL⁻¹day⁻¹) and group 4 exposed to zeolite only (20 mgL⁻¹day⁻¹). After 60, 90, 120 150 and 180 days of exposure, fishes were sacrificed, their liver, kidney and gills were removed and processed for estimation of soluble protein (Lowry method), glycogen (Anthon method) and cholesterol (Libermann-Burchard method) contents. All experiments were reported three times and average values were summarized in tables (10 to 18).

(a) Protein contents in liver, kidney and gills:

In control fishes, protein contents in liver, kidney and gills were almost similar to that of initial control fish. A slight increase with increasing age has been observed. As evident from table no. 10, 11 and 12, sublethal concentration of Pb(NO₃)₂ (20 mgL⁻¹day⁻¹) causes significant decrease in the protein contents of all three experimental tissues. In case of liver, kidney and gills the maximum decrease has been observed after 60 days of exposure i.e. 6.27, 15.07 and 16.8 percent respectively. While it was minimum after 180 days of exposure i.e. 5.09, 8.22 and 10.2 percent respectively. The reason behind minimum decrease after 180 days of exposure, it seems that the fishes become a little resistant to lead toxicity. The toxic effects of lead poisoning in fishes have been reviewed from time to time with reference to haematological and biological variables Jackim (1973), Johansson-Sjoberg and Larsson (1979), Haux and Larsson (1982). The overall decrease in the soluble protein contents is probably due to enzyme inhibition, responsible for protein synthesis Sastri and Gupta (1978). Liver is the
most important target for lead and cadmium toxicity, Holcombe et al. (1976) and Dallinger et al. (1978). Lead accumulates chiefly in bones, scales, gills, kidney and liver, Holcombe et al. (1976), Patterson and Settle (1977), Hodson, (1978a), Varanasi and Gmur (1978), Bollinberg and Johansen (1979) and Hodson et al., (1982). The chronic toxicity of lead increases with increasing lead concentration in the tissues, Hodson et al. (1982) and Hodson (1986). Lead is unique in its physiological toxicity and a series of responses due to lead intoxication has been well characterised by Davies et al. (1976) and Hodson et al. (1984). Exposure of fish to high concentration of aqueous lead can elicit several biochemical responses, Spry et al. (1981) and Hodson et al. (1984). According to Mass Oliva (1989) and Rajanna et al. (1990), metal binds strongly with sulphahydryl groups to form mercaptides, inhibiting large number of enzymes containing functional thiol groups as also observed with other heavy metals such as cadmium, Paier et al. (1993), Mercury, Visser et al. (1976) and gold, Mandel (1995). Mercury cause depletion in soluble protein, glycogen, RNA content and hepatic enzymes, Shakorri et al. (1994). Significant decrease in total blood serum protein along with increase in free amino acids (FAA) due to the toxicity of mercury, indicates break down of total blood serum proteins which in turn increase the FAA pool, Farah Aziz and Shakkorri (1992).

When fishes exposed to Pb(NO₃)₂ along with zeolite (each 20 mg L⁻¹ day⁻¹), the maximum improvement in soluble protein contents in liver, kidney and gills
have been observed i.e. 3.47, 8.74 and 13.8 percent after 180, 60 and 60 days of exposure respectively. The minimum improvement was observed 2.77, 3.32 and 11.2 percent after 120, 150 and 180 days of exposure in liver, kidney and gills respectively. When experimental fishes exposed to zeolite only, the picture improved further. As summarized in table no. 10, 11 and 12, the values of soluble protein contents were more in comparison to control values. The range of improvement due to only zeolite exposure has been 0.32-0.53, 0.67-0.83 and 0.78-0.95 percent in liver, kidney and gills respectively.

Zeolites (natural and synthetic) are ion exchangers. The sodium of zeolite is exchanged by lead ions in their molecular sieve Jain et al. (1996, 1997). The possible reaction is shown in Fig. no. IIIb-1. The improvement in the values of soluble protein in the experimental tissues exposed only to zeolite, suggests that zeolite is beneficial to biological system, as zeolites are also used as feed additive in case of cattles Jain et al. (1996). There are many metal chelators and other means used for remediation of metal toxicity Graziano et al. (1985), Klassen, (1985), Chislom (1971), Friedheim et al. (1978) and Hammond (1971). The effect of dietary supplementation of Iron and ascorbic acid have been reported in the treatment of lead toxicity Suzuki and Yoshida (1979). The chelators 2,3-dimercaptopropane 1-sulfonic acid (DMPS) and meso 2,3-dimercapto succinic acid (DMSA) have been used in the treatment of lead poisoning Apshian (1983), Friedheim et al. (1978), Chislom and Thomas (1985) and Graziano et al. (1985).
Potential lead antagonists, diethyl dithiocarbonate (DDC) Gale et al. (1986) and L-cysteine and N-acetyl-L-cysteine (NAC) Llobel et al. (1985, 1986), have also been reported.

(b) Glycogen contents in liver, kidney and gills:

In control fishes, glycogen contents in liver, kidney and gills observed almost similar to that of initial control fish. A slight increase with increasing age has been observed. As evident from table no. 13, 14 and 15, sublethal concentration of Pb(NO₃)₂ (20mg L⁻¹ day⁻¹) causes significant decrease in the glycogen contents of all three experimental tissues. In case of liver, kidney and gills the maximum decrease in glycogen contents has been observed after 60 days of exposure i.e. 42.98, 28.38 and 17.14 percent respectively, while the minimum decrease was observed after 180 days of exposure in liver, kidney and gills i.e. 30.93, 14.64 and 9.81 percent respectively. Minimum decrease after 180 days of exposure, is perhaps due to resistance develops in fishes against lead toxicity. Inhibition of glycogen contents is perhaps due to glycogenolysis as also reported by Shaffi (1979) and Johansson-Sjöbeck and Larsson (1979).

According to Mass oliva (1989) and Rajaina et al. (1990), metal binds strongly with sulphahydryl groups to form mercaptides, inhibiting large number of enzymes containing functional thiol groups has also observed with other heavy metals such as cadmium Paier et al. (1993), Mercury Visser et al. (1976) and gold Mandel (1995). Mercury also cause depletion in glycogen, contents and hepatic
enzymes Shakossi et al. (1994). Cadmium cause marked depletion of glycogen contents in liver and muscles Jinde and Niimi (1984). Decreased liver glycogen due to cadmium toxicity in Channa punctatus, has been observed by Dervale and Shah (1881). It is possible that increased glycogenolytic activity may be mediated through hormonal or neuromuscular changes Jinde and Niimi (1984).

When fishes exposed to Pb(NO₃)₂ along with zeolite (each 20mg L⁻¹ day⁻¹), the values of glycogen contents in liver, kidney and gills improved significantly. In case of liver, kidney and gills, the maximum improvement have been observed i.e. 19.7, 12.1 and 12.07 percent after the exposure of 60, 90 and 90 days respectively. The minimum improvement was observed i.e. 15.57, 3.42 and 8.54 percent after 120, 180 and 180 days of exposure in liver, kidney and gills respectively. When experimental fishes exposed to zeolite only, the picture improved further. As summarized in Table no.13, 14 and 15, in every case, the values of glycogen contents were observed more in comparison to control values. In fishes, exposed to only zeolite, the values of glycogen contents improved significantly, ranges between 1.08-1.12, 1.29-1.34 and 1.35-1.53 mg/g in liver, kidney and gills respectively.

Zeolites (natural and synthetic) are ion exchangers, as their sodium ions are exchanged by lead ions in their molecular sieve Jain et al. (1996, 1997). The possible reaction is shown in Fig.No.III b. The improvement in the values of glycogen in the experimental tissues exposed only to zeolite, suggests that zeolite
can be used in biological systems safely. There are many metal chelators and other means applied for remediation of metal toxicity, as reported by Graziano et. al. (1985); Klaassen (1985), Chisom (1970, 1971), Friedheim et al. (1978) and Hammond (1971).

(c) Cholesterol contents in liver, kidney and gills

In control fishes, cholesterol contents in liver, kidney and gills were almost similar to that of initial control fish. As evident from table no. 16, 17 and 18, sublethal concentration of Pb(NO₃)₂ (20mg L⁻¹ day⁻¹) causes significant decrease in the soluble cholesterol contents of all three experimental tissues. In case of liver, kidney and gills the maximum increase in cholesterol contents has been observed after 60, 180 and 180 days of exposure i.e. 8.2, 11.8 and 7.47 percent respectively, while the minimum increase was observed after 60 and 60 days of exposure in liver, kidney and gills i.e. 4.69, 7.55 and 4.78 percent respectively. The reason behind minimum increase of it seems that the fishes become a little resistant to lead toxicity. Inhibition of Hepatic cholesterol biosynthesis due to Mercury has been observed by Inoue et al. (1978). Liver is the important target for lead and cadmium toxicity Holcombe et al. (1976) and Dallinger et al. (1978). Lead accumulation is maximum in bones, scales, gills, kidney and liver Holcombe et al. (1976), Patterson and Settle (1977), Hodson (1978a), Varanasi and Gmur (1978), Bollinberg and Johansen (1979) and
Hodsen et al. (1982). The chronic toxicity of lead increases with increasing lead concentration in the tissues Hodson et al. (1982) and Hodson (1986).

Lead is unique in its physiological toxicity and a series of responses due to lead intoxication have been well characterised Davies et al. (1976) and Hodson et al. (1984). According to Mass oliva (1989) and Rajanna et al. (1990), metal binds strongly with sulphahydryl groups to form mercaptides, inhibiting large number of enzymes containing functional thiol groups are also observed with other heavy metals such as cadmium Paier et al. (1993), mercury Visser et al. (1976) and gold Mandel (1995). In lead workers, cholesterol and unsaturated fatty acids were reported comparatively higher. Matteo valentino et al. (1991). Lead produce an increase in cholesterol Karai et al. (1982) and unsaturated fatty acids Apostoli et al. (1985) and Donaldson (1985).

When fishes exposed to Pb(NO₃)₂ (20mg L⁻¹ day⁻¹) along with zeolite (20mg L⁻¹ day⁻¹) the values of cholesterol contents in liver, kidney and gills improved significantly. In case of liver, kidney and gills, the maximum improvement in cholesterol contents have been observed 5.02, 6.42 and 3.48 percent after the exposure of 60, 180 and 180 days respectively. The minimum improvement was observed 1.32, 5.0 and 2.73 percent after 180, 120 and 120 days of exposure in liver, kidney and gills respectively. When experimental fishes were exposed to zeolite only, the picture improved further. As summarized in table no. 16, 17 and 18, in every case, the values of cholesterol contents were observed
more in comparison to control values. The range of improvement due to only zeolite exposure (20mg L⁻¹ day⁻¹) ranges between 0.27-0.69, 0.63-0.80 and 0.68-0.85 in liver, kidney and gills respectively.

According to Jain et al. (1996, 1997), the lead ions are replaced by sodium, present within the molecular sieve of zeolite (fig.IIIb-1). Observations of the present investigation suggests the safe use of zeolite in biological system.

HISTOCHEMICAL STUDY

ACUTE STUDY:

Study of acute toxicity is generally carried out to determine the level of toxic agents which produce adverse effects on experimental animals in a short period of time. The acute dose of heavy metal is such that causes a biological system to deviate from its normal range of variation. The toxicological effects at acute stress generally occur due to the direct action of heavy metal on target tissue. It is also known that living organisms have ability to adapt themselves when exposed to new environment, but severe changes in the environment cause damage to fish life by affecting its resistance mechanism. Due to stress of heavy metal toxicity, biochemical changes can be observed instantaneously which in turn affect bound biochemcals, studied histochemically.

In the present study, protein contents in liver, kidney and gills were inhibited due to lead toxicity as also observed in biochemical study. Initially for 7 and 14 days no change has been observed, but protein depletion in liver, kidney
and gills in 21 days is due to enzyme inhibition as also reported by Sastri and Gupta (1978). Toxic effects of aqueous lead on biochemical responses in fishes has been well characterised by Davis et al. (1976) and Hodson et al. (1984) which in its turn leads to histochemical changes. After 28 and 35 days of exposure to lead nitrate, no further histochemical alternation in the experimental tissues is observed which is perhaps due to adaptation acquired by the fish for its surroundings.

When experimental fishes of group three and four exposed to the mixture of lead nitrate and zeolite and only zeolite respectively, improvement in the protein contents has been observed in comparison to the fishes of group one. Jain et al. (1995, 1996, 1997) have already reported protective action of zeolite on lead toxicity. Zeolites (both natural and synthetic) are ion exchangers. Sodium of zeolite is exchanged by lead in their molecular sieve. According to Chislem (1970, 71), Graziano et al. (1985) and Kloassen (1985), there are many metal chelators and other means used for remediation of metal toxicity. Suzuki and Yoshida (1979) suggested the use of iron and ascorbic acid for the treatment of lead toxicity.

In the present study after 28 and 35 days of exposure of Pb(NO₃)₂ depletion of glycogen contents was observed in liver, kidney and gills. The responses of liver glycogen to cadmium treatment is different in flounders and rainbow trout. In cadmium treated brackish water flounders, the observation of
hyperglycemia and increased liver glycogen suggested an increased glycogenolysis Larsson (1975), Larsson and Haux (1982). When fishes were exposed to the mixture of leadnitrato+zeolite, a further improvement has been observed in glycogen contents in the experimental tissues. Depurationof lead in liver, kidney and gills in brook trout has been observed by Holcombe et al. (1976). Prolonged exposure to cadmium induces the synthesis of metallothioneins in liver Kagi and Nord berg (1979). To evaluate the toxicity by different pollutants, considerable amount of literature is available on the toxicity of metal ions. Gupta and Rajbanshi (1979) reported that gill filament become completely covered by thick mucous layer in *Heteropneustes fossilis* exposed to copper.

After the sublethal concentration of Pb(NO₃)₂ it has been observed that a significant increase in lipid contents in all three experimental tissues (Liver, kidney and gills). In the present study a maximum increase in lipid contents has been observed after 28 and 35 days of exposure. A further improvement has been observed when fishes exposed to the mixture of lead nitrate and zeolite. It is to be noted that zeolites had inflicted the damages in tissues, that are by the toxicity of heavy metal.

II. Chronic study:

The toxicity of lead is systemic and chronic toxicity increases with increasing lead concentration in the tissues Hodson et al. (1982), Hodson (1986). Lead is unique in its physiological toxicity, and a series of responses to
lead intoxication has been well characterised Davies et al. (1976), Hodson et al. (1984). In present study, after 150 and 180 days of exposure of Pb(NO₃)₂ (20mgL⁻¹ day⁻¹) to the experimental fish, the depletion in protein contents in liver, kidney and gills has been observed. The over all decrease in the protein contents is probably due to enzyme inhibition, responsible for protein synthesis Sastri and Gupta (1987). It has been reported that some metals other than lead inhibit erythrocyte ALAD activity in vitro, although this enzyme is the most sensitive to lead Tomokuni (1979). Increase in the activities of hepatic enzymes has been reported to the mixture of Pb(NO₃)₂+zeolite and zeolite only improvement in protein contants has been observed. Protective action of zeolite on lead toxicity in fish has been reported of Jain et al. (1995).

The concentration of lead nitrate produced a mark deplition of glycogen contents in different tissues of fish. In present study after 150 and 180 days of exposure of Pb(NO₃)₂ in liver, kidney and gills, the depletion in glycogen contents has been observed. Black et al. (1966) reported a profound reduction of liver glycogen in rainbow trout. Decreased liver glycogen and decreased liver glucidic content were observed in cadmium treated channa punctatus Dabale and Shah (1981). It is suggested that the highest cadmium dosage induced increased glycogenolytic activity. Inhibition of glycogen contents, as also reported by Shaffi (1979) since lead cause glycogemolysis. When fishes exposed to the mixture of Pb(NO₃)₂+zeolite, a further improvement in glycongen contents has been
observed. The improvement in glycogen contents has been observed. The improvement in the glycogen contents is due to zeolite, suggests that zeolite can be used in biological system safely.

In the present study, the sublethal concentration of Pb(NO₃)₂ in liver, kidney and gills, the maximum increase in lipid contents has been observed after 150 and 180 days of exposure. Lead produce an increase in cholesterol Karai et al. (1981) and unsaturated fatty acids Apostoli et al. (1985) and Donaldon (1985). When fishes exposed to the mixture of lead nitrate+zeolite, the improvement has been observed towards the normal condition. Only zeolite treated, the liver, kidney and gills shows the further recovery. Zeolite can interfere with the toxic activity of heavy metals. From these results it has been concluded that zeolite has come as a boon to counter the toxic effects the heavy metal can inflict on the body systems. Zeolite is a vividly reliable chemical substance which can be employed with confidence to fight against heavy metal toxicity.

**Histopathological study**: 

Liver is an important centre for the disposal of excretory products. It is bound to be affected by the pollutants of any kind as these pollutants become the part of blood. Many workers have studied the histopathology of liver in respect of different pollutants like DDT King (1962), Verma et al. (1974), DDT, BHC, Lindane and Dieldrin, Mathur (1962a,b, 1965, 1976), Endrin Eller (1971), Bhattacharya et al. (1975), Sastry and Sharma (1978), Dutta (1979), Shafi and
Choudhary (1979). Considerable amount of literature is available on the histopathological changes in liver of fish induced by heavy metal toxicity. Extreme vacuolation of liver tissue were observed by Baker (1969) in fish exposed to copper. Gupta and Rajbanshi (1982) showed coagulated appearance, swelling of nuclei, cacluization with hydropic degeneration and necrosis of liver of *Heteropneustes fossilis* exposed to cadmium. In present study the changes were observed in respect of peripherial and central areas of the liver. The clumping of cytoplasm resulting into the appearance of vacoules in the hepatocytes. Due to clumping of cytoplasm the nucleus was displaced in certain stages. Toxicity of mercury of *Sarotherodon mossambicus* induced vacuolization with ruptured hepatocytes, cloudy swelling and degeneration of hepatocytes, hypertrophical nuclei and necrosis, Naidu et al. (1983b).

The haemopoinetic tissue forms a support matrix for the nephrons particularly of the posterior kindney. This support matrix is largely affected and was found degenerated leaving claeer spaces around the tubules. In present study haemorrhage in haemopoietic tissues was observed. Haemorrhage has been found in the observations made by, Sastry and Agrawal (1977) in case of *Ophiocephalus punctatus* exposed to mercuric chloride. Saxena (1981) in *Channa punctatus* and Shrivastava and Pandey (1986) in puntius sophore have also reported degeneration of haemopoietic tissue exposed to cadmium chloride Bowman's capsule exhibit loose shrunken capillaries tufts has also observed in present study. Glomeruli are
primarily concerned with filtration of water, the glomerular filtration rate is bound to be affected by the distortion of the glomeruli and this may also hamper the function of the tubules which are concerned with reabsorption of filtered water. Shrivastava and Pandey (1986) have also observed increase in the size of glomerulus after the exposure of *Puntius sophore* of cadmium chloride. When the influence of zeolite on the histopathological changes induced by heavy metals is taken into consideration, it is noticed that the changes were observed in *Heteropneustes fossilis* exposed to heavy metal toxicity and zeolite combination.

Considerable amount of literature is available on the histopathological changes in the gills of fishes exposed to different concentration of various pollutants. During respiration the gills remain in constant contact with water and considered as the best indicator of any pollution in water. In the gills of *Hilsalisha* and *Rita rita*, Dutta Munshi (1960) has identified mucous glands, basophilic mast cells and acidophilic mast cells, while in *Labeo rohita* has distinguished undifferentiated small cells, large rounded eosinophillic cells and mucous gland cells.

In the present study, histopathological changes in the gills of *Heteropneustes fossilis* exposed to lead nitrate have been observed. Gross histopathological changes were observed in primary and secondary gill lamellae and particularly in microcirculatory system of secondary gill lamellae. Haemorrhage was found in the primary gill rays. The epithelial lining was
eroded and the cells were seen accumulated at the bases of secondary gill lamellae. This was followed by odematous seprration of secondary gill lamellae from primary gill rays. The changes were rapid and gradually extended to secondary gill lamellae where the arrangements of epithelial covering was makedly affected. Skidmore and Tovell (1972) has studied the toxic effects of zinc in rainbow trout, *Salmo gairdneri* and noticed that the epithelial covering of the secondary gill lamellae has lifted away from the pillar cells. Khangrot and Somani (1980) studied the mercury toxicity on *Puntius sophore* and reported that gill epithelium is separted from the basement membrane and pillar cells. In the present investigation the epithelial layers have reduced to a thin membrane. When fishes were exposed to the mixture of lead nitrate and zeolite, it was found that gills did not show much haemorrhage in the primary gill rays, fusion of secondary gill lamellae, degeneration of epithelial cells and odematous separation of secondary gill lamellae from primary gill rays. Only zeolite treated fishes, a further recovery has been observed, shows that zeolite is not toxic rather it is beneficial to biological system.